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in

Rogers C. (ed.), Basurco B. (ed.).
The use of veterinary drugs and vaccines in Mediterranean aquaculture

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 86

2009
pages 13-28

Control of the use of veterinary drugs and vaccines in aquaculture in the European Union

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Abstract. This paper attempts to explain as simply as possible the regulatory framework for veterinary medicines in the EU and in particular how this framework affects the availability of medicines in aquaculture. The ways in which these controls have been introduced, the reasons for them and how they have been developed, particularly in the last decade, are described. The requirements and costs of developing regulatory data for Marketing Authorisation for producers of pharmaceutical and immunological products are considered in the light of the limited potential for return of investment on their part.

Keywords. Regulatory framework – MRL – Marketing Authorization – Residue monitoring – Immunologicals.

Contrôle de l'utilisation de médicaments vétérinaires et de vaccins en aquaculture dans l'Union européenne

Résumé. *Cet article tente d'expliquer aussi simplement que possible le cadre réglementaire concernant les médicaments vétérinaires au sein de l'Union européenne et en particulier comment ce cadre influence la disponibilité de médicaments pour l'aquaculture. Sont décrits les modes d'introduction de ces contrôles, les raisons les motivant et les modalités de leur développement, en particulier lors de la dernière décennie. Les exigences et les coûts liés au développement de données réglementaires donnant lieu à une autorisation de mise en marché pour les producteurs de produits pharmaceutiques et immunologiques sont considérés à la lumière du potentiel limité de rentabilité de leurs investissements.*

Mots-clés. *Cadre réglementaire – Limite maximale de résidus – Autorisation de mise en marché – Surveillance des résidus – Produits immunologiques.*

I – Introduction

This review is effectively an update of one presented and published in the proceedings of the American Chemical Society's symposium on Xenobiotics in Fish which was held in Dallas, Texas in 1998 (Alderman, 1999). Both the original and the present update (to 2003) concentrate on the changing regulatory framework controlling the authorisation and use of veterinary medicines in aquaculture in the European Union (EU). To an updated consideration of the regulatory framework on fish veterinary pharmaceuticals are now added detail and comments on the regulation of veterinary immunologicals for aquaculture.

In 1998, when the previous review was written, EU legislation on veterinary medicines was at an intermediate stage in the development of a harmonised regulatory framework for the control of all aspects of veterinary medicines within the EU. This harmonisation process was being carried out at a time when the regulatory environment would in any case have inevitably had to become more demanding as greater consumer safety requirements were introduced during the 1990s. Thus the initial legislation laid a framework which was primarily directed towards protection for consumers of food animals to which was added increased provision for protection

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of the environment. The net result was the introduction of an increasing range of requirements for safety, quality and efficacy data on veterinary medicines at a time when all areas of animal husbandry were becoming less profitable. Veterinary medicines for aquaculture form only a very small part of the animal health market, but must conform to all of these regulatory requirements, which are costly and laborious to satisfy, even for areas of veterinary medicine that have a more extensive customer base. It is appropriate to draw attention to the costs and profitability, and the effects of increased regulatory demands, at the beginning of this overview of European legislation because of the impact they have on the availability of aquaculture medicines. The result, not just in aquaculture, but in all areas of veterinary medicines for food animal species was a dramatic fall in numbers of applications for Marketing Authorisations (MAs), combined with a failure on the part of the pharmaceutical industry to support many existing products. Imposition of major increases in costs in a limited market produced these inevitable results. By 1998, it was increasingly being recognised that the good intentions of the original legislation had imposed a framework that was over ambitious both from timing and from the point of view of the costs imposed.

The harmonisation process commenced in 1981 with Directives 81/851/EEC and 81/852/EEC (CEC, 1981a,b). These two Directives and their subsequent amending Directives set the initial basic standards for approvals for veterinary medicines in the European Community. The term Marketing Authorisation (MA) replaced the term Licence. Veterinary immunological products were not covered by this initial legislation but were introduced into the process by Directive 90/677. The requirements of these initial pieces of legislation effectively limited the use of a veterinary medicine only to the disease and host species for which its approval had been granted. Directive 90/676 introduced the concept of the "cascade" system by which (for a few animals) medicines licensed for use in other food animal species could be used (the equivalent term in US is "off label use"). A standard withdrawal period of 500 days for cascaded and emergency use products in fish was introduced, but the problem of a definition for a "few animals" in the context of fish farming was, however, not addressed.

As part of the harmonisation process, a European Medicines Evaluation Agency (EMEA) was formed in 1993 (Council Regulation 2309/93/EC). It is responsible for both human and veterinary medicine and the EU's existing Committee for Veterinary Medicinal Products (CVMP) became a committee within its structure. The EMEA is responsible for the determination of applications for veterinary medicine authorisations submitted under Centralised Procedures (applications for Europe-wide MA) within the EU.

In 2001, all of these Directives, plus the numerous pieces of emending legislation defining data required and procedures for applying for Marketing Authorisation for veterinary medicines, were re-codified into a single all embracing Directive, 2001/82 (EPC, 2001). This did not change requirements or procedures but greatly simplified matters, avoiding the need to refer to multiple legal documents. At the time of writing, the European Parliament and Commission had published further draft proposals which modified and developed the laws codified in Directive 2001/82 (CEU, 2003). The preamble to these proposals recognised the conflict between the high costs of consumer safety requirements and the availability of veterinary medicines. It also recognised that the current borderlines between legal definitions of veterinary medicines and other products, such as feed additives, are sometimes unclear. Implementation of these good intentions can at least mitigate the present decline in veterinary medicine availability.

