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SCIENCE MEDICINES HEALTH

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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 guideline on demonstration of target animal safety and efficacy of veterinary medicinal products intended for use in farmed finfish (EMA/CVMP/EWP/459868/2008).

Keywords	***Veterinary medicinal products, target animal safety, efficacy, farmed finfish***

*The current revision consists of administrative changes made in order to align the guideline to the new definitions and terminology provided by Article 4 of Regulation (EU) 2019/6. The references to the legislation applicable and other scientific guidelines have also been updated. As no changes were made to the scientific content, no concept paper and no public consultation were deemed necessary.

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Guideline on demonstration of target animal safety and efficacy of veterinary medicinal products intended for use in farmed finfish

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Executive summary

The guideline on the demonstration of target animal safety and efficacy of veterinary medicinal products intended for use in farmed finfish provides guidance on the pre-clinical and clinical aspects of the assessment procedure for those who apply for the authorisation of such products.

This revision replaces the current guideline on demonstration of target animal safety and efficacy of veterinary medicinal products intended for use in farmed finfish (EMA/CVMP/EWP/459868/2008). The guideline only addresses finfish, as these are the main species kept under farmed conditions in Europe.

1. Introduction (background)

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

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

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

In principle the results of all trials performed may be used as documentation irrespective of where they have been carried out. However, the applicant should take into account the various conditions (e.g. climatic, disease situation, water temperature and salinity) as these may influence the outcome and hence the applicability of the studies.

2. Scope

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

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

3. Legal basis

This document should be read in conjunction with Regulation (EU) 2019/6. Applicants should also refer to other relevant European and VICH guidelines, including those listed among the references at the end of this document.

4. General considerations

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

If the product is intended for in-feed administration, the possible impact of the feed composition should be considered and investigated, if appropriate. The feed composition and manufacturing process may influence the medicinal product with regard to physico-chemical compatibility. Fish have a very marked sense of taste and smell. To ensure that fish will accept the final product, it is recommended that the

palatability of the active substance and if relevant the excipients, are investigated prior to clinical trials.

Conditioning and pelleting are the main factors affecting stability during the manufacture of medicated feeding stuff. These processes can subject the medicated feed to high temperature and pressures, which can cause degradation of active substances, excipients or feed constituents. Other quality points for consideration are homogeneity and segregation of these products. For further information the applicant is directed to the CVMP Note for guidance on additional quality requirements for products intended for incorporation into animal feeding stuffs (medicated premixes), see references listed at the end of this guideline.

To account for fish being poikilothermic animals, the term "degree-days" should be used wherever relevant.

Several farmed fish species are kept in areas where the water temperatures may vary considerably during the year. Pre-clinical studies should therefore be carried out to cover the relevant temperature range. The optimal temperature for the disease should also be taken into consideration when planning the studies. Normally studies should be carried out at two water temperatures. The applicant should justify the choice of water temperature(s) in relation to the fish species/ product / indication. Exceptions from carrying out studies at two different temperatures should be justified by the applicant.

More limited investigations may be acceptable for a compound previously authorised for use in another relevant species. This is elaborated under the appropriate topic (see sections 5.4 and 6).

When justified, data from non-aquatic species can be used as supportive information.

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

4.1. Study reports

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

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

To enable a proper assessment of tolerance in the target animal, adverse events should be reported in sufficient detail. An explanation of non-specific mortalities and comments on any physical or behavioural abnormalities should be provided.

For clinical trials the applicant should clearly state the onset and the duration of relevant disease outbreaks. This information will allow the evaluation of coincidental mortality data and the potential threat to the statistical power of the study. An explanation should be provided showing how the data continues to be valid and fit for purpose.

4.2. General study design

The main purpose of the documentation of efficacy is to demonstrate the therapeutic value of a new veterinary medicinal product for farmed finfish and to define an optimal dose and dosage regimen.

Normally, data from both pre-clinical studies and full scale clinical trials will be required. Where appropriate the applicant should justify the lack of relevant data.

The efficacy of the veterinary medicinal product should be stated as a function of dose, frequency and duration of treatment. The criteria used for the evaluation of efficacy in the trials should be pre-determined. In confirmatory clinical trials one primary efficacy endpoint should generally be identified and one or more secondary endpoints may be reported. The primary endpoints should accurately reflect the intended benefit of the product. The results should be presented in a way that is suited for adequate statistical evaluation. The clinical trials should cover all claimed indications and each indication should be discussed and reported separately. Statistical analysis of the results should be performed whenever relevant.

The applicant should justify the observation unit (e.g. individual fish or cage) and the number of samples collected on each sampling occasion. The sample sizes should be sufficiently large and statistically justified and based on clinically relevant endpoints.

