26 January 2024
EMA/CVMP/IWP/303516/2023
Committee for Veterinary Medicinal Products (CVMP)

Overview of comments received on Guideline on plasmid DNA vaccines for veterinary use (EMA/CVMP/IWP/365817/2022)

Comments from:

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

Stakeholder no.	Name of organisation or individual
1	AnimalhealthEurope

1. General comments

Stakeholder number	General comment (if any)	Outcome (if applicable)
1.	AnimalhealthEurope welcomes the opportunity to comment on the draft revised guideline. The revised draft guideline (especially the section 4. General considerations) is written in a way which seems to (overly) insist on "additional risks" and "concerns" with DNA vaccines (using a single publication which is otherwise not very detailed – see below). This is rather atypical for a technical guideline and may act as a disincentive for Industry to develop such types of vaccines (especially as most of the points that need to be addressed have a direct impact on the development cost & timelines and will also lead to a higher vaccine production cost, also known as Cost of Goods). We would suggest that a more neutral wording is used, if possible. Since there are only limited authorised DNA vaccines in the EU, having a detailed guideline might be more of a hindrance instead of an incentive for innovation. The layout of this draft GL leans closely to European Pharmacopoeia Monograph format, which again is unusual. Developing a targeted EP Monograph on DNA vaccines would be a(nother) step beyond (and is not supported by AnimalhealthEurope at this time).	In light of AhE comments Section 4. General Considerations is updated to reflect more 'neutral' language. Contrary to the comments, and as there is a limited number of authorised DNA vaccines in the EU, guidance is proposed to be advisory and not act as a hindrance to innovation. A risk awareness approach is retained in the General Considerations section and throughout the document. The layout of the guideline closely reflects other EU guidance documents.

2. Specific comments on text

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
Throughout the guideline	1	**Comment:** The guideline refers to "MCS" and "WCS", which might have perception implications. it is proposed to use the terms "Master seed bacteria" or "Master seed lot" and "Working seed bacteria" or "Working seed lot" for clarity. **Proposed change: change "**MCS" and "WCS" to "Master seed bacteria" or "Master seed lot" and "Working seed bacteria" or "Working seed lot", respectively.	Acceptable to change 'MCS' and 'WCS' to 'master seed lot' and 'working seed lot'.
43, 60, 106, 144, 146, 149, 189, 224	1	**Comment:** The terms bacterial and synthetic DNA plasmid is used in the guideline, but it is unclear how these are defined. Most, if not all, plasmids relevant for DNA vaccines are constructed at least partly by chemical synthesis. However, they may be manufactured in bacterial host cells or by synthetic means (*e.g.,* Doggybone). A clear definition is important for the interpretation of the quality section and should be included in the guideline. **Proposed change:** Include definitions of bacterial and synthetic DNA plasmid and align with text in the guideline.	Based on the comments received, a clearer meaning of the terms bacterial and synthetic DNA plasmids has been incorporated into the text. Definitions for these terms are not specifically developed for this guideline.
62-64	1	**Comment:** DNA vaccines may also be used to act against endogenous immunogens based on xenogeneic principle of vaccination. **Proposed change:** Plasmid DNA vaccines may be composed of more than one plasmid coding for different immunogens isolated from a single pathogen (virus, bacterium or parasite) or different pathogens or synthesised de novo **or endogenous antigens**.	Accepted.
77	1	**Comment:** Ph. Eur. 0784 is applicable to subunit vaccines where the antigen is produced using rDNA technology. This is stated in Ph. Eur. 0784 as follows: *"Products of rDNA technology are produced by genetic modification in which DNA coding for the required product is introduced, usually by means of a plasmid or a viral vector, into suitable micro-organisms such as bacteria and yeast, or a suitable cell line of mammalian (including human), insect or plant*	Ph. Eur. 0784 is removed from the legal basis and included in references. Although Ph. Eur. 0784 is not relevant on a legal basis, principles of the monograph will apply to the standards expected for the manufacture and