As suggested above, there is often a lay assumption that major profits are to be made from veterinary pharmaceuticals and, whilst for some companion animal products this may be true, for food species it is today far from reality, particularly in niche market areas such as that of aquaculture medicines. Fortunately, in salmonid culture at least, the last 15 years have seen the development and wide availability of efficient vaccines for the prevention of bacterial fish diseases. This has partly made the reduced availability of aquaculture pharmaceuticals less serious than it would otherwise have been, and has also driven a reduction in support of pharmaceuticals for fish by reducing industry demand, thus reducing sales and profit, that might

otherwise have supported continuing availability. The costs of developing the data package described below, which is required for pharmaceuticals, is considerably higher than for immunologicals, although neither is negligible, but they vary with the nature, complexity and novelty of the products concerned. Once data is generated, both at the stage of obtaining a Maximum Residue Limit (MRL) and at the final stage of MA, fees are required by the regulatory authorities, which, although small compared with the costs of generating the data, are still significant (Table 1).

Table 1. Fees charged for obtaining MRL and MAs. Centralised procedure fees: (EMEA) (April 2003)

Veterinary pharmaceuticals	
For establishing an MRL	50,000 €
For modifying an MRL	15,000 €
Full MA (EU wide) application	100,000 €
Veterinary immunologicals. Full MA application	50,000 €
Member States (e.g. UK, April 2003). Full MA Application UK	
Major	30,000 €
Complex	17,200 €
Standard	7,425 €

In addition to the European Union laws and bodies, there are two other organisations that have influence on approvals of veterinary medicines in Europe. These are the European Pharmacopoeia Commission (a Council of Europe body) and the Codex Alimentarius Commission (a United Nations body). They are referred to elsewhere in this document as EPC and CAC, respectively. The EPC is responsible for the preparation and maintenance of the European Pharmacopoeia which now includes Monographs for testing of some fish vaccines (q.v.). The CAC, amongst other responsibilities, sets Maximum Residue Limits for Veterinary Drugs (MRLVD), which are recognised internationally.

II – Veterinary medicine: Definition

Before discussing details of the veterinary medicines approvals process, perhaps the meaning of the term should be defined. A veterinary medicine is a product designed for administration (to animals) for a Medicinal Purpose. This is defined as:

(i) treating or preventing disease
(ii) diagnosing disease
(iii) contraception
(iv) anaesthesia
(v) otherwise preventing or interfering with normal operation of a physiological function

A veterinary medicinal product is therefore a product used by being administered (to animals) for a Medicinal Purpose. Within the EU such veterinary medicinal products may only be manufactured and supplied by the holders of a Marketing Authorisation.

Currently (2003), the EU consists of 15 member states with 10 more who will become members in May 2004, as well as 3 applicants in line to join, and all of the new Member States will have to adapt their existing legislation to comply with EU requirements. Much of the law in this area also has EEA application (i.e. it also applies to Norway, Iceland and Liechtenstein). The procedures and requirements for MAs (licensing) have now been simplified by codification into the single Directive 2001/82/EC. Although linked in many ways with Directive 2001/82/EC, provisions for the control of veterinary residues in edible tissues for consumer safety however remain as a separate and extensive body of legislation.

III – Marketing authorisation: Three scientific criteria

Applications for veterinary medicine MAs are assessed in the EU, and in most other regulatory regimes, against three basic scientific criteria. These are quality, efficacy and safety. The requirements for satisfying each of these criteria are presented in detail in the relevant volumes of "The Rules Governing Medicinal Products in the European Union" published by the European Commission. These documents are most easily accessed from the EU website http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/eudralex_en.htm where current versions may be downloaded.

Although these three regulatory criteria apply equally to veterinary pharmaceuticals and veterinary immunologicals, the specific details differ so that they are best considered separately.

1. Pharmaceuticals

For pharmaceutical products, quality criteria principally concern the chemistry and pharmacy of all components of the medicinal product, details of the processes of manufacture, packaging and stability under storage, especially in relation to formulation and intended route of application (i.e. bath or in feed). This includes details of the chemical nature and identity of excipients and, especially for medicated premixes, details of particle size and distribution.

Data are required to support manufacturer's claims of efficacy and this can sometimes be difficult to demonstrate. With bacterial and viral diseases, effective laboratory challenges can normally be developed, thus enabling the demonstration of comparative relative percent survivals (RPS) between protected and unprotected animals, although with some agents field challenges may be essential. Field challenges are also required to confirm that laboratory efficacy results are replicable under farm conditions, but major difficulties can arise in proving that an adequate challenge has occurred since no farm will allow numbers of animals to remain unprotected to prove challenge and serve as a focus of on-farm infection.

For therapeutants, efficacy studies need to demonstrate that the drug concerned is effective against the target pathogen and that an appropriate dose regimen has been demonstrated in terms of dose titration and duration. Studies of this type can be difficult and costly to carry out and there has been a tendency to use a standard therapeutic period, often 10 days with less than adequate scientific support. For fish, the great majority of therapeutants are supplied by the oral route which leads to additional requirements for medicated premixes and manufacture of medicated feeds.

Although much of the requirements of the quality components are in themselves safety considerations, safety itself is a separate specific consideration and forms by far the largest part of any application for approval. Applicants are required to demonstrate that the product is:

i) safe to the consumer;
ii) safe to the user – farm staff, during feed medication on farm or in the feed mill;
iii) safe to the target species (fish);
iv) safe to the environment.