In studies of products intended for use against aquatic one-host parasites (e.g. sea lice on salmon) sampling a limited number of fish from many cages instead of many fish from a small number of cages is recommended in order to take into account clustering which naturally occurs with such parasites.

In all studies the final formulation or an essentially similar formulation should be used and administered by the proposed route. Where a similar formulation is used, bioequivalence should be confirmed.

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

5. Pre-clinical studies

The objective of pre-clinical studies is to characterise the active component/formulation either to collect information of relevance when designing clinical trials, or to document that clinical data obtained previously could be used to comprise a new formulation, a new route of administration or administration in a new environment with regard to temperature or salinity.

When deciding which pre-clinical studies are relevant for the part 4 documentation, the mode of action (if known) and route of administration should be taken into account. For example, for waterborne products acting directly on ectoparasites, neither pharmacodynamic nor pharmacokinetic parameters in the target species are relevant from an efficacy point of view. It is however important to know the mode of action and the effective concentration for the parasite. On the other hand, for ectoparasiticides administered orally, pharmacokinetic parameters of the target species are of relevance, as the active substance needs to reach the site where it is presented to the parasites (e.g. blood, tissue fluids or mucus layer) in sufficient amounts.

Great care should be taken to ensure that the fish receive the intended dose. For single dose studies of orally administered products, it is recommended to administer the test substance orally by gavage. For repeated dose studies of premixes intended for medicated pellets, examples of control methods applicable to trials with small and large numbers of test fish, respectively, are given below:

- Small number of test fish:

  Count the number of pellets before they are given to the fish. After dosing, count the uneaten number of pellets and then calculate the average dose received.
- Large number of test fish:

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

5.1. Pharmacodynamics

The pharmacodynamic (PD) effects, including the mode of action of the active ingredient(s) as the basis for the recommended use of the product, should be described. In some cases, this information will be available in the safety part of the dossier. A cross reference to Section 3A could be made where appropriate.

All available relevant data (e.g. published references) should be presented, including data from other animal species, where appropriate.

For antimicrobials, microbiological studies *in vitro* should be carried out according to the principles in the CVMP guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances. The study parameters should be stated in detail. Notably, temperature and possibly salinity in the medium may affect MIC values and should be stated. *In vivo* studies to investigate the mode of action are normally not required.

For antiparasitics, the mode of action could be investigated *in vitro* according to standard protocols. For *in vivo* studies the principles of the VICH GL7 (Efficacy requirements for anthelmintics: General requirements) apply.

As fish are poikilothermic, the applicant should justify their choice of temperatures at which *in vivo* studies are conducted as significant temperature related effects can be expected. In addition other water quality parameters such as water salinity, pH and hardness needs to be taken into account in case of topically administered products.

5.2. Pharmacokinetics

It is recommended to carry out pharmacokinetic studies in finfish in accordance with the principles in the CVMP guideline on conduct of pharmacokinetic studies in target animal species, as far as they may be applied to fish. This guideline describes all relevant steps for pharmacokinetic studies, regardless of species. The main difference in fish is that repeated samples from the same fish may not be possible. Thus, samples from different fish at different time-points may be necessary. When modelling the results, all fish sampled are regarded as one "mega-fish". There are statistical methods available to determine confidence intervals for pharmacokinetic parameters, e.g. bootstrapping (e.g. Nordgreen *et al.* (2009)).

Pharmacokinetic studies are required for part 3 in all cases where there is a need to document the residue depletion profile. However, the applicant is also strongly recommended to consider pharmacokinetics as a tool to help reducing the total amount of clinical data – and hence the number of test fish – needed.

In pre-clinical studies where systemic pharmacodynamics or tolerance is documented, pharmacokinetic data recorded in the study will increase the total amount of information, as effects could then be linked to exposure rather than to dose only. Also, in studies where the individual dose is not known (as in case of oral group medication) this will allow estimation of an exposure/effect relationship on an individual level.

Pharmacokinetic data can also be used for bridging purposes. For instance, if clinical data are recorded in a study where a certain formulation is used, these data could be relevant also to support the therapeutic performance of another formulation, provided there are comparative pharmacokinetic data to show that the two formulations are similar in terms of exposure.

Comparative pharmacokinetic studies could also be considered to bridge data between different environments such as different salinity or different temperatures (high and low end of natural variation).

5.2.1. Performance of tests

Pharmacokinetic studies should be performed under relevant conditions (e.g. salinity, temperature), as kinetic parameters, such as bioavailability, may be significantly affected by such factors.

The choice of study design will depend on the objectives of the study. As it is difficult to collect a comprehensive set of data from an individual fish, analysis on group level is normally used. Standard pharmacokinetic parameters could be calculated based on data from groups of animals sacrificed at different time points.