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		origin. The DNA introduced can then be expressed as a protein. The desired product is then recovered by extraction and purification." As such, the relevance to plasmid DNA vaccines is not evident. **Proposed change:** Please delete the reference to Ph. Eur. 0784	control of DNA plasmid vaccines and in conjunction with the new guideline could be useful for reference.
88-91	1	**Comment:** We are not aware of any scientific support for the recommended limit of >80 % supercoiled plasmid with regard to either efficacy or safety. A general limit of >80 % supercoiled plasmid might limit innovation and prevent safe and efficacious vaccines from entering the European market. We agree that the minimum concentration of plasmid used to establish efficacy should be given and that if supercoiled DNA is used, the amount of supercoiled plasmid as a measurement should be stated (as proposed in part 5.1 Data requirements for Part 2 Quality, line 135-136). Consistent with general principles applied to the establishment of control test specifications for vaccines in EU, the limit should be established in each individual case based on quality, safety, and efficacy data. Lee et al, 2018 is used as reference for the recommendation that the pDNA should be supercoiled to more than 80% to help prevent the potential risk of insertional mutagenesis. Lee et al, 2018 is a review article claiming that the risk of integration is a function of the DNA being supercoiled but is not providing any reference to actual data in support of this statement. The WHO states in the more recent DNA vaccine guideline from 2021 (Annex 2, Guidelines on the quality, safety, and efficacy of plasmid DNA vaccines, WHO Technical Report Series, No. 1028, 2021) that *"Although chromosomal integration of the plasmid DNA was initially a major theoretical concern, the data obtained to date have not borne out this concern."* The WHO guideline refers to several recent scientific publications. As described in the "Guideline on plasmid DNA vaccines for veterinary use" line 325-334, the potential concern regarding chromosomal integration of the plasmid DNA will be addressed in each case in the mandatory integration studies.	Acceptable to delete the sentences based on the comment provided; the reference to *Lee et al* is removed. However, the following sentence is included instead at line 88– 'Integration studies, where relevant, should be undertaken with the finished product. The percentage of supercoiled plasmid should be stated.' Based on the comments received the following, at line 91, is also removed as unnecessary text 'Additionally, the use of minicircle and ministring DNA reduces the risk further as the unmethylated CpG (cytosine-phosphate-guanine) repeats are removed (Lee et al, 2018).'

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		Lee et al, 2018 also claims that *"The guidelines for industrial production of DNA vaccines published by the FDA recommend that the pDNA is supercoiled to more than 80% to help prevent insertional mutagenesis."* It is true that the FDA Guidance for Industry, Considerations for plasmid DNA vaccines for infectious disease indications, guideline, Nov 2007, recommends the plasmid DNA to be preferably >80% supercoiled. However, the FDA guideline recommends >80% supercoiled plasmid DNA as a bulk release criterion without mentioning insertional mutagenesis. The proposed recommendation based on Lee et al, 2018 therefore appears to be stricter than the FDA guideline since it can be interpreted to apply for finished product at end of shelf life. It can further be mentioned that intact supercoiled plasmid DNA inevitably will be degraded by nucleases starting shortly after vaccination, and it is therefore unclear how a general recommended limit of >80% supercoiled plasmid can contribute to increased safety beyond what is already required. Based on all the above, we suggest removing the following sentences *"The use of supercoiled DNA (scDNA) can reduce the potential risk of insertional mutagenesis. It is recommended that the pDNA should be supercoiled to more than 80% to help prevent the potential risk of insertional mutagenesis (Lee et al, 2018)."* **Proposed change:** ~~The use of supercoiled DNA (scDNA) can reduce the potential risk of insertional mutagenesis. It is recommended that the pDNA should be supercoiled to more than 80% to help prevent the potential risk of insertional mutagenesis (Lee et al, 2018).~~	
98-101	1	**Comment:** With respect to the risk on integration, albeit each product must be assessed on a case-by-case basis, data and supportive evidence from existing registered plasmid backbones / DNA vaccine platforms (lines 51-53) should also be taken into consideration in the risk assessment. This would strengthen the confidence when evaluating new candidates based on	Acceptable proposal.