A. Consumer safety: The Maximum Residue Limit and Regulation EEC/2377/90

For the consumer, the primary safety aspects concern the prevention of hazards of consuming unsafe residues in the edible tissues of farmed animals. Primary control is exercised via Maximum Residue Limits (MRL), established by Council Regulation EEC/2377/90 (CEC, 1990). Readers should perhaps be reminded that whilst EU Directives must be implemented in the laws of Member States, EU Regulations are Community law applying across the EU. The MRL defines the maximum level of residues of any component of a veterinary medicine that may be present in foodstuffs of animal origin without presenting any harm to the consumer. With MRLs

defined, improvements in analytical methodology will not mean that detection of previously undetectable residues suddenly renders unsafe an animal which with previous analytical methods had been regarded as safe.

When enacted, Regulation 2377/90 provided for a period in which the required new data on existing products could be developed. After the end of that period, if no allocation to an Annex of 2377/90 (see below) had been made it was intended that products would have to be withdrawn for use in food species. This intention was too ambitious, and further extensions were granted for products for which manufacturers had indicated an intention to support by submission of preliminary data. All such extensions have now elapsed and the use of substances for which an MRL allocation has not been made results in illegal residues in any animal treated with such substances. The MRL for any substance is determined from data submitted to the Safety of Residues Working Party, a sub committee of the Committee for Veterinary Medicinal Products (CVMP). This determination is ratified by the CVMP and adopted into law by a Regulatory Committee in the form of Commission Regulations. This means that, although an MRL is generic and not proprietary, commercial support for the generation of data is essential. The MRL is determined by an iterative process from a range of safety data, the most important of which is the Acceptable Daily Intake (ADI). The ADI is defined as the level of a substance that may be consumed daily without presenting a hazard to the consumer. This is based on a suitable no-observed adverse effect level (NOAEL) or from observations in humans, divided by a safety factor, often 100. For a discussion of MRL determination and ADI relationships see Woodward (1996).

As indicated above, all pharmaceutically active components of veterinary medicines must be allocated to an Annex in 2377/90 before they can be considered for MA. The Annexes to Regulation 2377/90 are as follows:

Annex I. Full MRL set.
Annex II. Safe, no MRL needed to protect the consumer.
Annex III. Sufficient data to set a Provisional MRL, but additional data needed to allocate full MRL.
Annex IV. On consumer safety grounds no MRL can be set. Substances placed in this Annex are prohibited for use in food animal species.

Substances once of interest to aquaculture now placed in Annex IV include chloramphenicol, the nitrofurans (including nitrofurazolidone and nifurpirinol) and dimetridazole. Malachite green, once extensively used in aquaculture, has not been allocated to an Annex under 2377/90 because published data are inadequate to allow a modern assessment of its safety. This lacuna in the legislation can present significant difficulties, since, in this case, detection of malachite green (and metabolite) residues constitutes detection of illegal residues.

MRL data packages consist of two main parts, the safety file and the residue file. Although MRLs were originally intended to be species specific, much of the data in the safety file (e.g. mammalian toxicity studies and the ADI) may be reused for new species MRL applications. It should be noted that, in the EU as in the USA, for fish, edible tissues are defined as muscle with adherent skin in natural proportions (CEC, 2002). A factor for consumption of 300 g (substituting for red meat as a dietary component) is taken to determine the fish meat contribution to the ADI in the calculation of the MRL. Muscle and skin, in natural proportion, is therefore the target tissue for determining withdrawal periods in fish.

The unique part required for each species MRL is the residue study. This requires the determination of the marker residue which may either be the original drug or the metabolite most characterising the depletion of the drug from the edible tissues. Marker residue studies in general are conducted using radiolabelled drug. This is always extremely expensive to manufacture and as a safety study must be conducted in fish in GLP compliant conditions. There are very few such facilities capable of conducting such studies.

Initially, the intention was to require metabolite data for each active and excipient in each animal species for which the manufacturer intended to list in its MA. The rapid loss of veterinary medicines resulting from such requirements brought about a reassessment and EMEA published a Note for Guidance (Committee for Veterinary Medicinal Products, 1997) which recognised Salmonidae as "major species" and that any MRL for Salmonidae could be extended to other fin fish species. This was accompanied by a further Note for Guidance (Committee for Veterinary Medicinal Products, 1998) stating that, although only a limited number of MRLs have been established for fish, where these have been evaluated, the marker residue determined in Salmonidae has been identical to that established in other animal species. This pragmatic approach would allow the use of an MRL established for a substance in muscle in a major mammalian species to be applied to Salmonidae and other fin fish as well.

This pragmatic approach from the EMEA recognises the difficulties imposed by the costs of developing data for an MRL against expectations of poor or slight financial returns by potential applicants. Although not specifically expressed, there was evident anxiety that, by imposing excessive demands on data for minor species medicines, a lack of such medicines was rapidly developing, which has since been confirmed in proposals for modification of Directive 2001/82/EC.

There is, however, one problem not properly covered by Regulation 2377/90/EEC, nor so far addressed by any other legislation, EU or national. It is that without an application to set an MRL a substance is effectively in "limbo". Its use in food species is not authorised; equally it is not a prohibited Annex IV substance. More than most areas of veterinary medicine, aquaculture has used a range of "traditional remedies" whose use has persisted to the present day. Malachite green is the classic case and it is most unlikely that anyone will make a formal application to establish an MRL for this product. It can hardly be expected that, even if the effort were to be limited to the cost of assembling published data (which is known to be inadequate for a proper assessment) and of submitting it for consideration by the Working Group on the Safety of Residues, any application for an MRL allocation could be funded by a commercial sponsor. However, without such an application and accompanying data package a substance like malachite green will not be scrutinised by the Working Group and therefore it will not be entered into Annex IV and will not become a prohibited substance. However, it is true that, in any tissue residue monitoring programme, substances such as malachite green, without MRL or authorisation in any food species, would be regarded as illegal residues.

