14 November 2011
EMA/CVMP/IWP/897330/2011
Committee for Medicinal Products for Veterinary Use (CVMP)

Overview of comments received on 'Guideline on the design of studies to evaluate the safety and efficacy of fish vaccines' (EMA/CVMP/IWP/314550/2010)

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

Stakeholder no.	Name of organisation or individual
1	IFAH Europe
2	European Coalition to End Animal Experiments (ECEAE)
3	PHARMAQ AS

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1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)
1	IFAH-Europe acknowledges the opportunity to provide comments to this draft guideline. As a number of fish vaccines have been or can be classified as MUMS products (cf. EMA/CVMP/IWP/123243/2006), a reference to the reduced data requirements for these products in the proposed guideline would be appropriate.	Agreed – the following text will be included in Paragraph 1 (Introduction) lines 51-53: *For some fish species / diseases reductions in the requirements may be acceptable as outlined in EMA/CVMP/IWP/123243/2006: 'Guideline on data requirements for IVMPs intended for minor use or minor species/limited market'.*
2	The European Coalition to End Animal Experiments (ECEAE) is the pan-European member of the International Council on Animal Protection in Pharmaceutical Programmes (ICAPPP). We are an umbrella organisation representing animal protection organisations across 19 member states who campaign peacefully to end animal experiments. This guideline refers to the conduct of studies in live animals. Hence ECEAE urges the CVMP to incorporate the principles of the 3Rs into the guideline where appropriate in the interests of animal welfare. Suggestions for additional text are made in the specific comments section below. ECEAE notes that guidance on quality requirements for fish vaccines is now outlined in "Requirements for the production and control of immunological veterinary medicinal products" and specific details of animal numbers etc. have been omitted. ECEAE welcomes this change as Guidance Document 7BIm9a specified the use of more fish in batch potency tests than have been required by the monographs of	

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	the European Pharmacopoeia. This lack of consistency may have encouraged manufacturers to use more fish than necessary rather than risk regulatory rejection.	
3	PHARMAQ welcomes the new guideline for the design of studies to evaluate the safety and efficacy of fish vaccines in order to give a better overview of the current requirements.	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
74 – 75	1	Comment: It is practically impossible to address all variations that might possibly occur in farms, especially for genetic variation. Proposed change: *"~~Any~~ Variation from those which will be encountered under commercial use conditions should be addressed. Fish used in studies should preferably be derived from stock used commercially."*	Agreed – text amended as suggested.
85 – 89	1	Comment: When referring to the environment where vaccination is done, this is feasible. However, if challenge also needs to be done in the environment where challenge exposure happens in the field (for salmon this is usually seawater) then this is not practically feasible for laboratory studies. Most challenge models in salmons are in fresh water on non smoltified salmon. Proposed change: *"Water quality including temperature and salinity (e.g. freshwater versus seawater) used in each laboratory study during vaccination must be relevant to the environment under which the vaccine will be used for commercial purposes. For challenge model development, consideration can ~~must~~ also be given to possible changes in the water conditions / environment which fish may be exposed to during the life cycle (e.g. changes associated with transfer to sea)."*	Agreed – text amended as suggested.
91 – 93, table 101	1	Comment: This section is unclear. Studies should be carried out at the water temperature '*relevant for the species of fish*'. For salmon 'relevant for the species' would range from 2 – 20 °C. The relation to the table with the 'examples of optimal water temperature' is not clear. Also the optimal water temperatures in the table seem open for discussion, as e.g. for salmon and rainbow trout the optimal temperature for the species is broader.	Agreed – table removed and lines 91-97 deleted (refer to next comment for details).

