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**COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE  
(CVMP)**

**GUIDELINE ON DEMONSTRATION OF TARGET ANIMAL SAFETY AND EFFICACY OF  
VETERINARY MEDICINAL PRODUCTS INTENDED FOR USE IN FARMED FINFISH**

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This guideline replaces the current "Guideline on the efficacy of veterinary medicinal products for use in farmed aquatic species"; last update September 1994, published in Vol. 7 (7AE22a) of "The Rules governing medicinal products in the European Union".

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**KEYWORDS**

Fish, veterinary medicines, dossier requirements

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## 34 EXECUTIVE SUMMARY

35 The revised guideline on the demonstration of target animal safety and efficacy of veterinary  
36 medicinal products intended for use in farmed finfish provides updated guidance on the preclinical and  
37 clinical aspects of the application procedure for those who seek approval of such products.

38 The revision replace the “Efficacy of veterinary medicinal products for use in farmed aquatic species”  
39 and takes into account the development during recent years, and feedback obtained from users of the  
40 previous guideline which was last revised in 1994.

### 41 1. INTRODUCTION

42 Veterinary medicinal products intended for use in finfish will have to satisfy all the usual requirements  
43 for approval. This includes documentation of quality, demonstration of safety for the consumer, the  
44 user and the environment, and demonstration of efficacy and tolerance in the target species.

45 This document provides special guidance in respect of the documentation required to confirm efficacy  
46 and tolerance for medicinal products for use in finfish.

47 The procedures outlined should be considered for all submissions, but may not be applicable for all  
48 veterinary medicinal products for use in aquaculture. If certain aspects are modified or omitted,  
49 justification should be provided.

50 In principle the results of all trials should be applicable irrespective of where they are carried out;  
51 however, the applicant should take into account the various conditions (e.g. climatic, disease situation,  
52 water temperature and salinity) as these may influence the outcome of the studies.

### 53 2. SCOPE

54 The aim of this guideline is to provide guidance regarding the demonstration of efficacy and target  
55 animal safety for veterinary medicinal products intended for use in farmed finfish.

56 Immunological veterinary medicinal products are excluded from the scope of this guideline.

### 57 3. LEGAL BASIS

58 This document is intended to provide guidance on the demonstration of efficacy and target animal  
59 safety for veterinary medicinal products intended for use in farmed finfish.

60 It should be read in conjunction with Directive 2001/82/EC, as amended. Applicants should also refer  
61 to other relevant European and VICH guidelines, including those listed among the references at the  
62 end of this document.

### 63 4. GENERAL CONSIDERATIONS

64 The applicant is encouraged to standardise study protocols and study reports as far as possible to  
65 facilitate the comparison of study results and the possible extrapolation between species.

66 If the product is intended for in-feed administration, the possible impact of the feed composition  
67 should be considered and investigated, if appropriate. The feed composition and manufacturing  
68 process may influence the medicated product with regard to physico-chemical compatibility.

69 Conditioning and pelleting are the main factors affecting stability during manufacture of medicated  
70 feed. These processes can subject the medicated feed to high temperature and pressures, which can  
71 cause degradation of active substances, excipients or feed constituents. Other quality points for  
72 considerations are homogeneity and segregation of these products. For further information the  
73 applicant is directed to the guideline on additional quality requirements for products intended for

74 incorporation into animal feedingstuffs (medicated premixes) among the references listed at the end of  
75 this guideline.

76 To account for the fish being poikilothermic animals, the term “degree-days” should be used wherever  
77 relevant.

78 All laboratory studies should be carried out at both the optimal and the maximum or minimum water  
79 temperatures relevant for the species of fish and the disease. The applicant should justify their choice  
80 of maximum or minimum temperature(s) in relation to the choice of product / indication. Exceptions  
81 from carrying out studies at two different temperatures as described here should be justified by the  
82 applicant. The table below gives examples for optimal water temperatures for some fish spp.