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		comparable registered plasmid backbones / DNA vaccine platforms with known safety profiles for similar applications (target species, target tissue, route of administration, amount of plasmid, age of animals etc.). **Proposed change:** Please include that supportive evidence from registered comparable plasmid backbones / DNA vaccine platforms will be taken into consideration in the risk assessment.	
123-124	1	**Comment:** Antibiotic resistance genes are used for the selection of bacterial host cells carrying the plasmid DNA during production. Such a gene should thus be functional in the bacterial host cells. However, it should not be functional in the animal being vaccinated with the plasmid DNA. It is noted that in view of the common route of administration of DNA vaccines (intramuscular) and based on literature the risk of the plasmid DNA being shed by the vaccinated animal and subsequently being taken up by environmental bacteria is negligible. The sentence as it is written now suggests that the presence of antibiotic resistance genes in the plasmid DNA is not acceptable at all. **Proposed change:** ~~In principle,~~ ~~t~~**T**he presence of ~~(functional)~~ antibiotic resistance genes in the finished product ~~is not acceptable.~~ **may be allowed for selection purposes in the bacterial host; however, such genes must not be functional in the vaccinated animal.**	Accepted with some amendments. It is agreed that the original text was too restrictive as the presence of antibiotic resistance genes in the plasmid is possible. The final text reads: "*The presence of antibiotic resistance genes in the finished product should be avoided, wherever possible; if used for selection purposes in the bacterial host, it should be assured that any antibiotic resistance or other residual genes in the finished product are non-functional in vaccinated animals and not transferable to other organisms.*"
135-136	1	**Comment:** In these lines it is stated that the minimum concentration of plasmid DNA used to establish efficacy should be given (if supercoiled DNA is used, the amount of supercoiled plasmid as a measurement of the potency should be stated). This suggests that the concentration of total plasmid DNA (pDNA) rather than supercoiled plasmid DNA (scDNA) may be used for	If the potency is determined by measuring scDNA then the percentage of scDNA (biologically active) should be stated.

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		quantification of active substance. In lines 89-90 it is recommended that the pDNA should be supercoiled to more than 80% and lines 280-281 state that a quantitative assay to determine the amount of scDNA needs to be established. The text in lines 135-136 seems in conflict with this. **Proposed change:** Please clarify the requirements with respect to quantification of plasmid DNA, *i.e.,* total pDNA and/or scDNA.	Proposed rewording at line 135-136; 'The minimum concentration of plasmid used to establish efficacy should be given (if supercoiled DNA is used, the **percentage** of supercoiled plasmid as a measurement of the potency should be stated)' See above, the sentence at line 89 – 90 is deleted. Rewording at line 279-280 for clarity; A quantitative assay to determine the amount of (sc) DNA, for example by HPLC or other suitably validated tests, needs to be established, **where supercoiled DNA is used to measure potency.**
161	1	**Comment:** It is stated that "DNA sequence homology checks of the plasmid with all published DNA sequence data of the target species should be performed and the information given in the application dossier." It may be difficult to be exhaustive in the published DNA sequence data and since no validated databases exist, and scientific knowledge evolves, such information can vary during the product lifetime. **Proposed change:** DNA sequence homology checks of the plasmid with ~~all published~~ DNA sequence data of the target species should be performed and the information given in the application dossier.	Accepted.