		Proposed change: Please remove the table: *"~~The table below gives examples of optimal water temperatures for some fish species.~~ The laboratory studies should be carried out at the water temperature(s) relevant for the species of fish, the disease and the data to be obtained from each study."*	
92 – 100	1	Comment: The proposed text suggests onset of immunity should be documented for each (low/high) temperature. This is not practical and also not relevant as the onset of immunity is expressed in degree-days. It does make sense to evaluate different situations in case there are different production practices: e.g. for Salmon two production systems are used, with S0 or S1 smolts, which are stocked at different seasons and different temperatures. When feasible, field trials should preferably be performed for both situations. However, the farmer should be responsible for making sure the fish are raised in the optimum environmental conditions in order to avoid stress that may impact the physiological response to the vaccine. Proposed change: *"The different climatic conditions and water temperatures within the European Union should be considered, when relevant for the fish species/disease in question. Some studies may need to be performed <u>following the main production practices for the species (e.g. S0 and S1 stocking for salmon)</u> ~~both at the high and the low end of the temperature span for the relevant fish species~~."*	Partially agreed. As OOI is expressed in degree days it is accepted that a specific reference to the temperature at which OOI studies are conducted is not necessary in the guideline. In addition, the first paragraph of section 4.2 states that water temperature in the study should be relevant to the normal use conditions for the vaccine. Lines 91-97 therefore deleted. However, as disease profile may vary with climatic conditions / water temperatures and also for parenteral vaccines, higher water temperatures are associated with increased local reactions, vaccine performance at high – low end of temperature range is an important aspect to be considered. The g/line text does not require studies to be performed at both high & low temperatures only that this should be considered. A sentence has been added that the chosen *conditions* should be justified. Text amended as follows:

			'The different climatic conditions and water temperatures within the Community should be considered, when relevant for the fish species/disease in question. Some studies may need to be performed both at ~~the~~ high and low ~~end of the~~ temperature ~~span~~ for the relevant fish species / disease distribution. Similarly, some studies may need to be performed following the main production practices for the species (e.g. S0 and S1 stocking for salmon). The chosen conditions should be justified by the applicant for each study.'
102 – 105	1	Comment: We agree that onset of immunity documented in laboratory trials should be expressed in degree-days. However, this is less relevant and less informative for duration of immunity. Furthermore it is too complex to document degree-days in field studies. Therefore the duration of immunity should be expressed in weeks (or months) under normal farming conditions. Proposed change: *"To account for the fish being poikilothermic animals and taking into account the fact that immunity in fish is temperature dependant and that the frequency and intensity of injection site reactions increases with higher water temperatures, all comparative data from safety and efficacy studies (~~both~~ from laboratory ~~and field~~ studies) involving fish should be based on "degree-days", except the duration of immunity."*	Partially agreed. Accepted that in general calculation of degree days from field data is complex. However, to allow for situations where the DOI is short and therefore may be studied under laboratory conditions, the text has been revised as follows: *"To account for the fish being poikilothermic animals and taking into account the fact that immunity in fish is temperature dependant and that the frequency and intensity of injection site reactions increases with higher water temperatures, ~~all~~ comparative data from safety and*

			efficacy laboratory studies ~~(both laboratory and field studies)~~ involving fish should be based on "degree-days"'.
135 – 140	1	Comment: We suggest that consideration should be taken when requesting studies in different fish species that can be affected by the same pathogen. Some diseases are not restricted to fishes from the same genus or family and therefore, taxonomic classification may not be the best scientific basis to compare physiological response to vaccination. The disease agent and its pathogenesis and the rearing conditions of the fishes are more appropriate criteria for assessment of safety and efficacy. For example: ▪ *Tenacibaculum maritimum* (bacterial marine skin disease) affects all major tropical marine species raised in Asia, cold water marine Salmonids and temperate species such as Mediterranean Sea Bass/bream; ▪ Iridovirus has been isolated from various fish species: Tilapia (*Oreochromis niloticus*), Barramundi (*Lates calcarifer*), Japanese Yellowtail (*Seriola spp*), Red Sea Bream (*Pagrus major*) and Grouper (*Epinephelus fuscogutatus*). Proposed change: *"Studies performed in one species of fish may be considered relevant for the evaluation of safety in a second species of fish ~~of the same genus or taxonomic family~~, provided that they are kept under the same environmental conditions. In such case, there should be supportive data from studies in the second species. It may for example be considered unnecessary to carry out laboratory safety studies in trout if such studies have been carried out on other species (e.g. salmon) ~~of salmonids~~, and if field studies in trout are available."*	Partially agreed. For parenteral vaccines, factors such as the size of the fish/ temperature/ water quality etc at vaccination can contribute to local reactions. In order to consider a study in one fish species as representative of the safety profile in other species, it is important that these factors are similar for vaccination of both species. Text amended as follows: *"Studies performed in one species of fish may be considered relevant for the evaluation of safety in a second species of fish ~~of the same genus or taxonomic family~~, provided that ~~they are kept under~~ the ~~same environmental~~ recommended conditions for use of the vaccine in both species are similar e.g. similar fish size/ water temperature and quality etc at the time of vaccination. In such case, there should be supportive data from studies in the second species. It may for example be considered unnecessary to carry out laboratory safety studies in trout if such studies have been carried out on other species (e.g. salmon) ~~of~~*