83

<b>Fish species</b>	<b>Latin name</b>	<b>Optimal water temperature (s) (°Celsius)</b>
Atlantic salmon	<i>Salmo salar</i>	12-15
Rainbow trout	<i>Oncorhynchus mykiss</i>	14-18
Cod	<i>Gadus morrhua</i>	8-14
Sea bass	<i>Serranidae</i>	8-28
Carp	<i>Cyprinidae</i>	20-23
Halibut	<i>Hippoglossus hippoglossus</i>	8-12
Turbot	<i>Psetta maxima</i>	14-18

84

85 The origin/varying genetics of the experimental fish is important to obtain valid reproducible results,  
86 and any variation should be addressed. All finfish species shall be identified by their colloquial name  
87 followed in parenthesis by the Latin or Linnean description.

88 Extensive testing may be required for compounds with a novel molecular structure or compounds not  
89 previously approved for aquatic species. More limited investigations may be acceptable for a new salt  
90 or ester of a compound previously approved for the same or other relevant species.

#### 91 **4.1 Study reports**

92 To facilitate the evaluation of the documentation of efficacy and target animal safety, all experimental  
93 techniques should be described in such detail as to allow them to be reproduced. The investigator  
94 should establish their validity. Each experimental trial or field trial and the conditions under which  
95 they are performed should be described in detail. Separate reports on all trials, whether favourable or  
96 not, should be provided. Adequate summaries of groups of trials based on the same protocols may be  
97 provided.

98 The applicant is directed to the “Guidelines for reporting the results of experiments on fish” (Brattelid  
99 and Smith, 2000) for detailed guidance on the contents of the study report.

100 Adverse events, side effects and target animal tolerance should be reported. An explanation of non-  
101 specific mortalities and comments on any physical or behavioural abnormalities should be provided.

102 For clinical studies the applicant should clearly state the onset and the duration of relevant disease  
103 outbreaks. This information will allow censoring of irrelevant mortality data, potentially threatening  
104 the statistical power of the study.

#### 105 **4.2 General Study Design**

106 As water quality has been identified as an important element for maintaining healthy fish and ensuring  
107 valid experimental results, the water quality parameters temperature and salinity should be addressed  
108 in detail.

109 The efficacy of the veterinary medicinal product should be stated as a function of time, dose,  
110 frequency and duration of treatment. The criteria used for the evaluation of efficacy in the trials should  
111 be pre-determined. The results should be presented in a way that is suited for adequate statistical

112 evaluation. The clinical trials should cover all claimed indications and each indication should be  
113 discussed and reported separately.

114 Statistical analysis of the results should be performed whenever relevant.

115 The applicant should justify the observation unit (e.g. individual fish or cage) and the number of  
116 samples collected on each sampling occasion. The sample sizes should be sufficiently high to allow  
117 for the results to be statistically significant and clinically reliable.

118 In studies of products intended for use against aquatic one-host parasites, like for example sea lice on  
119 salmon, sampling a limited number of fish from many cages instead of many fish from a small number  
120 of cages is recommended. This is to take into account clustering which naturally occurs with such  
121 parasites.

## 122 **5. PRECLINICAL STUDIES**

### 123 **5.1 General considerations**

124 Great care should be taken to ensure that the fish receives the required dose. For single dose studies of  
125 orally administered products it is recommended to administer the test substance orally by gavage.

126 For repeated dose studies of premixes intended for medicated pellets, examples of control methods  
127 applicable to trials with small and large numbers of test subjects, respectively, are given below.

#### 128 *5.1.1 Small number of test subjects*

129 Count the number of pellets before they are given to the fish. After dosing, count the uneaten number  
130 of pellets and then calculate the actual dose received.

#### 131 *5.1.2 Large number of test subjects*

132 Small X-ray-dense glass beads (ballotini) may be incorporated when manufacturing medicated feed  
133 pellets for the trial, at a known concentration of beads per pellet. This can be determined by X-raying  
134 the pellets. When the number of beads per pellet is known, a representative number of fish may be X-  
135 rayed to reveal the average number of pellets ingested by the fish. It is also possible, by using small  
136 and large pellets every other day, to reveal how many pellets were ingested two different days in a row  
137 (Horsberg, Hoff and Nordmo, 1996).