When new MRLs are approved, details of the assessment are published on the EMEA website (http://www.emea.europa.eu/) but the legal status is announced in a Commission Regulation modifying Regulation 2377/90. Naturally a large number of such emending laws have been issued since 2377/90 has been in place, and occasional Regulations providing a recodified list have been published, but none has been issued for several years.

B. The CAC and MRLVD

In the twelfth edition of the Procedural Manual of the Codex Alimentarius Commission (CAC, 2001), the maximum limit for residues of veterinary drugs (MRLVD) is defined as "the maximum concentration of residue resulting from the use of a veterinary drug (expressed in mg/kg on a fresh weight basis) that is recommended by the Codex Alimentarius Commission to be legally permitted or recognised as acceptable in or on a food". CAC set MRLVDs are accepted internationally. The procedures by which the CAC sets MRLVDs are complex and, owing to the inevitable international involvement, slow. Data are analysed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which meets only once a year. When a recommendation is reached (after much JECFA consideration), the conclusions are passed to CAC's own expert committee, the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF), for further evaluation.

Currently, the only full CAC MRLs for aquaculture species are for the administration of

oxytetracycline at 100 µg/kg to "fish" and "giant prawn", but several additional MRL proposals from JECFA are now within the CAC system. From this, it is clear that it will be many years before the CAC sets a usable list of MRLs relevant to aquaculture; national or market-area MRLs will therefore predominate in the protection of consumers within their areas.

C. Withdrawal periods

The establishment of an MRL allows the setting of a withdrawal period for the product. The withdrawal period is a set time between last medication and earliest date of slaughter and must be sufficient to ensure that veterinary residues have depleted to levels below the MRL. A withdrawal period is effectively proprietary since it refers to the specific formulation and presentation of a complete product. The MA applicant normally proposes a withdrawal period based on data generated in the species for which the product is intended. The licensing authority concerned assesses the data against the proposal and agrees with the proposal. Alternatively, the licensing authority may discuss and agree an alternative with the applicant, or may refuse on grounds of inadequate data. In most food animal species, withdrawal periods are defined in elapsed days, but because fish are poikilotherms whose metabolic rate is determined by environmental temperature, the EU has taken the approach of requiring data to be presented from trials conducted with at least two water temperatures relevant to the proposed conditions of use. If depletion is found to be temperature dependant then a withdrawal period in degree days will be set, thus making the withdrawal period a function of temperature and time. If the data does not indicate a temperature effect on depletion then a day-based withdrawal can be accepted.

D. Target species safety

Tolerance studies must be carried out to determine the safety of the product to the target fish species. Details are provided in the "Rules" for both therapeutants and immunologicals (CEC, 1998b). In general these rules require the application of a double dose of the product followed by a period of close observation. Where a pharmaceutical is applied over a number of days, as is the case for most oral antimicrobial therapeutants, application of indicated dose for twice the recommended time period and for double the dose for the recommended time period are required. In some regulatory environments (e.g. USA), data from a safety test using up to 10x the manufacturer's recommended dose may be required.

E. User safety (farm and feed mill staff)

Medicines' legislation requires that user safety be assessed in the safety package and that the product label must include advice and warnings to the user giving guidance for safe use. Hazards associated with feed medication, whether in feed mill or on farm, must be considered, as must any hazards to the final user (fish farm staff). Such hazards include dust, the need for use of gloves, masks and other protective clothing for volatile liquids and for most formulations, the risks of skin absorption and hand contamination. Advice on these hazards must be provided on packaging labels and inserts and details of these labels are assessed as part of the approval process. The normal Health and Safety legislation protecting workers exposed to potential hazards in the workplace also applies, and requires the employer to have in place proper assessments of the hazards associated with the use of veterinary medicines in the farm environment. The user safety data included on label and package inserts should provide sufficient information for such occupational safety assessments to be made.

F. Environmental safety

Requirements to assess environmental safety of the use of any veterinary medicine product passing through the authorisation process formed part of the original harmonisation legislation and is now included in Directive 2001/82, with more extensive guidelines provided (CVMP, 1996). A twofold approach is required, Phase I consisting of an environmental impact

assessment which can be largely literature based, followed by Phase II involving studies based on the findings of Phase I. This guideline requires a three tiered approach in which the results obtained in each tier dictate the need for and type of tests needed in the next tier. If the product is demonstrated to be free of problems in a lower tier, then there is no need to carry out further tests at the higher tier level. Specific areas of concern at each tier are physico-chemical, environmental fate and ecotoxicity.

Acceptable results under the medicines' authorisation process will mean that a Marketing Authorisation (subject to all other necessary data) will be granted for a product to be marketed. However, as with user safety, further considerations, this time those of environmental protection, are then involved once the product is on the market and in use by farms. Authorities responsible for protection of the aquatic environment may determine how it is used, either generally or in specific localised environments. Since, in the process of Marketing Authorisation, only general environmental aspects may be taken into consideration, control of local environmental impact is necessary. There is considerable opportunity for regulatory conflict and, where environmental authorities are regionally- rather than nationally-based, there are opportunities for unequal interpretation of regulations to occur. Conflict may also occur between requirements of any environmental agency and the need to conduct preliminary field trials on substances still under development, where field trials are a necessary part of developing the full environmental assessment package.

At the inter-member state level there are also opportunities for uneven application of controls, since the harmonisation of environmental protection legislation across the EU is far less developed than it is for veterinary medicines. As well as EU and Member State environmental impact controls, multinational agreements such as the OSPAR Convention (The Convention for the Protection of the Marine Environment of the Northeast Atlantic, 1992) may also have a role in the control of environmental impacts, including those of veterinary medicines.