			~~salmonids~~, and if field studies in trout are available."
152 – 153	1	Comment: Systemic and local reactions are evaluated macroscopically using Speilberg scoring. There are no methods/criteria in place for microscopical examination and the need for this is unclear. We propose to remove this requirement. Proposed change: At the end of the monitoring period, the fish should be slaughtered and examined for systemic and local reactions~~, both macroscopically and microscopically~~.	Partially agreed. While not relevant for existing fish vaccines, microscopic examination may be relevant for vaccines in the future. Text revised as follows: At the end of the monitoring period, *when appropriate,* the fish should be slaughtered *humanely using a method described by Directive 2010/63/EC* and examined for systemic and*/or* local reactions, ~~both~~ macroscopically and*/or* microscopically *as appropriate*.
166 – 167	1	Comment: See comment on lines 135 – 140 and lines 236 – 239; there should not be a need to perform field trials on each species. Proposed change: *"It may be more appropriate to evaluate the long term safety effects of vaccine administration over the life span ~~of each fish species~~ in field studies as discussed in section 6 below."*	Agreed – text revised as recommended.
185 – 186	1	Comment: Demonstration of efficacy by means of an alternative method based on antibody response should be mentioned as well to reduce the number of challenge studies needed. Proposed change: *"~~Challenge~~ Efficacy data are required for each proposed indication and for all target species in which efficacy is claimed. Challenge studies can be replaced by an alternative method based on antibody response when the correlation with efficacy has been demonstrated."*	Agreed. Text revised as follows: *"~~Challenge~~ ~~data~~ Data are required for each proposed indication ~~and~~ for all target species ~~in~~ for which efficacy is claimed. Where justified, challenge studies can be replaced by an alternative method based on antibody response when a correlation with efficacy has been demonstrated."*

216 – 219	1	Comment: From an animal welfare point of view, it is generally not acceptable to keep unvaccinated fish on site as 'indicators of exposure' when a positive control is used. Proposed change: "~~*If a positive control (e.g. a comparator vaccine) is used, consideration should be given to maintaining a (small) group of non vaccinated fish in a separate test pen to serve as indicators of exposure to disease(s) at farm level. Once the relevant infection has been diagnosed in the controls they can be slaughtered.*~~"	Not agreed. The use of a non-vaccinated group to monitor the occurrence of natural exposure is considered to be of benefit to field studies where positive controls are used. The guideline recommendation is to give 'consideration' to the use of a 'small' group of non-vaccinated fish. Line 218-219 has been amended to include a reference to humane slaughtering of these fish as follows: 'Once the relevant infection has been diagnosed in the controls they can be slaughtered humanely *using a method described by Directive 2010/63/EC.* In addition, lines 226-227 have been revised to highlight the importance of using a clinically relevant indicator of disease as follows: *"The method of identification and confirmation of the presence of the causal agent(s) for the natural challenge in each group is an important factor for field studies involving fish. The method used must be relevant to the disease situation and should be recorded for a representative number of fish in each group".*
236 – 239	1	Comment: See also comments on lines 185 – 186; the use of serology as alternative for challenge should be mentioned. Proposed change: *"Omission of field studies and submission of ~~challenge~~*	Agreed – text revised as recommended.