### 138 **5.2 Pharmacology**

139 All studies should be performed according to the expected method of use of the product in the field.

140 Studies in target species are as a general principle required for the assessment of the pharmacological  
141 effects. However, the mode of action and route of administration should be taken into account. For  
142 example, for an extension to a new target animal species for a waterborne sea lice product acting  
143 directly on sea lice, pharmacological parameters in the target animal would be of no importance with  
144 regard to efficacy. However, they may be relevant to target animal safety and residue data. For sea lice  
145 products given orally, pharmacological parameters would be relevant to efficacy.

146 The changes in concentration of the active substance should be determined as a function of time, dose,  
147 frequency and duration of treatment, and of the route of administration of the test substance. Any  
148 observed changes should be described. From the application of appropriate models or from model-free  
149 calculations, the pharmacokinetic parameters should be derived and presented. If ED<sub>50</sub> or EC<sub>50</sub> are  
150 known or established from model studies, these can serve as a basis for the selection of the treatment  
151 dose.

152 As fish are poikilothermic, temperature related pharmacology trials should be conducted as significant  
153 temperature related effects can be expected. The applicant should justify their choice of temperatures  
154 at which the studies are conducted.

155 Depending on the nature of the veterinary medicinal product in question, studies on interactions with  
156 other veterinary medicinal products should be undertaken.

### 157 **5.3 Pharmacodynamics**

158 The pharmacodynamic effects, including the mode of action of the active ingredient(s) as the basis for  
159 the recommended use of the product, should be described. Desired effects as well as adverse effects  
160 and any dose dependency of these should be investigated. If relevant, the frequency of adverse effects  
161 should be reported.

162 If feasible, model studies should be carried out. Effects found in such model studies should be  
163 reported as a function of dose and ED<sub>50</sub> or EC<sub>50</sub> values should be provided, as they can serve as a basis  
164 for the selection of the treatment dose.

### 165 **5.4 Pharmacokinetics**

#### 166 *5.4.1 General considerations*

167 It is recommended to carry out pharmacokinetic studies in finfish according to the principles in the  
168 CVMP guidelines for the conduct of pharmacokinetic studies in target animal species, as far as they  
169 may be applied to fish.

170 When the product is intended for fish kept in areas with varying water temperatures, pharmacokinetic  
171 studies should be carried out at different water temperatures (high end and low end of the natural  
172 variation). When the product is intended for fish kept in both seawater and freshwater,  
173 pharmacokinetic studies should be carried out in both types of water.

#### 174 *5.4.2 Performance of tests*

175 Due to the high degree of inter-individual differences observed in fish, samples from several fish per  
176 timepoint (at least 10 are recommended) are required for analyses. For repeated blood sampling  
177 analysis results from at least 4 individuals are required. The investigator shall justify the chosen  
178 number of fish samples per time point.

179 It is recommended that the group consists of a sufficient number of animals, as this will allow for  
180 sampling small numbers of fish without stressing the remaining fish in the group. Sampling fish out of  
181 a small group may cause stress symptoms like decreased food uptake among the remaining ones. For  
182 many species of fish the group should preferably consist of at least 1000 individuals.

#### 183 *5.4.3 Bioavailability*

184 Bioavailability for premixes should be determined by administration of a medicated feed prepared by  
185 the procedure recommended by the manufacturer. The possible influence of different feed types  
186 should be considered.

187 Data describing the bioavailability of oral products should be provided, as this is important in the  
188 evaluation of environmental effects. Water salinity may significantly affect the bioavailability, and  
189 products must be tested under relevant conditions (fish kept in seawater and/or freshwater).

#### 190 *5.4.4 Distribution*

191 Methods using radiolabelled substance, e.g. whole-body autoradiography, can be useful for studies of  
192 distribution. This may be relevant in pharmacokinetic studies of antibiotics that are intended to exert  
193 an effect on specific sites or organs. For waterborne sea lice products acting directly on the sea lice,  
194 distribution studies are less relevant.

195 5.4.5 *Metabolism*

196 As fish generally metabolise drugs at a lower rate than mammals and the number of metabolites are  
197 usually lower, there is less need for studies on metabolism, especially for substances where  
198 information relating to other species is available.

199 5.4.6 *Elimination*

200 The most important factors influencing the rate of elimination should be determined and discussed,  
201 e.g. water temperature, salinity, O<sub>2</sub> content, feeding and physiological status of the fish.

