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Available chemotherapy in Mediterranean fish farming: Use and needs

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Abstract. The availability of aquaculture treatments has changed significantly over the past several years. The European legislation regarding veterinary pharmaceuticals has evolved since 1981 towards important restrictions in the use of medicines to treat food producing animals. The establishment of maximum residue levels (MRLs) for all active substances has raised concerns about the availability of many fish medicines. However, great progress has been made since then and a large number of compounds remain available for the treatment of diseased fish. Currently available and authorised fish medicines are tabulated. Some promising new products have also been developed in recent years.

Keywords. Aquaculture treatments – Drugs – European legislation – MRL – Bioavailability – Antibacterials – Anaesthetics.

La disponibilité de chimiothérapie en aquaculture méditerranéenne : Utilisation et besoins

Résumé. *La disponibilité en médicaments aquacoles a changé de façon significative au cours de ces dernières années. La législation européenne sur la pharmacie vétérinaire a évolué depuis 1981 dans le sens d'une restriction importante de l'utilisation de substances médicamenteuses pour traiter les animaux destinés à la consommation humaine. L'établissement de limites maximales de résidus (LMRs) pour toutes les substances actives avait alors suscité des craintes quant à la disponibilité de nombreux médicaments aquacoles. Néanmoins, des progrès certains ont été accomplis entretemps et un grand nombre de molécules restent disponibles pour le traitement des poissons malades. Un tableau des médicaments aquacoles actuellement disponibles et autorisés est dressé. Quelques produits innovants ont également été développés ces dernières années.*

Mots-clés. *Traitements aquacoles – Médicaments – Législation européenne – LMR – Biodisponibilité – Antibactériens –Anesthésiques.*

I – Introduction

A decade ago, research into aquaculture treatments largely focused on the use of oral chemotherapeutants, mainly antibiotics. However, there was increasing concern about potential drug resistance, both in terms of its impact on the aquatic environment and its potential influence on consumer health.

The formulation of the "MRL" (Maximum residue limit) regulation in 1990, together with the development of monitoring programs for residuals from aquaculture drugs, made it clear that important restrictions in the use of medicines in fish farming could be anticipated. At the same time, noteworthy development was seen in the field of fish vaccine research that provided an alternative solution to chemical treatments, in most cases for bacterial diseases, leading at least to sizeable reductions in the quantities of antibiotics used in aquaculture. However, no licensed vaccines are available for some important marine pathogenic bacteria such *as Flexibacter maritimus* or *Rickettsia* sp.

In addition to bacterial disease treatments, strong headway was made in the field of antiparasitic treatment, in particular for the treatment of salmon lice and other crustacean parasites, with the emergence of oral treatments, instead of organophosphates, whose toxicity

and attached risks were becoming unacceptable. In the same way, some highly questionable substances, such as malachite green, have been gradually disappearing from the fish farming therapeutic arsenal and promising innovative products should be available to replace them in the near future.

II – The impact of European legislation on veterinary pharmaceuticals

Following the determination to harmonize the market within the European Union, a major evolution occurred in the legislation governing veterinary pharmaceuticals, particularly antibiotics. The basic European legislation for harmonization of laws relating to veterinary pharmaceuticals was adopted more than 20 years ago (CEC, 1981a,b, 1987). The main change, which mostly affected the availability of aquaculture medicines, has undoubtedly been the regulation for the establishment of maximum residue limits (MRL), the so-called "MRL regulation" adopted in 1990 (CEC, 1990). When it came into force in January 1992, there was some apprehension that many existing products might well disappear. This regulation states that "Member States can only grant a marketing authorisation for a veterinary medicinal product intended for a food-producing animal if for each contained active substance an MRL has been set". The initial concept of MRLs for the "pharmacologically active" substances has also been extended to include every excipient. The establishment of an MRL required new studies. For already old compounds, no longer protected by any commercial patent, no company was ready to bear the rather high costs related to these studies. Most commonly used antibiotics, such as flumequine and, even later, oxolinic acid, were at risk of being prohibited because of a lack of sponsors.

In addition, monitoring programs to detect and prevent the presence any residues of illegal products or of residues above the MRL have been established (CEC, 1996). For legislation purposes, all these rules have recently been repealed and codified in a synthetic Directive (EPCEU, 2001).