When studies of distribution are used to support efficacy (e.g. substances that require distribution to skin or mucus for effect), relevant part 3 studies should be planned with the combined use in mind. Either standard chemical analyses or methods using radiolabelled substance (such as whole body autoradiography and scintillation counting) could be used.

Sampling fish out of a small group may cause stress, e.g. decreased food uptake among the remaining ones, which may affect the outcome of the study. Measures should be taken to reduce stress. It is recommended that the group size is large enough to avoid stressing the remaining fish during all sampling procedures throughout the study. The applicant should justify the group size chosen for each study.

5.3. Resistance

The mechanism for, and frequency of, resistance should be discussed including information on possible transmission. Possible cross-resistance to other active substances used in farmed fish should be stated.

For ectoparasiticides, experience of development of antiparasitic resistance should be included, if relevant. Experience related to development of resistance also from other areas of use (e.g. as pesticides) should be included when available.

5.4. Tolerance in the target species

Target animal safety should be determined in all of the target species, as defined by the investigator, unless otherwise justified. Studies performed in one species of fish may be considered relevant for the evaluation of tolerance 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 a case there must be supportive safety data from clinical trials in the second species. It may for example be considered unnecessary to

carry out formal target animal safety studies in trout if such studies have been carried out on other species of salmonids, and if supportive safety data from clinical trials in trout are available.

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

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

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

The following points apply to all target animal safety studies:

5.4.1. Test product

It is recommended to use the final formulation of the medicinal product. Where the formulation used in studies differs from the final commercial formulation the applicant must demonstrate that the bioavailability of the formulations is the same. Substances administered by gavage should have a suitable formulation, e.g. solution, suspension, capsule or in feed. All formulations used in the tests should be assayed for the concentration of the active substances before the start of the study(ies).

5.4.2. Negative control groups

Studies with in-feed medication should be carried out with the medicated group (preferably using the final formulation of the test product), and an untreated "feed-alone" group.

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

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

5.4.3. Holding

The fish to be tested should be in a normal physiological condition and be feeding well during two weeks of acclimatisation. The allocation of fish in groups should be done randomly the day prior to administration of the test product, using an appropriate method. To reduce stress caused by handling of the fish, and for practical reasons, it could be acceptable to allocate and/or mark fish groups immediately before or during administration of the test product, if justified.

Acclimatisation is not applicable for embryonal stages.

The following conditions of exposure are recommended:

Stocking density:

Semistatic test:

- Waterborne administration: max. 1 g fish/litre of water
- Oral administration: max. 5 g fish/litre of water
- Parenteral administration: max. 5 g fish/litre of water

Flow through:

Higher loading is acceptable.

Group size and number:

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

Fish size:

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

5.4.4. Necropsy histopathology examinations and blood analyses

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

In relation to the pre-clinical studies on target animal safety, haematology and blood chemistry should be performed. If blood chemistry and haematology parameters are unremarkable in the highest dose group, there is no need for testing in the lower dose groups.

The parameters chosen for testing should be justified by the applicant. For substances already approved for other animal species, the decision on whether blood chemistry and haematology are performed should be based on the previous findings in these other species. The applicant should include in the documentation a discussion/justification of the decision on whether blood chemistry is done, and, if so, which parameters are chosen for testing.

5.4.5. Dose justification and duration of dosage

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

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

For single dose studies at least 3 dose levels should be tested. The selection of dose levels should be based on the proposed therapeutic dosing regimen.

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

5.4.6. Oral administration

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

For solutions and suspensions given by gavage, the concentration of the active ingredient should be adjusted so that, if possible, no more than 0.5 g or ml test product per 100 g fish is used to achieve

the required dose. These maximum dosage recommendations are given as an advice to the applicant on the practical dosage limitations in fish.

5.4.7. Waterborne administration

Dipping and bathing are methods of administration considered as waterborne administration.

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

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

5.4.8. Parenteral administration

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

In some cases, investigating fewer than three dose levels could be justified since there are practical limitations to the volume that can be injected into fish. In addition, the test solution may have limited solubility which restricts the maximum concentration in a restricted volume. This can make it difficult to obtain more than two dose levels with a significant difference.

5.5. Laboratory studies

The test conditions can be controlled and standardised in land or sea-based test facilities. Pre-clinical studies should be performed for the main target species.

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

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

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

If negative controls are used:
Studies with in-feed medication should be carried out with the medicated group (using the test product), and an untreated "feed-alone" group. For products intended for other routes of administration one negative control group is usually sufficient.

5.5.1. Challenge studies

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

The test animals should not previously have been exposed to the challenge organism. Specific justification should be given for the use of test animals that have previously been exposed to the challenge organism, since such exposure has the potential to alter study results. The challenge organism should be of a strain relevant for the current disease situation, and be isolated and characterised by the most appropriate method, preferably a standard method used by the national reference laboratory, which should be described in detail. The timing and performance of the challenge and the design of the study should be justified by the investigator. The results of the introduction of the challenge organism should be reported, based on parasite counting, microbiological analyses or other pertinent investigations. If appropriate a statistical analysis should be provided.