G. Pharmacovigilance and residue monitoring

Member States are required to operate a system for pharmacovigilance, to detect and report any adverse reactions in consumers, target species or environment (CEC, 2001a). This applies to both pharmaceutical and immunological products.

There is, of course, little value in developing an elaborate and expensive system for ensuring that no unacceptable residues are present in food of animal origin (i.e. Regulation 2377/90) if no action is taken to confirm and enforce compliance. The complimentary stage of the EU veterinary medicines harmonisation programme took the form of Directive 96/23/EC (CEC, 1996). This Directive introduced fish meat, poultry meat, milk and honey into Member States monitoring programmes that had previously been limited to red meat. It requires member states to produce a monitoring programme to search for illegal or excessive drug residues in fish meat. It is also a third country Directive (Article 29) in that countries exporting to the EU have to demonstrate that they are able to ensure that no unacceptable residues are present in fish meat exported to the EU by means of a suitable residues control and monitoring programme.

Any existing non-statutory national residue monitoring programmes have been replaced by a statutory programme in compliance with Directive 96/23. For farmed fish, a monitoring level of one sample (=one or more fish) per 100 tonnes of production is required. A maximum of two thirds of samples may be taken at a wholesale level provided traceability to farm is guaranteed, and the rest must be collected from the farm itself. Farms must keep full records of medications used and provide access to these during sampling inspections. Samples will be analysed for:

(i) the presence of residues of approved substances in excess of the MRL;

(ii) residues of illegal substances and for substances which it is believed may be in use under the provisions of the cascade system or for other reasons (e.g. traditional remedies such as malachite green) but for which there is no MRL in fish;

(iii) the presence of Annex IV substances.

Table 2 presents a full list of substances to be monitored for under Directive 96/23. As indicated above, this is a third country Directive, and would be exporting states must satisfy the European Commission that they have in place a residue monitoring programme equivalent to that in place in EU MS. Article 29(1) of Directive 96/23/EC states that inclusion and retention on the lists of third countries provided for in Community legislation from which Member States are authorised to import animals and animal products covered by this Directive shall be subject to submission, by the third country concerned, of a plan setting out the guarantees which it offers regarding the monitoring of the groups of residues and substances referred to in Annex I of the Directive. Article 8(3) of the Directive requires that by no later than 31 March each year, Member States (MS) shall forward to the Commission their monitoring plan results and that third countries must also comply.

Table 2. Directive 96/23 list of substances

Group A – Substances having anabolic effect and unauthorised substances	
(A1)	Stilbenes, stilbene derivatives, and their salts and esters
(A2)	Antithyroid agents
(A3)	Steroids
(A4)	Resorcylic acid lactones including zeranol
(A5)	Beta-agonists
(A6)	Compounds included in Annex IV to Regulation (EEC) 2377/90
Group B – Veterinary drugs† and contaminants	
(B1)	Antibacterial substances, including sulphonamides, quinolones
(B2)	Other veterinary drugs
(B2a)	Anthelmintics
(B2b)	Anticoccidials, inc. nitroimidazoles
(B2c)	Carbamates and pyrethroids
(B2d)	Sedatives
(B2e)	Non-steroidal anti-inflammatory drugs (NSAIDs)
(B2f)	Other pharmacologically active substances
(B3)	Other substances and environmental contaminants
(B3a)	Organochlorine compounds including PcBs
(B3b)	Organophosphorus compounds
(B3c)	Chemical elements
(B3d)	Mycotoxins
(B3e)	Dyes
(B3f)	Others

†Including unlicensed substances which could be used for veterinary purposes.

The most recent list of such approved monitoring plans was given in the Annex to Commission Decision 2001/487/EC (CEC, 2001b) which includes a listing of those third countries whose residue monitoring plans for aquaculture products had been approved. Subsequent monitoring of these plans when they are underway by the EU FVO may lead to reconsideration of approvals.

Plans and results of veterinary residue monitoring programmes must be submitted to the European Commission for approval and, additionally some MS publish the results of their monitoring programmes. One readily accessible example of this are the results of the UK monitoring programme that were routinely published before and since the implementation of Directive 96/23 and are now available on the internet at http://www.vmd.gov.uk/. Very brief summaries of the MS residues monitoring programmes for 1998 and 1999 are at http://europa.eu.int/comm/food/fs/sfp/fcr/reports/reports_en.html. The reports of the European

Commission's Food and Veterinary Office which carries out audits and on-the-spot checks on food safety controls in MS and in countries exporting to the EU are at http://europa.eu.int/comm/food/fs/sfp/fcr/reports/reports_en.html.

It should also be understood that the requirements of Directive 96/23 are minimum requirements and MS may, and do, carry out additional monitoring programmes both on national and imported products. In some MS, both the responsible Veterinary Medicines authorities and Food Safety bodies may have such additional monitoring programmes, normally carried out at wholesale or retail rather than farm levels. Provided that identical standards are required for monitoring national and imported food animal products, and that the definitions of safety comply with the current Annexes to Regulation 2377/90, then any level of monitoring above the minimum set by Directive 96/23 (and its amendments) may be used.

If, in any of the residue surveillance programmes, illegal residues are detected (i.e. residues of Annex I or III substances above the MRL at slaughter, or Annex IV or other specified residues at any time), MS authorities are required to take follow up action to prevent re-occurrence. Legal action against violation may ensue, although the tendency within any MS will be towards action to prevent re-occurrence unless the violation is clearly as a result of deliberate action. Additional analytical and monitoring costs are likely to be charged to the violator.

In the case of third country imports, violation may result in banning imports either at the MS level or at the European level, followed by inspection by veterinary officials. Imports will be barred until the cause of the violation is dealt with and proper assurances to prevent re-occurrence are in place. Reports of Inspections by the Food and Veterinary Office are available at: http://europa.eu.int/comm/food/fs/inspections/vi/reports/index_en.html.