202 **5.5 Pharmacological studies of antimicrobials and antiparasitic products**

203 For antimicrobials, pharmacological studies in finfish and the PK/PD analysis should be carried out  
204 according to the principles in the CVMP guideline for the demonstration of efficacy for veterinary  
205 medicinal products containing antimicrobial substances. For anthelmintics, the principles of the VICH  
206 overall guideline for efficacy requirements for anthelmintics apply. For other antiparasitic products,  
207 the general pharmacokinetic guideline should be used.

208 5.5.1 *Development of resistance*

209 The mechanism for, and frequency of development of resistance should be discussed. Possible  
210 development of chromosomal or plasmid mediated resistance to other active substances used in  
211 farmed fish should be stated. For ectoparasiticidals, experience of development of antiparasitic  
212 resistance should be included, if relevant.

213 5.5.2 *Transfer of resistance*

214 The potential hazard of transferring microbial resistance to wild fish pathogens, other waterborne  
215 pathogens or human pathogens by using the medicinal product as indicated by the manufacturer  
216 should be considered.

217 **5.6 Tolerance in the target species**

218 Target animal safety should be determined in all the target species, as defined by the investigator,  
219 unless otherwise justified. Studies performed in one species of fish may be considered relevant for the  
220 evaluation of tolerance in a second species of fish of the same genus or taxonomic family, provided  
221 that they are kept under the same environmental conditions. In such a case there must be supportive  
222 safety data from clinical trials in the second species. It may for example be considered unnecessary to  
223 carry out formal target animal safety studies in trout if such studies have been carried out on other  
224 species of salmonids, and if clinical studies in trout are available.

225 Excipients normally used in pharmaceutical products for terrestrial animals may not be well tolerated  
226 by aquatic species. Safety of excipients should be determined and lack of appropriate data justified.

227 It is important to take into account possible adverse effects on development (malformations) if the  
228 medication is applied to young fish (embryos, larvae and juveniles), and where these products can  
229 easily interfere in the growth. It is important to indicate the range of sizes and weights of fish which  
230 are recruited for the trial since the same treatment may not have the same effect in different sizes of  
231 fish.

232 Studies of repeated dose tolerance are relevant only for products intended for repeated dose  
233 administration.

234 The following points apply to all target animal safety studies:

235 5.6.1 *Test product*

236 It is recommended to use the final formulation of the medicinal product. Where the formulation used  
237 in studies differs from the final commercial formulation the applicant must demonstrate that the

238 bioavailability of the formulation is the same. Substances administered by gavage should have a  
239 suitable formulation, e.g. solution, suspension, capsule or in feed. All formulations used in the tests  
240 should be assayed for the concentration of the active substances before the start of the trial(s).

#### 241 5.6.2 *Negative Control Groups*

242 Fish have a very marked sense of taste and smell. Consequently, studies with in-feed medication  
243 should be carried out with the medicated group (using the test product), a placebo group (using the test  
244 formulation without active substance) and an untreated “feed-alone” group (i.e. 2 forms of control),  
245 otherwise the feed effect cannot be differentiated from the formulation and medication effect.

246 In all tests, the test product and placebo should be administered in the same manner as intended for the  
247 finished product. Untreated controls should be handled identically to treated fish.

248 For studies of other than in-feed medication, the control substance should be either saline or finished  
249 product deprived of the active substance. The applicant should justify their choice of control  
250 substance, taking into account that the excipients may have some effects of their own.

#### 251 5.6.3 *Holding*

252 The fish to be tested should be in a normal physiological condition and be feeding well during two  
253 weeks of acclimatisation. The allocation of fish in groups should be done randomly the day prior to  
254 administration of the test product, using an appropriate method. Acclimatisation is not applicable for  
255 embryonal stages.

256 The following conditions of exposure are recommended:

#### 257 **Stocking:**

##### 258 *Semistatic test:-*

- 259 • waterborne administration: max. 1 g fish/litre of water
- 260 • oral administration: max. 5 g fish/litre of water
- 261 • parenteral administration: max. 5 g fish/litre of water

##### 262 *Flow through:*

263 Higher loading is acceptable

#### 264 **Group size and number:**

265 The numbers of fish per group should be justified, and should not be less than 10 with a  
266 minimum of 2 tanks per dose and 2 control tanks.