		laboratory studies only may be accepted if adequately justified by the investigator. For example, in case of a second species closely related to a first species for which the product is fully documented and where recognised ~~challenge~~ models to establish vaccine efficacy (challenge or antibody response) exist, ~~challenge~~ laboratory studies may be sufficient to document efficacy also in the second species."	
244 – 246	1	Comment: We suppose that not so much food safety but farming-economic reasons are behind this proposed requirement. The requirement is not justified as field studies covering the whole life span may take unacceptably long periods (years), whereas sufficient safety information can be obtained from the period over the studies or under conditions that the fish are most sensitive to potential adverse effects of the vaccine. Moreover, Guideline EMA/CVMP/IWP/123243/2006 clearly indicates that field safety studies are not required for MUMS products, but may be asked as follow-up measure (e.g. through pharmacovigilance). Proposed change: *"For injection vaccines to be used for fish intended for human consumption and that are not covered by Guideline EMA/CVMP/IWP/123243/ 2006 (MUMS products), safety data from field studies (weight gain, local reactions) covering the whole study period ~~life span~~, should however always be available."*	Partially agreed. It is important that the field studies reflect the safety of vaccine use over the life cycle of the fish species. The text in lines 244-246 is revised as follows: *"For injection vaccines to be used for fish intended for human consumption and that are not covered by Guideline EMA/CVMP/IWP/123243/ 2006 (MUMS products), safety data from field studies (weight gain, local reactions) which are predictive of the safety over the life cycle ~~covering the whole study period life span~~, should however always be available."* For consistency, lines 158-165 in section 5.1 have been amended as follows: *On this basis, the safety studies ~~which include an evaluation of~~ should be capable of allowing a prediction to be made of the safety profile over the average life span of the fish species ~~should be conducted. Such studies should include measurement of~~. For*

			example weight gain over the life span (for food producing fish) and ~~assessment at slaughter time of~~ the for parenteral vaccines, the percentage of fish down-graded on quality grounds due to adhesions / pigmentation at slaughter time etc are important aspects to be considered.
250 – 252	1	Comment: Please consider the use of serology as alternative for challenge. Proposed change : *"Studies conducted under semi-field conditions where groups of fish are taken from the holding tank / cage / pen etc at different intervals and subjected to challenge infection or evaluation of specific antibodies level are useful in evaluating the DOI."*	Agreed. Text revised as follows: *"Studies conducted under semi-field conditions where groups of fish are taken from the holding tank / cage / pen etc at different intervals and subjected to challenge infection or evaluation of a specific ~~antibodies level~~ antibody response (where a suitable correlation with efficacy has been established) are useful in evaluating the DOI."*
263 – 264	1	Comment: The DOI claims are evaluated during the procedure and approved by the member states involved. It is not feasible to document the duration of immunity for every condition which can occur in the field. Specifying in the SPC the design and conditions (into how much detail?) under which the DOI has been established is of little relevance as farmers cannot and will not adapt their production method to the method followed in the DOI study/studies. Proposed change: *"Unless for specified reasons (e.g. existence of different production methods for the target species, the DOI not being determined for all target species or the DOI being different for the different target species), ~~T~~the DOI claims proposed for the SPC ~~should~~ do not have to*	Agreed. Text revised as follows: *"In general for the DOI claims proposed for the SPC, it is not necessary to refer to the design and the conditions used in the studies ~~(e.g. challenge / field studies; freshwater / salt water; water temperatures~~ unless for specified reasons (e.g. different production methods exist for the target species,*

		refer to the design and the conditions used in the studies ~~e.g. challenge / field studies; freshwater / salt water; water temperatures~~."	*DOI has not been determined for all target species or different DOI for different target species)."*
275	1	Comment: Reference is from 1996. Proposed change: Speilberg scoring system (Midtlyng *et al*. ~~1986~~ 1996).	Agreed
57-62	2	Comment: This guideline relates to animal studies. Hence it is appropriate to remind applicants of their obligation to adhere to the principles of the 3Rs, and to refer to legislation relating to the protection of animals used for scientific purposes. Proposed change (if any): This document is intended to provide guidance on the conduct of studies to demonstrate the target animal safety and efficacy for immunological veterinary medicinal products intended for use in farmed finfish. The 3Rs principles should be adhered to in all animal studies and every effort should be made to replace, reduce and refine animal use where scientifically and practically possible. It should be read in conjunction of Annex I of Directive 2001/82/EC as amended by Directive 2009/9/EC and relevant European Pharmacopoeia (Ph.Eur.) monographs (e.g. Ph. Eur monograph 0062 and 5.2.6). Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes also applies. This will be replaced by Directive 2010/63/EC which also incorporates the guidelines for husbandry and care covered in Appendix A of Council of Europe Treaty ETS 123 European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.	Agreed. Lines 57-62 have not been revised as recommended however a new paragraph on animal welfare concerns has been included in Section 4 'General considerations for studies involving fish' similar to the text proposed for the revised version of the guideline on "Requirements for the production and control of immunological veterinary medicinal products" . In addition, lines 60-62 have been revised to include a reference to Directive 2010/63/EC.
78-79	2	Comment: In vaccination-challenge studies, non-vaccinated control animals will develop disease and experience associated distress. Hence, in accordance with the principles of the 3Rs, the number of non-vaccinated control fish	Agreed. The reference to 'high' in line 79 has been removed.