As already explained, the programmes described above are designed to meet the requirements of the EU, they are therefore minimum "statutory" requirements. European MS (and agreed exporting country) monitoring programmes are designed to assure that national food production is safe and meets EU standards and thus there is no requirement to test imports from other MS or from approved exporting countries.

However, MS own food safety agencies and, for that matter, private bodies within those MS (e.g. major supermarket chains and food processing companies) are free to carry out additional monitoring of food across the whole range of food safety matters and normally will do so at the wholesale or retail level. Samples collected for inclusion in such surveillance programmes will consist of foods from across the full range of foods available to consumers and will therefore include foods of animal origin with national, European and third country origin. If illegal residues (e.g. of Annex IV substances) are detected, MS are required to advise the European Commission and, once aware that such residues may be present, it is likely that additional highly targeted testing will be commissioned, and action taken against the source of the problem.

Two recent cases of such MS non-statutory monitoring have led to trade upsets. One referred to the detection of chloramphenicol (an Annex IV substance) in a range of animal products from China, and the other to detection of nitrofuran (also Annex IV substances) residues in prawns imported from SE Asia. In both cases, imports were halted until the situation had been remedied in a way acceptable to the European Commission and FVO inspections had been carried out.

2. Immunologicals

As with pharmaceuticals, the basic information required in support of MA applications for immunologicals concerns quality, safety and efficacy. In the case of immunologicals, quality additionally includes quality of any excipients and adjuvants plus the microbiological aspects of the master and seed cultures, fermentation facilities and inactivation or attenuation procedures, etc. In practical terms, for fish, these requirements do not differ from the requirements for other veterinary vaccines.

As for pharmaceuticals, details of the supporting documentation required is listed in Volume VI of the "Rules" (CEC, 1998a) and the required safety and efficacy tests are further defined in Volume VII of the "Rules" (CEC, 1998b). Recently, the European Pharmacopoeia Commission (EPC) has published three fish vaccine test monographs defining safety and potency tests (EPCM, 2002a,b,c). The EU requires that where an EPC Monograph exists, it must be followed and a tendency has developed amongst regulators to expect the Monograph approach to be followed for testing all fish vaccines. Unfortunately, the three EPC Monographs published so far (Table 3) are judged by many in the field to be scientifically inadequate and with significant ambiguities in presentation, a critical failure for documents intended for international application. In addition, the three monographs are written as applying only to monovalent vaccines for use to prevent a single disease, whereas single antigen vaccines against the three monograph pathogens are now rarely used, since multivalent vaccines are the rule. It is to be hoped that these problems are properly addressed in the monographs on *Moritella viscosa* and *Yersinia ruckeri*, which are understood to be in preparation and that the existing monographs are redrafted to remove the more obvious flaws.

Table 3. European Pharmacopoeia Commission. Vaccine Test Monographs

Pathogen	EPC reference
Aeromonas salmonicida	01/2002:1521
Vibrio anguillarum	01/2002:1581
Vibrio salmonicida	01/2002:1580
Moritella viscosa (draft)	
Yersinia ruckeri (proposed)	

The safety tests required by EPC Monographs do not differ significantly from the standard double dose test specified in the earlier Volume VII Guidelines. The 21 day double dose safety test on at least three batches of vaccine has been long established, but there are ambiguities in cross references to EPC general vaccine safety provisions (European Pharmacopoeia, Section 5.2.6) requiring single and double dose safety tests. The Monographs also lack uniformity in definitions of the number of animals required to be used in each safety test. For injection vaccines, this is a minimum of fifty at the development stage, and ten at the routine batch test stage. The numbers are not justified scientifically, nor is there a requirement that mock vaccinated control fish be included in the tests. At a time when animal testing is strictly regulated, a proper scientifically validated justification for the numbers of animals specified for these tests should be provided.

In the EPC furunculosis vaccine monograph, there is a description of adverse reactions, primarily peritoneal adhesions, in a description of field safety trials, but for laboratory trials and for the two Vibrio Monographs "No abnormal local or systemic reaction [must] occur" and if more than 6% of fish die (from causes not attributable to the vaccine) the trial is invalid. Adhesions do occur in fish within the 21 day period of a double dose safety trial and the phraseology of the monographs is such that mortalities attributable to the vaccine are not a reason for the vaccine to be regarded as unsafe.

Of course, manufacturers and test facilities do include mock vaccinated fish in safety trials to ensure that non-vaccine cause can be separated from vaccine cause and a vaccine or vaccine batch that was clearly associated with excessive adverse reaction and mortality would not be regarded as safe.

The EPC monographs also require that the fish used in these tests are "from a population that does not have specific antibodies against the relevant serovars of [pathogen] and which has not been vaccinated against nor exposed to [pathogen]". Seronegativity does not necessarily demonstrate that fish have not previously been exposed to a specific pathogen or that they are

not immune. The requirements of Volume VII of "The Guidelines" defining freedom from disease and site disease history provided better guarantees that fish were suitable for use in safety and potency tests and demonstrated a better understanding of fish than do the present EPC monographs.

For vaccines, efficacy/potency must be demonstrated after manufacture in the target species against challenge by the target pathogen. Secondly, the duration of immunity (the period for which a vaccine offers protection) must be supported either by field or laboratory data, as must the claimed shelf life (the maximum time between manufacture and use when the product is stored under recommended conditions). In the development stage, data must be presented to show that at least three batches of vaccine are safe and effective, and field efficacy studies are also required. Once an MA has been granted, each production batch needs to be checked to confirm safety and potency.