5.5.2. Dose determination studies

Dose determination studies are normally pre-clinical studies with or without challenge. The purpose of the studies is to determine the optimum dose, dosage interval and total period of treatment for the claimed indications. By integrating PK and PD-data as a basis for the preselection of a treatment dose, the need for more extensive studies such as traditional dose determination studies might be reduced. The estimated dose should be confirmed by dose confirmation studies.

A dose/response relationship for therapeutic effect and, if possible, for adverse effects, should be established. Dose determination studies may be performed as clinical trials. Data from well controlled pre-clinical studies are preferred wherever relevant models are available, and clinical trials could then serve to confirm the findings from the controlled trials. The final dosage recommendations should be supported by documentation showing that satisfactory efficacy is obtained within the relevant temperature range.

Tests must be carried out in seawater and freshwater, if relevant to the proposed use, unless it is documented that the pharmacokinetics of the active substance is not affected by salinity.

In case there are validated endpoints/models available, dose determination could be performed only as PK/PD modelling. In those cases it is important that a sufficiently large exposure range is investigated implying that normally more than one dose level should be investigated.

For antimicrobials, MIC data could be used together with pharmacokinetic parameters to estimate appropriate level of exposure. Such data could be used for dose determination provided the PK/PD surrogate marker used is adequately validated. Note that these parameters differ between antimicrobials and bacteria implying a necessity to justify the choice of parameter in each case. The pharmacokinetic endpoints should be derived from plasma, and the free (non-protein bound) fraction of active substance concentration should be used for calculations.

5.5.3. Dose confirmation studies

Separate dose confirmation studies can be replaced by clinical trials performed with the final formulation of the veterinary medicinal product administered in the recommended dosage regimen.

6. Clinical trials

The scope of the clinical trials is to ensure that the veterinary medicinal product is efficacious and safe in the diversified conditions for aquaculture found in the EU. Clinical trials should be performed in accordance with GCP.

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

For products intended against diseases representing a potential threat to animal welfare, negative controls should only be used if no product is authorised for the claimed indication. The control group can be treated once an adequate estimation of difference in effect can be established.

Clinical trials are required for each proposed indication and for all target species in which efficacy is claimed. For some products, such as waterborne treatments which act directly on ectoparasites and which are independent of the pharmacokinetics in the fish, clinical trials in a second species may not be required if the clinical data obtained for the main fish species can be shown to be relevant to the second species. In such cases, sufficient justification for the omission of clinical trials, together with documentation of target animal tolerance is necessary. All trials should be performed under appropriate conditions according to the proposed method of use of the product. Trials should for example be carried out in water temperature(s) in which the test product is likely to be used considering the different climatic conditions within the community. The trials should be blinded, unless otherwise justified.

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

The clinical trials should include control groups. Applicants should justify the choice of control group (positive or negative). In case a positive control is used, the study design should be justified with regard to sensitivity to detect effects above placebo level. The applicant should consider/discuss all variables likely to confound results and the methods that will be used to reduce/avoid them.

Fish should be removed from the trial when showing definitive signs of disease and/or when there has been pathological confirmation of disease in the holding unit rather than waiting for death to occur. Only where the removal of fish showing clinical signs would significantly disrupt the value of the data should animals showing such signs be left in the enclosures. In all circumstances, humane endpoints should be applied.

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

For oral medication the daily uptake of medicated feed should be recorded together with the daily dose of the active substance, if possible. Premixes should be administered as medicated feed prepared by the procedure recommended by the manufacturer, preferably using a standardised feed.

6.1. Selection of farms

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

6.2. Selection of groups

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

6.3. Trial procedure

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

6.4. Diagnostic criteria

The presence of the investigated diseases should be confirmed in all groups included in the trial. The criteria for establishing an accurate diagnosis should be given. Standard diagnostic methods should preferably be used. The same criteria are to be used in all trials and should include post mortem examination of a sufficient number of fish, at least six fish from each group. The precise disease condition and identification of any pathogenic organism should be provided.

Diseases caused by microorganisms should be diagnosed by isolating and characterising the pathogen by the most appropriate microbiological method, preferably a standard method which should be described in detail. Samples from at least six fish per group are recommended.

Definitions

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

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

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.

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

Negative control: A negative control group is a group treated with placebo, either saline or vehicle (finished product deprived of the active substance) or left untreated, for comparison with the test product under evaluation.

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CVMP Note for guidance on additional quality requirements for products intended for incorporation into animal feeding stuffs (medicated premixes) (EMEA/CVMP/080/95)

CVMP Guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals) (EMA/CVMP/EWP/81976/2010)

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