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168-170	1	**Comment:** It is stated that it should be demonstrated that the host cell if free from bacteriophage and other extraneous agent contamination in line with Ph. Eur., requirements. According to Ph. Eur. 5.2.5 (Management of extraneous agents in immunological veterinary medicinal products), extraneous agents testing follows a risk assessment-based approach. **Proposed change:** ~~It should be demonstrated that the host cell is free from bacteriophage and other extraneous agent contamination~~ **The risk of potential extraneous agents of the host cell should be assessed** in line with Ph. Eur. Requirements **(including bacteriophages) and tested accordingly where applicable**.	Acceptable, with amendment for clarity: '**A risk-based approach can be used to** demonstrate that the host cell is free from bacteriophage and other extraneous agent contamination in line with Ph. Eur. requirements.'
173-174	1	**Comment:** Investigation of the expression of prokaryotic genes, such as a selection marker, in a eukaryotic cell line is not straight forward. **Proposed change:** It would be preferable to have this aspect covered in a risk assessment.	Accepted with amendment: '**On a risk basis** the expression of prokaryotic genes, such as a selection marker, in a eukaryotic cell line should be investigated.'
175-177	1	**Comment:** It is stated that the likelihood of any cross-contamination *e.g.*, by recombination with endogenous sequences in the cell substrate used during the construction or production of the DNA plasmid should be evaluated. It is assumed that this may be achieved by sequencing of the plasmid DNA of the MCS. **Proposed change:** The likelihood of any cross-contamination e.g., by recombination with endogenous sequences in the cell substrate used during the construction or production of the plasmid DNA should be evaluated. **This may be demonstrated by sequencing of the plasmid DNA of the master seed lot.**	Accepted proposal.
195	1	**Comment:** Clearance capacity should be sufficiently established based on the difference before and after the purification steps in general (not necessarily assessed after each step).	Accepted with further amendment: 'Clearance capacity for the removal of contaminants will be established for the purification process by the

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		Proposed change: Clearance capacity for the removal of contaminants will be established for the purification process by the difference in contaminant levels before and **after** ~~each~~ **purification steps**.	difference in contaminant levels before and after **critical** purification steps.'
218-221	1	**Comment:** It is stated that "*the integrity of the plasmid DNA sequence should be demonstrated by validating the proposed storage and recovery conditions of WCS as the worst-case scenario*." It is not clear what is meant by this, and this appears redundant with the paragraph immediately above (lines 212-217): if the sequence of the plasmid is kept 100% identical between Master Seed, Working Seed and finished product (as per paragraph above) this paragraph has no added value (versus paragraph above). **Proposed change:** Please remove the entire paragraph (lines 218-221).	Removal of the paragraph is not accepted. The text is retained and elaborated further: 'The integrity of the plasmid DNA sequence should be demonstrated by validating the proposed storage and recovery conditions of the working seed lot as the worst-case scenario, **i.e. at more extreme periods/temperatures of storage proposed, unless otherwise justified.'**
222-223	1	**Comment:** It is stated that it should be demonstrated that MCS and WCS/starting molecules for *de novo* synthesis are free from extraneous microbial agents in accordance with the Ph. Eur. requirements. According to Ph. Eur. 5.2.5 (Management of extraneous agents in immunological veterinary medicinal products), extraneous agents testing follows a risk assessment-based approach. **Proposed change:** ~~It should be demonstrated that MCS and WCS/starting molecules for de novo synthesis are free from extraneous microbial agents in accordance with the Ph. Eur. requirements.~~ **The risk of potential extraneous agents of the Master Seed Lot and Working Seed Lot/starting molecules should be assessed in line with Ph. Eur. 5.2.5 and tested accordingly where applicable.**	Accepted with amendment: 'The risk of potential extraneous agents of the master seed lot and working seed lot/starting molecules should be assessed in **accordance** with Ph. Eur. requirements.'
224-225	1	**Comment:** It is stated that bacterial plasmids should be demonstrated to be free from potential contamination, for example with endogenous viruses,	Accepted with amendment:

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		including wild-type from of any viral vectors. It is assumed that this may be achieved by sequencing of the plasmid DNA of the Master Seed lot. **Proposed change:** Bacterial plasmids should be demonstrated to be free from potential contamination, for example with endogenous viruses, including wild-type forms of any viral vectors. **This may be demonstrated by sequencing of the plasmid DNA of the Master Seed Lot.**	'Bacterial plasmids should be demonstrated to be free from potential contamination, for example with endogenous viruses, including wild-type forms of any viral vectors, for example **by sequencing of the plasmid DNA of the master seed lot.'**
245-248	1	**Comment:** For the batch potency test, it is specified that an appropriate level of functional activity should be demonstrated at least during development and that a qualitative expression assay may not be required for routine testing. In the same way, we suggest specifying for identity that confirmation of the expressed antigen should be documented at least during development and that the assay may not be required for routine testing. **Proposed change:** Confirmation of the identity of the expressed antigen should also be documented **at least during the development phase** through the use of specific assays, such as Western Blot, immunofluorescence assay (IFA) and/or ELISA. For vaccines containing plasmids encoding non-antigenic biologically active molecules, confirmation of the identity of the expressed molecule should be assessed with an appropriate bioassay. **The assay may not be required to be performed on a routine basis for identity.**	Accepted. In line with Ph. Eur. 0062 identification testing is carried out on each batch using suitable methods. Therefore, it is the responsibility of the applicant to provide a suitable method for routine testing for identity of the active substance in the finished product, albeit this may differ from the test performed during development (identity of the expressed antigen in the finished product) but should be deemed appropriate. Text is reworded (line 267 -282) and split into in process and finished product testing. Text amended to remove 'and/or ELISA'.
265-266	1	**Comment:** For acceptance criteria of impurities during manufacture and at release, the option to quantify these per ml final product should be added.	Accepted.

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		Proposed change: "For all impurities, acceptance criteria based on consistency batches and safety studies should be established per quantity of total DNA, **per ml final product**, or EU/ml for endotoxins (as per Ph. Eur. requirements)."	
274-275	1	**Comment:** It is stated that "*Whatever the assay, an approved in-house reference preparation should be established, where possible, from an appropriately characterised batch of vaccine that has been shown to be efficacious (or of the same quality as the efficacious batches)*". Flexibility should be given in the choice of the in vitro method for potency test with other options than an approved in-house reference. **Proposed change:** Please delete.	Not accepted. The sentence is considered to allow for flexibility however suggest to amend the sentence in light of the comment: **Where possible,** an approved in-house reference preparation should be established, ~~where possible,~~ from an appropriately characterised batch of vaccine that has been shown to be efficacious (or of the same quality as the efficacious batches).
288	1	**Comment:** For vaccines in general, we are not aware that there is a specific requirement to provide consistency data from 3 different batches of antigen/active substance. Typically, two batches of antigens are accepted in EU applications. We wonder why there seems to be additional requirements for plasmid DNA vaccines in this regard. We would suggest revising the wording accordingly. **Proposed change:** Preferably, the three batches of vaccine should be prepared from ~~three separate~~ **more than one** active substance batches.	Accepted in principle, with amendment: The three batches of vaccine should be prepared from **at least two different** active substance batches.
303-304	1	**Comment:** We suggest removing the word "functional" from the sentence "*The potency test should be used to determine if the product is efficacious (functional) over the proposed shelf life of the product*.". It should be unnecessary to include the word "functional" since the potency test performed during stability should be appropriate to determine if the product is efficacious over the proposed shelf life. The word "functional" could	Accepted.