		used should be the minimum possible to achieve a statistically significant result. In view of animal welfare and consistency with EMA/CVMP/IWP/206555/2010, we recommend the additional text below Proposed change (if any): The numbers of fish per group should be justified and the sample size in each group should be sufficiently high to allow for the results to be statistically significant and clinically reliable without using more than are necessary. However, for vaccination-challenge studies, the possibility of reducing the number of control non-vaccinated fish should be investigated as these fish will also suffer disease and associated distress.	The new paragraph on animal welfare concerns in Section 4 addresses the issue of reducing the number of non-vaccinated fish.
152	2	Comment: Applicants should be reminded of their obligations to adhere to the principles of the 3Rs when slaughtering fish. Proposed change (if any): At the end of the monitoring period, the fish should be slaughtered humanely using a method described by Directive 2010/63/EC and carcasses examined for systemic and local reactions, both macroscopically and microscopically.	Agreed. Text revised as follows: At the end of the monitoring period, when appropriate the fish should be slaughtered humanely using a method described by Directive 2010/63/EC and examined for systemic and / or local reactions macroscopically and / or microscopically as appropriate.
181-184	2	Comment: Lethal endpoints in efficacy studies inevitably involve animal suffering. ECEAE recognises the difficulties associated with developing validated humane endpoints for fish. However, applicants should be encouraged to adopt such refinements should they become available. Proposed change (if any):	Agreed. Text revised as recommended.

		In the case of claims for protection against mortality, it is important that the evaluation period is of sufficient duration to reveal the total development of the mortality curve, both in control and vaccinated animals as vaccination may delay the onset of mortality. In efficacy studies where mortality is expected, the use of validated humane endpoints should be considered.	
57-59	3	Comment: 1. The sentence in lines 57-59 is almost identical to the sentence in lines 54-55 and could be deleted.	Agreed – text revised as recommended,
57-62	3	Proposed change (if any): This document is intended to provide guidance on the conduct of studies to demonstrate the target animal safety and efficacy for immunological veterinary medicinal products intended for use in farmed finfish. It should be read in conjunction with Annex I of Directive 2001/82/EC as amended by Directive 2009/9/EC and relevant European Pharmacopoeia (Ph. Eur.) monographs (e.g. Ph. Eur. monograph 0062 and 5.2.6).	
67-69	3	Comment 2. The requirement to use fish free from antibodies against any of the antigens which the vaccine is intended to protect against is described in specific monographs for some individual antigens. In addition, Ph.Eur 5.2.6 lists three applicable immune status categories which can be applied where there is no specific monograph. In principle, it is important that fish are free of antibodies but the level at which this is documented may vary, especially between fish used in lab trials and field trials. For lab trials certificates for the particular batch of fish may be provided, however, when several hundred thousand fish are included in field trials the immune status of the entire population is not documented to the same extent. Proposed change (if any): The fish to be used must not have been vaccinated against any of the antigens in the vaccine and should not have specific antibodies against any of the vaccine antigens against which protection is claimed, unless	Partially agreed. The inclusion of the term 'unless justifiable' suggests that seropositive or vaccinated fish can be used which is not the intention. Text revised as follows: The fish to be used must not have been vaccinated against any of the antigens in the vaccine and should be from a population shown to be free from not have specific antibodies against any of the vaccine antigens against which protection is claimed.

		justifiable.	
70		Comment 3. What is meant by 'physiological status' of fish?	For clarification, text has been amended as follows: The range of species, ages, sizes, weights and physiological status (e.g. smoltification, sexual maturation) of fish used in the studies must be representative of those to which the vaccine will be administered in the field for commercial purposes.
72	3	Comment 4. Proposed change (if any): For laboratory studies, fish of the minimum recommended vaccination age / or size should be used.	Agreed – text revised as recommended.
80-82	3	Comment 5. PHARMAQ agrees that tank effects should be overcome but thinks the guideline should allow for different approaches to overcome this effect. Proposed change (if any): If vaccinated and control fish are not housed in the same tank, to overcome tank effects which may be experienced between groups of fish which are kept under identical conditions but in different tanks, measures should be taken to overcome such effects e.g. a minimum of two tanks should be used for each of the vaccinated and control groups.	Agreed. – text revised as recommended.
85-87	3	Comment 6. The guideline refers to 'water quality including temperature and salinity'. The intended meaning of 'water quality' is unclear. It is further stated that the 'Water quality (...) must be relevant to the environment under which the vaccine will be used for commercial purposes'. Taking into account the varying temperatures and salinity along the Norwegian coastline, it is suggested to make the requirement less absolute by replacing 'must' with should. Proposed change (if any): Water quality including temperature and salinity (e.g. freshwater versus	Agreed. Text revised in accordance with EMA/CVMP/459868/2008 'Guideline for S+E of VMPs for use in farmed finfish' i.e. 'Water quality parameters such as temperature and salinity (e.g.