#### 267 **Fish size:**

268 It is recommended to use fish of the most sensitive category for which the product is intended  
269 (size/age and physiological status).

#### 270 5.6.4 *Necropsy and histopathology examinations*

271 As a minimum, tissues from all fish in the highest dose group and control group should be examined  
272 macroscopically and microscopically. Where the toxicity of the test product is anticipated to be  
273 relatively high, different necropsy schemes may be required, to include gross and microscopic  
274 examinations for all fish or for randomly pre-selected fish. If lesions are found in any tissue from the  
275 highest dose group, then samples from fish in the second highest dose group of the test product should  
276 be examined macroscopically and microscopically, until a no-observable-adverse-effect-level is  
277 determined. In addition, tissues from all fish showing systemic clinical signs should be examined  
278 macroscopically and microscopically.

279 5.6.5 *Dosage and duration of dosage*

280 The choice of dose levels and duration of exposure must be justified by the applicant.

281 The chosen levels must be adequate for demonstration of a sufficient margin of safety for the  
282 veterinary medicinal product when used under field conditions. This means that the choice of dosage  
283 levels should be sufficiently high to account for the fact that varying degrees of unintended overdosing  
284 will commonly occur in practice with such types of medicinal products intended for waterborne or in-  
285 feed treatment.

286 For single dose studies at least 3 dose levels should be tested. The selection of dose levels and duration  
287 of the treatment period should be based on the proposed therapeutic dosing regimen.

288 For repeated dose studies the selection of dose level(s) and duration of the dosing period should be  
289 based on the proposed therapeutic dosing regimen and on results from single dose studies.

290 5.6.6 *Oral administration*

291 Detailed records on feed uptake and concomitant daily dose should be given.

292 The maximum dose should usually not exceed 2000 mg/kg fish. For solutions and suspensions given  
293 by gavage, the concentration of the active ingredient should be adjusted so that, if possible, no more  
294 than 0.5 ml test solution per 100 g fish gives the required dose. These maximum dosage  
295 recommendations are given as an advice to the applicant on the practical dosage limitations in fish.

296 5.6.7 *Waterborne administration*

297 Dipping, bathing or “top dressing” on the water surface are methods of administration considered as  
298 waterborne administration.

299 Waterborne treatment must usually have a very broad margin of safety due to the difficulty of accurate  
300 dosing/estimation of water volume in raceways or sea cages.

301 The duration of treatment should be equal to or longer than the proposed length of treatment. Dosage  
302 of the veterinary medicinal product – like in mammals – is principally a function of treatment  
303 concentration and exposure period. For sedatives and anaesthetics for use in finfish the length of  
304 exposure period is the main parameter available for adjustment during treatment.

305 5.6.8 *Parenteral administration*

306 Both the test and the control product should be administered by injection. The same volume of test  
307 solution should preferably be administered to the fish in both the test and the control group. Also, the  
308 maximal volume of the veterinary medicinal product administered in one injection site and assessment  
309 of the reaction in the injection site should be provided.

310 **6. CLINICAL**

311 The main purpose of the documentation of efficacy is to prove the therapeutic value of a new  
312 veterinary medicinal product for aquatic species and to define an optimal dose and dosage regimen.

313 Clinical trials are required for each proposed indication and for all target species in which efficacy is  
314 claimed. For some products, such as waterborne treatments which act directly on ectoparasites and  
315 which are independent of the pharmacokinetics in the fish, clinical trials in a second species may not  
316 be required if the clinical data obtained for the main fish species can be shown to be relevant to the  
317 second species. In such cases sufficient justification for the omission of clinical studies, together with  
318 documentation of target animal tolerance is necessary.

319 All studies should be performed under appropriate conditions according to the proposed method of use  
320 of the product. The study/studies should for example be carried out in (a) water temperature(s) in  
321 which the test product is likely to be used considering the different climatic conditions within the  
322 community.

323 The studies should be blinded unless otherwise justified.

324 Normally, data from both laboratory and full scale field trials will be required. Where appropriate the  
325 applicant should justify the lack of relevant data.