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		perhaps be interpreted to add excess restriction and reduce flexibility with regard to the potency test used in the stability study. **Proposed change:** The potency test should be used to determine if the product is efficacious ~~(functional)~~ over the proposed shelf life of the product.	
310		**Comment:** For safety, it is mentioned that batches of the "maximum dose should be used." For the purposes of clarity, it should be further specified which dose is in question (*i.e.*, if it is only potency/DNA content, or also purity (*e.g.*, protein content)/endotoxin content). Talking about worst case scenarios for safety in the same approach/reasoning of the general considerations (section 4), would a minimum % of scDNA be a better approach to determine certain risks rather than maximum dose?	For safety, batches with maximum potency should be used and consideration given to the maximum levels of impurities. Therefore, the text is amended for clarity; 'Unless otherwise justified, batches with the maximum dose **(i.e. maximum titre, antigen content or potency, also considering maximum level of relevant impurities e.g. endotoxins)** should be used.'
315-318	1	**Comment:** It is considered relevant to investigate the biodistribution of the plasmid DNA by the most sensitive method available (or rather, an appropriately sensitive method). However, it is not considered necessary to also investigate the cellular uptake of the plasmid DNA. The risk for cellular uptake and subsequent integration is considered to be sufficiently addressed by studies required for integration risk analysis. According to WHO guidelines on the quality, safety, and efficacy of plasmid DNA vaccines (Annex 2, Guidelines on the quality, safety, and efficacy of plasmid DNA vaccines, WHO Technical Report Series, No. 1028, 2021), biodistribution of the plasmid DNA vaccine outside of the injection site is not expected to occur at clinically relevant levels. Therefore, any cellular uptake outside of the injection site is not expected to occur at clinically relevant levels either. Further, as cellular uptake of the plasmid DNA at the injection site is a pre-requisite for the vaccine to be efficacious, cellular uptake at the injection site will be	Acceptable proposal with amendment; 'Using **suitably** sensitive methods, the extent of **plasmid** DNA distribution to the target and surrounding tissue including the draining lymph nodes should be analysed at various time points.'

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		satisfactorily demonstrated through immunogenicity trials. As investigation of cellular uptake is not required for evaluating aspects related to neither the safety nor efficacy of the plasmid DNA vaccines, the reference for requirement to investigate cellular uptake as part of the biodistribution study should be removed. **Proposed change**: Using ~~the most~~ **appropriately** sensitive methods available, the extent of **plasmid** DNA distribution to ~~and the cellular uptake by~~ the target and surrounding tissue including the draining lymph nodes should be analysed at various time points.	
319-320	1	**Comment:** The timing of sampling should be justified based on the biodistribution and persistence data of the plasmid, independent of a requirement of gene expression. Investigation of the persistence of the plasmid in the tissues is likely to be a more sensitive method for plasmid detection than investigation of expression of genes. Although the potential risk for tumorigenicity at the injection site (although considered to be very low) relates to persistence of the plasmid DNA rather than expression of genes by the plasmid, it is the persistence of the plasmid that should determine the timing for sampling. Also, it is important to note that previous concerns related to integration, autoimmunity and immunopathology caused by DNA vaccines have not been borne out (Annex 2, Guidelines on the quality, safety, and efficacy of plasmid DNA vaccines, WHO Technical Report Series, No. 1028, 2021). **Proposed change:** The timing of sampling should take into account information on the ~~duration of gene expression and~~ persistence of the **plasmid** DNA in the body of the vaccinated animal.	Acceptable proposal. The need for integration studies should be based on risk assessment. Amended text: 'The timing of sampling should take into account information on the **biodistribution and** persistence of the plasmid DNA in the body of the vaccinated animal.'
324-329	1	**Comment**: It is not straightforward to study integration of plasmid DNA into the host genome. The text in lines 326-329 indicates such testing is needed if plasmid DNA is detected at the site of administration which, assuming	Line 325 states; 'These studies, where relevant, should be undertaken with the finished product.' Therefore, studies are only needed where