		seawater) used in each laboratory study must should be relevant to the environment under which the vaccine will be used for commercial purposes.	freshwater versus seawater) used in each laboratory study must during vaccination should be relevant to the environment under which the vaccine will be used for commercial purposes'
90	3	Proposed change (if any): The water quality temperature and salinity should be documented in each study report.	Agreed. Text revised as follows: The Water quality parameters such as temperature and salinity should be documented in each study report.
91-105	3	Comment 7. In our opinion, the table with optimal water temperatures should be removed. In a general guidance document like this it would be better to have less detail on required temperatures. The reasons are partly given in the guideline itself by the several factors impacted by temperature; • onset of immunity • different climatic conditions • the disease itself • local adverse reactions The guideline states that 'Some studies may need to be performed at both the high and low end of the temperature span for the relevant species'. PHARMAQ agrees with this concept, but taking into account the narrow temperature range for Atlantic salmon (12-15°C) we do not believe that the guideline succeeds in giving good advice. Under field conditions in Norway, Atlantic salmon will experience temperatures ranging from approximately 3-17°C. However, instead of proposing to change the temperature interval, it is suggested to remove the table of optimal water temperatures completely as it is perceived to complicate rather than simplify the guideline with respect to choosing relevant water temperature in studies. It is strongly believed that guidance on the choice of temperature can be given generally.	Agreed.
91-101	3	Proposed change (if any): The table below gives examples of optimal water temperatures for some fish species. The laboratory studies should be carried out at the water temperature(s) relevant for the species of fish, the disease and the data	Agreed. As OOI is expressed in degree days a specific reference to the temperature at which OOI studies are conducted is

		to be obtained from each study. The temperature at which the vaccine will be administered under normal practice should be taken into consideration when designing the study. For instance, studies intended to document time to onset of immunity for vaccines given at low temperatures (e.g. in the spring) should be performed at equally low temperatures. The chosen temperature(s) should be justified by the applicant for each study. The different climatic conditions and water temperatures within the European Union should be considered, when relevant for the fish species/disease in question. Some studies may need to be performed both at the high and low end of the temperature span for the relevant fish species. (Table)	not necessary. In addition, the first paragraph of section 4.2 states that water temperature in the study should be relevant to the normal use conditions for the vaccine. Therefore lines 91-97 are deleted. To account for situations where there are different production practices: e.g. for Salmon two production systems are used, with S0 or S1 smolts, which are stocked at different seasons and different temperatures, text of lines 98-100 are amended as follows: 'The different climatic conditions and water temperatures within the Community should be considered, when relevant for the fish species/disease in question. Some studies may need to be performed both at the high and low end of the temperature span for the relevant fish species / disease distribution. Similarly, some studies may need to be performed following the main production practices for the species (e.g. S0 and S1 stocking for salmon). The chosen conditions should be justified by the applicant for each study.
102-105	3	Comment 8. The guideline states that 'all comparative data from safety and efficacy studies (both laboratory and field studies) involving fish should be based	Accepted that degree days difficult to calculate for field studies.