For potency (efficacy) trials the EPC monographs again lack clarity. Fish must be seronegative to enter the trial, and equivalent numbers of mock vaccinates are also required. Mock vaccinates are fish of the same stock injected only with sterile saline. Mock and vaccinate groups must be held in the same tank and challenged using a validated challenge after time has been allowed for immunity to develop. At the development stage, at least three batches of the vaccine must be tested for efficacy. The requirement that vaccinate and mock vaccinate fish must be held together means that more animals must be used than would be the case if one group of control mock vaccinated fish were held separately, thus acting as controls for all three vaccine batch groups. The reason for the requirement for control and vaccinate fish to be mixed appears to arise from the belief that otherwise major tank to tank variation may affect the validity of the trial. With properly designed test systems this should not be the case and the presence of control and vaccinated fish can result in other problems for trial validity, namely that as control fish die, they may well produce a secondary co-habitant challenge to the vaccinated fish. Figures 1 and 2 present examples where secondary and even tertiary co-habitant challenges affect the outcome of a vaccine potency trial.

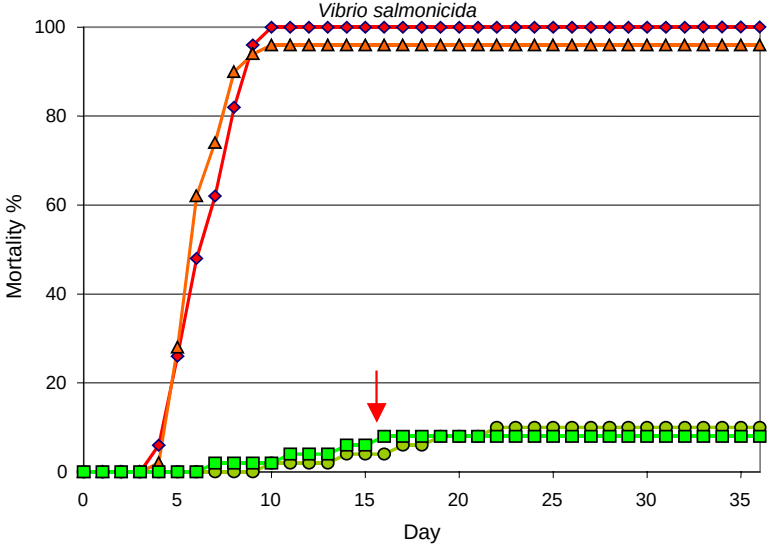

Fig. 1. Vaccine potency trial in which the effect of a secondary cohabitant challenge deriving from the control fish held in the same tank is seen at day 16 (arrow).

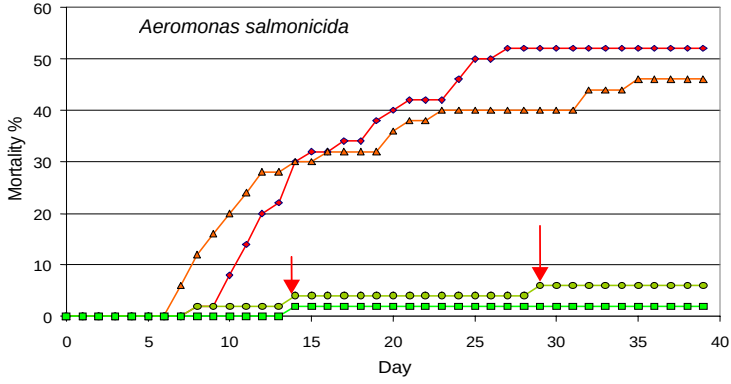

Fig. 2. Vaccine potency trial in which the effect of secondary and tertiary cohabitant challenge deriving from the control fish held in the same tank are seen at days14 and 29 (arrows).

For fish vaccines, potency has long been expressed in the form of Relative Percent Survivals (RPS), which is the number of vaccinated fish that remain alive in the face of a pathogen challenge compared to the numbers of surviving control fish. The EPC Monographs have taken this tool and have attempted to develop it, possibly with the idea of improving the welfare of the fish tested. The term RPS60 has been introduced, which is the percentage of vaccinates alive when 60% of the control fish have died. The RPS60 is therefore calculated as 1-(M/60)*100, where M = vaccinate mortality at 60% control mortality. In theory, this would allow the trial to terminate earlier than under previous guidelines in which potency tests were run for a minimum of 21 days or for 3 days after the last mortality, if longer. However the 60% control fish mortality has to be demonstrated as specific (i.e. pathogen caused). This is generally viewed as recovery of the pathogen from the dead fish, but this takes a minimum of 2 days and in practice does not result in shorter trials.

Results, however, can be misleading, thus, in Fig. 3 with final 80% control mortality, the vaccine continues to offer good protection irrespective of whether the RPS occurs at 60% control mortality or at any other time. Figures 4 and 5 with a different trial present a different view, although both present the same data with the same RPS60 of 87%, but at 21 days the vaccine is clearly less protective with the RPS having stabilised at 64%.

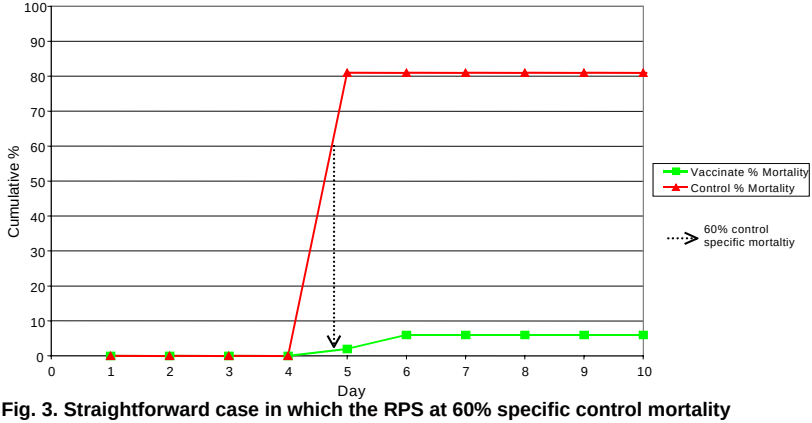

Fig. 3. Straightforward case in which the RPS at 60% specific control mortality does not differ significantly from the RPS later in the trial.