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		plasmid DNA will remain at the site of administration for some time, means integration studies are always needed. **Proposed change:** More guidance on the circumstances under which integration studies need to be conducted should be conducted would be helpful. Integration studies should be waived if biodistribution and persistence studies indicate that plasmid DNA is not persisting over time at high levels. In this case, a risk assessment should suffice.	relevant, the need for integration studies is based on risk assessment.
85 and 324-334	1	**Comment:** The differentiation between tumorigenesis/tumorigenicity and oncogenesis/oncogenicity is unclear? If a potential induction of tumour of the cells of the target animals, **from an acellular agent** (such as nucleic acid), is expected, then this would be **oncogenicity**. As usually with DNA vaccines no cells are injected to the animal (purification process of the harvest), it is not the tumorigenicity that should be investigated but the oncogenicity? As per FDA/WHO definitions (e.g.: **FDA Guidance for Industry (February 2010)** Oncogenicity is defined as the property of certain biological agents (e.g., viruses) or materials (e.g., nucleic acids) that are capable of immortalizing cells and endowing them with the capacity to form tumors. **WHO** Annex 3, TRS No 978 : Oncogenicity is defined as The capacity of an acellular agent – such as a chemical, virus, viral nucleic acid, viral gene(s) or subcellular element(s) – to cause normal cells of an animal to form tumours. The tumours that arise in an oncogenicity test are of host origin, whereas in a tumorigenicity test, the tumours are derived from the inoculated cells. Design for tumorigenicity tests (such as described for *e.g.,* in human Ph. Eur. 5.2.3) may not be suitable. **Proposed change:** Please consider for e.g., L 330: "tumourigenicity' to be replaced by "oncogenicity' (twice), also for L85.	Acceptable proposal. Text is updated with 'oncogenicity'.

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324-335	1	**Comment:** The obligation to perform a carcinogenicity study is considered as relative if the target species is not destined for consumption (like broiler or salmon) -> "life expectancy of target animal". If the principles of VICH GL28 (carcinogenicity) are applied, we speak of a 24-month rat study and/or an 18-month mice study. Obtaining an 80% supercoiled DNA should allow the absence of such a study.	Current text is considered acceptable.
331-333	1	**Comment**: A requirement for recording the incidence of tumours at the end of every safety and efficacy experiment is not considered to be justified. It is proposed to limit estimation of this prevalence only to the pivotal target animal safety studies, supported by pharmacovigilance data post marketing. (See proposed change below). Also, it is not sufficiently clear from the text that the requirement for estimating prevalence of tumours only applies in cases where integration is detected or suspected from the results of the integration study, despite the initial comment in line 326 about "a step-by-step analysis should be carried out". The pre-requisite should be made clearer. **Proposed change:** Further, the incidence of tumours in the target species, particularly at the site of injection and in the target tissues, should then be recorded at the end of each **pivotal target animal** safety ~~and efficacy~~ study	A partially accepted proposal with amendment. The length of the study is important therefore reference to efficacy studies, as often longer studies, should be included. The text has been amended to increase clarity and also provide flexibility in the approaches, as follows: 'If integration is detected or suspected, and a risk of oncogenicity due to the life expectancy of target animals is identified, a test for oncogenicity in a susceptible laboratory animal system could be carried out. Alternatively, the incidence of tumours in the target species, particularly at the site of injection and in the target tissue, could be recorded at the end of pivotal target animal safety and relevant efficacy studies (e.g. duration of immunity). In case any undesirable results are obtained in the laboratory

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			animal system, an investigation in the target animal is required.'
343	1	**Comment:** It may not be easy to design studies to address the adverse effects on the immune system and this should not be a pre-requisite for registration if there is no particular risk identified. **Proposed change:** "Specific studies **may** be conducted"	Accepted, however text is amended; '**Where relevant, specific** studies **should be** conducted to address the possibility of adverse effects on the immune system, particularly if cytokine or other immunomodulatory genes are used as adjuvants.'
343-344	1	**Comment**: Some clarity on the type of study that may be considered to address examination of immunological functions would be helpful. We would propose the addition of a "classic example", see below. **Proposed change**: Specific studies should be conducted to address the possibility of adverse effects on the immune system, particularly if cytokine or other immunomodulatory genes are used as adjuvants. **A suitable study may be a side-by-side comparison assessing a possible negative impact (of the vaccine under development) on a serological response induced by another vaccine in the target species.**	Acceptable proposal.

Please add more rows if needed.