326 Omission of field studies and submission of challenge studies only may be accepted if adequately  
327 justified by the investigator. For example, in case of a second species closely related to a first species  
328 for which the product is fully documented, challenge studies may be sufficient to document efficacy  
329 also in the second species.

330 In all studies the final formulation or an essentially similar formulation should be used and  
331 administered by the proposed route. Where a similar formulation is used, it should be justified with  
332 regard to bioavailability.

333 The trials should include control groups.

334 Applicants should justify the choice of control group (positive or negative). If a placebo is used, the  
335 applicant is directed to the text regarding control product in section 5.6 (Tolerance in the target  
336 species). If a positive control is chosen, a veterinary medicinal product authorised according to the  
337 relevant EU requirements should preferably be used. The daily uptake of medicated feed should be  
338 recorded together with the daily dose of the active substance, if possible. Premixes should be  
339 administered as medicated feed prepared by the procedure recommended by the manufacturer,  
340 preferably using a standardised feed.

341 The applicant should consider/discuss all variables likely to confound results and the methods that will  
342 be used to reduce/avoid them.

343 If feasible and without disrupting the value of the data obtained, fish should be removed from the trial  
344 when showing definitive signs of disease and/or when there has been pathological confirmation of  
345 disease in the holding unit rather than waiting for death to occur.

346 The nature and frequency of adverse drug reactions should be monitored and recorded.

347 Signs of interactions with other medicinal products or with feed should be carefully observed during  
348 the clinical trials.

## 349 **6.1 Laboratory studies**

350 The test conditions can be controlled and standardised in land or sea-based test facilities. Experimental  
351 trials should be performed for the main target species.

352 The fish to be included in the trials should be of similar age and size, be susceptible to the disease in  
353 question and be of known origin and health status. The allocation of fish in groups should be done  
354 randomly, using an appropriate method.

355 Every study should be designed to allow for appropriate statistical evaluation. A sample size analysis  
356 should be presented. Significant differences might be experienced between different groups of fish  
357 which are kept under identical conditions owing to the fact that they are kept in different tanks.  
358 Therefore, at least two groups kept under identical conditions but in different tanks should always be  
359 used. The experimental unit should be justified.

360 The parameters to be recorded for evaluation should be justified. The applicant should justify the  
361 statistical evaluation methods.

### 362 *6.1.1 Challenge studies*

363 Challenge models (cohabitant, waterborne, injection) and their relevance to natural conditions (time of  
364 challenge/time of treatment/infection pressure etc.) should be discussed.

365 The test animals should not previously have been exposed to the challenge organism, if possible. The  
366 challenge organism must be of a strain relevant for the current disease situation, and be isolated and  
367 characterised by the most appropriate method, preferably a standard method used by the national  
368 reference laboratory, which should be described in detail. The timing and performance of the  
369 challenge and the design of the study must be justified by the investigator. The results of the

370 introduction of the challenge organism should be reported, based on parasite counting, microbiological  
371 analyses or other pertinent investigations. If appropriate a statistical analysis should be provided.

#### 372 6.1.2 *Dose determination trials*

373 The purpose of the trials is to determine the optimum dose, dosage interval and total period of  
374 treatment for the claimed indications. A dose/response relationship for therapeutic effect and, if  
375 possible, for adverse effects, should be established. Dose determination trials can be performed as a  
376 combination of experimental studies and field trials. Data from well controlled experimental studies is  
377 preferred wherever relevant models are available, and field studies should serve to confirm the  
378 findings from the controlled trials. Where no or only poor models exist, more emphasis should be  
379 placed on field studies.

380 The dosage recommendations should be supported by studies showing the adjustments necessary to  
381 retain a satisfactory effect at the lowest and highest water temperature recommended.

382 Tests must be carried out in seawater and/or freshwater, as relevant to the proposed use.

#### 383 6.1.3 *Dose confirmation trials*

384 Separate dose conformation trials can be replaced by field trials performed with the final formulation  
385 of the veterinary medicinal product administered in the recommended dosage regimen.

### 386 **6.2 Field studies**

387 The scope of the field trials is to ensure that the veterinary medicinal product is efficacious and safe in  
388 the diversified conditions for aquaculture found in Member States. The field studies are to be  
389 performed in established farms. A satisfactory number of sites with conditions representative for the  
390 normal in-use conditions should be used. The applicant should justify the number of sites. The field  
391 studies should be performed in accordance with GCP as far as possible.