		on "degree-days"'. With regards to field studies it may be practically challenging to obtain the same number of degree days from study site to study site since the temperature may vary greatly. In our opinion it is more relevant to compare the field results for a new vaccine with control groups at the same study site. With regards to local adverse reactions, it is considered that degree days may not give all the information, as the maximum temperature which the fish are exposed to may also be important. Proposed change (if any): To account for the fish being poikilothermic animals and taking into account the fact that immunity in fish is temperature dependant and that the frequency and intensity of injection site reactions increases with higher water temperatures, all comparative data from safety and efficacy studies (both laboratory and field studies) involving fish should be based on "degree-days", unless other comparison is justified. Safety and efficacy data from field studies will normally be compared with control groups within each participating study site.	Text revised to indicate that degree days calculation is relevant only to laboratory studies as follows: 'To account for the fish being poikilothermic animals and taking into account the fact that immunity in fish is temperature dependant and that the frequency and intensity of injection site reactions increases with higher water temperatures, all comparative data from safety and efficacy studies (both laboratory and field studies) laboratory studies involving fish should be based on "degree-days".
111	3	Comment 9. That the vaccine dose must reflect both dose volume and amount is considered self-evident and can be deleted. Proposed change (if any): The vaccine dose (i.e. dose volume and amount) and administration method(s) employed in the safety (...)	Not agreed. Development studies may have used a different dose volume / amount and therefore such studies can only be considered supportive.
114-117	3	Comment 10. Three definitions are given (parenteral administration, immersion administration and oral administration) in section 4.3. We suggest moving these to the Definitions section starting on line 267. Proposed change (if any): Parenteral administration: the vaccine is administered by injection. Immersion administration: vaccine is administered by dipping or bathing the fish in an immersion bath/tank. Spray vaccination is a form of immersion vaccination. Oral administration: vaccine is administered via the feed	Agreed

For inclusion after line 273	3	Proposed change (if any): Parenteral administration: the vaccine is administered by injection. Immersion administration: vaccine is administered by dipping or bathing the fish in an immersion bath/tank. Spray vaccination is a form of immersion vaccination. Oral administration: vaccine is administered via the feed	Agreed
150-152	3	Comment 11. According to Ph.Eur. general monograph 5.2.6 the acute safety examination period should be minimum 14 days. However, monograph 1521 (furunculosis) states that the fish should be observed for 21 days. Thus, although not wrong, the advice to daily monitor the fish for a minimum of 14 days may be misleading. Proposed change (if any):To assess the acute safety characteristics of the vaccine, the fish should be monitored daily for mortality / morbidity over a minimum of a 14 day period taking into account the optimal water temperature for the target species. Confer Ph.Eur for detailed advice on duration of acute safety periods for specific antigens.	Agreed: Text revised to read: To assess the acute safety characteristics of the vaccine, the fish should be monitored daily for mortality / morbidity over a minimum of a 14 day period (or as recommended in the relevant Ph. Eur. monograph for specific vaccines) taking into account the optimal water temperature for the target species.
152-153		Comment 12. It should not be required that fish are slaughtered. Furthermore, we think the investigations can be defined for each study and all investigations may not be relevant. Proposed change (if any): At the end of the monitoring period, the fish should, when appropriate, be slaughtered and examined for systemic and/or local reactions, both macroscopically and/or microscopically.	Partially agreed. Text revised to include a reference to humane slaughtering as follows: At the end of the monitoring period, , when appropriate, the fish should be slaughtered humanely using a method described by Directive 2010/63/EC and examined for systemic and/or local reactions, both macroscopically and/or microscopically as appropriate.
155	3	Comment 13. Misspelling of Speilberg.	Agreed.

		Proposed change (if any): Spieilberg	
158-167	3	Comment 14. Monitoring of safety over the life span of the target fish species is not primarily done in lab studies. It is also referred to section 6 which deals with field studies. In our opinion, the sections concerning life span effects should be deleted. Proposed change (if any): It is important to take into account the possible adverse effects of vaccine administration on development over the life span of the target fish species. This is particularly important in the case of parenteral vaccines as adhesions may have a negative effect on spawning, and adhesions / pigmentation may result in rejection or down-grading of fish at slaughter. On this basis, studies which include an evaluation of the safety profile over the average life span of the fish species should be conducted. Such studies should include measurement of weight gain over the life span (for food producing fish), assessment at slaughter time of the percentage of fish down-graded on quality grounds due to adhesions / pigmentation etc. It may be more appropriate to evaluate the long term safety effects of vaccine administration should be evaluated over the life span of each fish species in field studies as discussed in section 6 below.	Not agreed. It is important to emphasise that safety over the life span of the fish should be considered. This section identifies the aspects to be considered for this evaluation. The text clearly indicates that this evaluation may be done under field conditions i.e. it is not a requirement to investigate these parameters under laboratory conditions. Lines 162-165 have been amended to outline items which need to be considered as follows: 'On this basis, the safety studies should be capable of allowing a prediction to be made which include an evaluation of the safety profile over the average life span of the fish species should be conducted. Such studies should include measurement of For example, weight gain over the life span (for food producing fish) assessment at slaughter time of and for parenteral vaccines the percentage of fish down-graded at slaughter time on quality grounds due to adhesions / pigmentation etc are important aspects to be considered..