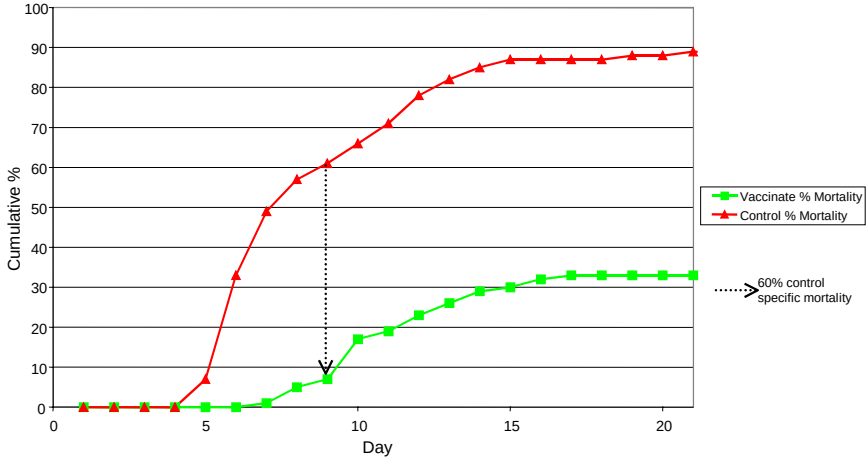

Fig. 4. By presenting only data relevant to the 60% control mortality the RPS appears high.

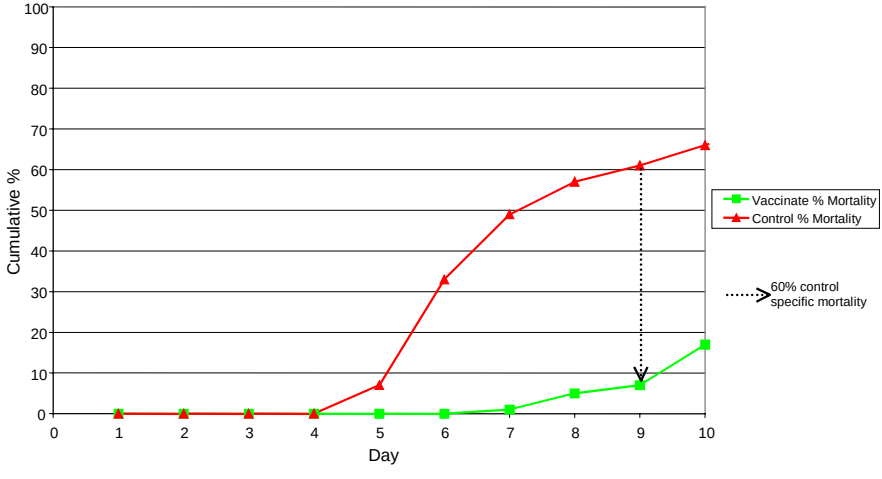

Fig. 5. If the results of the full 21 day trial are presented, the final RPS is seen to be less good than at the 50% control mortality point.

The EPC monographs specify minimum control mortalities for tests to be valid and minimum RPS60 levels for vaccine batches to pass (Table 4). No scientific justification is presented for these figures. The 80% RPS60 for *Aeromonas salmonicida* was certainly not attained by any of the early *Aeromonas salmonicida* vaccines (an RPS of 25% was common) whose introduction and uptake by the industry lead to the present range of very effective vaccines. Additionally, a tendency to work to "monograph style" to satisfy regulators leads to problems with pathogens such as *Moritella viscosa* where demonstration of specific mortality depends more on clinical pattern than reliable recovery of a pathogen from fish.

Table 4. European Pharmacopoeia Commission Vaccine Potency RPS60 pass requirements

	Minimum control mortality	Monograph RPS pass requirement
Aeromonas salmonicida	60	80
Vibrio anguillarum serotype 01	60	75
Vibrio anguillarum serotype 02	60	75
Vibrio salmonicida	60	90

Overall, therefore, whilst the EPC monographs represent an attempt to impose uniformity in tests on fish vaccines, they, in fact, represent a reduction in scientific test quality. No evidence for the reasons for setting the various numbers and parameters imposed are given, which presents those carrying out the tests that are essential to the availability of effective vaccines with the dilemma of how to justify their application. Additionally, they are as much, if not more, open to yielding inaccurate or misleading results.

IV – Conclusions

This paper presents an updated overview of the procedures and requirements for authorisation for sale of veterinary medicines in the European Union, concentrating on medicines for use in aquaculture. These requirements are complex and impose costs which can be difficult to justify for the limited aquaculture market. This has been reflected in a decreased availability of therapeutants for use in aquaculture in the last 15 years. Fortunately, the regulatory requirements for vaccines are less demanding and, given the difficulties of use of therapeutants in the aquatic environment, the rapid development of aquaculture vaccines in the last ten years has helped to solve many of the problems of bacterial disease in aquatic species.

The changing regulatory environment of the 1990's has now begun to stabilise and it is to be hoped that the proposals for further development of that environment, as foreseen in proposals from the European Parliament and Council (CEU, 2003), will not result in further demands for additional data.

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