392 A product authorised according to relevant EU requirements should preferably be used in the control  
393 group(s) (positive control).

394 Negative controls should only be used if no product is authorised for the claimed indication. The  
395 control group can be treated once an adequate estimation of difference in effect can be established.

#### 396 6.2.1 *Selection of farms*

397 The number and suitability of the sites selected for clinical trials should be justified by the applicant.  
398 These should be geographically well distributed to optimise the possibility of diversified  
399 environmental conditions, disease situation and management practices. Each site should have several  
400 pens or tanks with fish of the relevant size/age and physiological condition (e.g. smoltification, sexual  
401 maturation) for the proposed use of the veterinary medicinal product. At least two of the pens or tanks,  
402 and preferably several pairs of pens/tanks should be used in the trial. The farmer should preferably be  
403 experienced in keeping detailed records on all important factors concerning the farm and its fish.  
404 Records on the source of fish and the disease history in different pens or tanks must be kept. Previous  
405 medication, use of chemicals and vaccines should be known. Daily records of outbreaks of disease,  
406 mortality and medication are required, as well as known and stable management practice concerning  
407 e.g. hygiene, feeding, handling and use of feed additives and chemicals. Weekly records may be  
408 accepted for water temperatures below 8 °C, if justified by the applicant.

#### 409 6.2.2 *Selection of groups*

410 All fish in one tank or pen are considered as one group. A minimum of two groups must be used in  
411 each trial, one of which must be a control group, which in most cases will be a positive control group.  
412 The allocation of the groups should be done randomly, using an appropriate method. The prevalence  
413 of disease, daily mortality, clinical symptoms and other relevant parameters should be comparable in  
414 the treated and control groups at the start of the study.

415 6.2.3 *Trial procedure*

416 Field trials in commercial fish farms should preferably be performed in spontaneous outbreaks of the  
417 diseases for which efficacy is claimed. Trials should thus be conducted at the time of year and under  
418 conditions where a “successful natural challenge” must be defined by the investigator, and should  
419 include the method of identification of the causal agent. Information from trials performed with  
420 unsuccessful natural challenge may be provided with an explanation of the failures. All trials should  
421 be performed with adequate controls. Field trials with anaesthetics or other “non-therapeutics” should  
422 be performed with healthy fish. All trials must be planned so that suitable data are available for  
423 statistical analysis. Clinical endpoints of relevance for the proposed indication should be chosen, and  
424 primary and secondary endpoints should be specified.

425 6.2.4 *Diagnostic criteria*

426 The presence of the investigated diseases must be confirmed in all groups included in the trial. The  
427 criteria for establishing the diagnosis should be given. The same criteria are to be used in all trials and  
428 should include post mortem examination of at least six individuals from each group. The precise  
429 disease condition and identification of any pathogenic organism should be provided. Bacterial diseases  
430 should be diagnosed by isolating and characterising the pathogen by the most appropriate  
431 microbiological method, preferably a standard method which should be described in detail. Samples  
432 from at least 6 fish per group are recommended.

433 **7. DEFINITIONS**

434 For the purpose of this guideline, the following definitions apply:

435 Finfish: A term used to separate true fish from shellfish, crayfish, jellyfish etc. All the  
436 species of fish mentioned in this guideline are examples of true finfish.

437 Degree days: Is a measure of cooling or heating. The amount of degree days is determined by  
438 multiplying the water temperature each day with number of days. For example,  
439 10 days with 5° C equal 50 degree days.

440 Positive control: A positive control group is a group treated with an authorised reference product  
441 approved for the same indication and used according to the label instructions, for  
442 comparison with the test product under evaluation.

443 Negative control: A negative control group is a group treated with placebo (either saline or test  
444 formulation without active ingredient) or left untreated, for comparison with the  
445 test product under evaluation.

446 ED<sub>50</sub>: The dosage that produces a desired effect in half the test population.

447 EC<sub>50</sub>: The concentration of a drug where 50% of maximal effect is observed.

448 **8. REFERENCES**

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