			In addition, lines 244-246 have been amended to clarify that the field studies should be predictive of the safety profile over the life cycle (i.e. it is not specifically required to conduct field studies that cover the entire life cycle) as follows: "For injection vaccines to be used for fish intended for human consumption and that are not covered by Guideline EMA/CVMP/IWP/123243/ 2006 (MUMS products), safety data from field studies (weight gain, local reactions) which are predictive of the safety over the life cycle covering the whole study period life span, should however always be available."
205-208	3	Comment 15. Suggestion to include a sentence on marking of fish. Proposed change (if any): At least two of the pens or tanks, and preferably several pairs of pens/tanks should be used in the study per vaccinated and control group. If fish are to be marked, the least harmful technique for the fish should be chosen. The study site personnel farmer should preferably be experienced in keeping detailed records on all important factors concerning the farm and its fish.	Partially agreed. For animal welfare reasons the aim is to move away from markings such as fin clipping etc. Including a reference to 'markings' could suggest that this is an acceptable practice. A new paragraph on animal welfare concerns has been included in Section 4 which includes the following sentence: The method used to identify vaccinated and controls fish should involve the least harmful technique.

214-218	3	Comment 16. It is considered unlikely that commercial fish farms will agree to keeping a group of unvaccinated fish at their farms. In Norway, the Norwegian Animal research Authority (NARA) / Forsøksdyrutvalget (FDU) who approves field studies from an animal welfare point-of-view, may not approve of keeping unvaccinated fish in the studies. Proposed change (if any): The type of control group used (i.e. mock-vaccinated, non-vaccinated or positive control) should be justified. If a positive control (e.g. a comparator vaccine) is used, consideration should be given to maintaining a (small) group of non vaccinated fish in a separate test pen to serve as indicators of exposure to disease(s) at farm level.	Not agreed. The use of a non-vaccinated group to monitor the occurrence of natural exposure is considered to be of benefit to field studies involving positive controls. The guideline recommendation is to give 'consideration' to the use of a 'small' group of non-vaccinated fish. Line 218-219 has been amended to include a reference to humane slaughtering of these fish as follows: 'Once the relevant infection has been diagnosed in the controls they can be slaughtered humanely using a method described by Directive 2010/63/EC. In addition, lines 226-227 have been revised to highlight the importance of using a clinically relevant indicator of disease as follows: "The method of identification and confirmation of the presence of the causal agent(s) for the natural challenge in each group is an important factor for field studies involving fish. The method used must be relevant to the disease situation and should be recorded for a representative number of fish in each group".

230-231	3	Comment 17. The wording describing the situation that natural challenge has not caused disease, and that an explanation of the failures should be provided, is unfortunate and implies that the study has failed. Proposed change (if any): Information from studies where natural challenge has not caused disease, should discuss the non-conclusive efficacy results. In addition, a full evaluation on the safety data should be provided. performed with unsuccessful natural challenge should be provided with an explanation of the failures, as they would still be relevant for the safety evaluation.	Agreed. Text revised as follows: Information from studies performed with unsuccessful where a natural challenge was not detected should be provided with an explanation of the failures, as they would still be relevant for the safety evaluation. The evaluation along with a discussion of the relevance of the parameters chosen as endpoints should be discussed and justified with regard to their relevance for the proposed claims. A full evaluation of the safety data from these studies should be provided.
250-252	3	Comment 18. PHARMAQ agrees that taking fish for challenge at different intervals are useful in evaluating the DOI. However, in practice this is very difficult to do as the fish are negatively affected by moving from a cage / pen into a (usually) smaller tank. In this new environment fish tend to feed less, and they are more prone to disease e.g. due transport damage to the external mucus membrane (skin).	Not accepted. The guideline refers to such studies being 'useful' i.e. they are not 'required'. Text unchanged.
271	3	Comment 19. Suggestion to change the definition of degree days. Proposed change (if any): Degree days: Is a measure of cooling or heating. The amount of degree days is determined by multiplying the water temperature each day with number of days. For example, 10 days with 5° C equal 50 degree days.	Agreed.