Interestingly, third countries exporting products into the EU are also required to demonstrate that they comply with these regulations (Schnick and Smith, 1999). The situation is no better in the United States where there are only four chemicals fully approved for use as therapeutants for fish, and only for a few species and particular uses (Table 1).

Eventually, important consequences also occurred for unauthorized compounds, because the detection of residues by monitoring programs gives evidence either of the use of a non-authorized product (such as furazolidone) or of an insufficient withdrawal period to allow for the elimination of the pharmaceutical.

The setting up of an MRL is only a preliminary step towards the achievement of a full marketing authorisation, which is ever more expensive. With perhaps the exception of Norway, marine aquaculture is a narrow market compared with land animal farming. This small market can hardly cover the costs of official registration under the current regulations, except for already well documented products which would only require additional safety and efficacy data for aquatic species (Alderman, 2002). Therefore, not surprisingly, a concentration effect occurred in parallel with the pharmaceutical industry. While most of the aquaculture medicine and vaccine suppliers were rather small start-up enterprises, they were eventually absorbed by larger firms. At the moment, all aquaculture suppliers belong to major international groups: Schering-Plough (AVL), Akzo-Nobel (Intervet Norbio), Novartis (Aquahealth, Vericore), Alpharma (AL, Biomed), and Bayer (Microtek).

Table 1. USDA-FDA animal drugs approved for aquaculture

Active drug	Trade name	Species	Approved uses	Dosage regimen	Withdrawal
Tricaine methane sulfonate	Finquel, MS 222, TMS	Fish and other aquatic poikilotherms	Temporary immobilization (sedation/anesthesia) for Ictaluridae, Salmonidae, Esocidae, and Percidae (for approved uses for other poilkilothermic animals, refer to the product label)	15 - 330 mg/l (fish) 1:1000 to 1:20,000 (other poikiloterms)	
Formalin	Formalin-F, Paracide-F, Parasite-S	Salmonids, catfish, largemouth bass, bluegill	Control of external protozoa and monogenetic trematodes	Tanks and raceways: up to 170 ml/l up to 1 h at above10℃ (50℉); up to 250 ml/l indefinitely at below 10℃ Earth ponds: 15 to 25 ml/l indefinitely	
		Salmonid and esocid eggs	Control of fungi of the family Saprolegniaceae on salmon, trout, and esocid eggs	Tanks and raceways: 50 to 100 ml/l up to 4 h daily; Earth ponds: 15 to 25 ml/l single treatment	
	Parasite-S	Penaeid shrimp	Control of external protozoan parasites on cultured penaeid shrimp	15 to 25 ml/l single treatment	
Sulfadimethoxine - ormetoprim	Romet 30	Salmonids	Furunculosis	50 mg/kg/day for 5 days	42 days
		Catfish	Enteric septicemia		3 days
Oxytetracycline	Terramycin	Pacific salmon	Marking of skeletal tissues	250 mg/kg/day for 4 days	Salmon <30 g: 7 days
		Salmonids	Ulcer disease, furonculosis, bacterial hemorrhagic septicemia, pseudomonas disease	2.5 to 3.75 g/100lb/day for 10 days	21 days
		Catfish	Bacterial hemorrhagic septicemia and pseudomonas disease		
		Lobsters	Bacterial hemorrhagic septicemia and Gaffkemia	1 g/lb medicated feed for 5 days	30 days
Sulfamerazine	*Not currently available*	Rainbow trout, brook trout and brown trout	Furunculosis	10 g/100 lb/day for up to 14 days	21 days

In the meantime, increasing concerns have been raised about the use of antimicrobials in all food producing animal species and their potential risks for consumer safety. However, the real risks originating from the use of aquaculture drugs in general have been moderated largely by some authors showing that it has been a bit of a non-issue. The World Health Organisation recently recognised that, basically, human health was not likely to be affected by the use of antibacterials in aquaculture (WHO, 1999).

Quite a large number of antibiotics have been tried in aquaculture and it is comforting to note that at the technical level there are treatments available for the majority of the bacterial fish pathogens (Austin and Austin, 1999). On a regulatory level, a great step forward was taken by the European Medicine Agency (EMEA) at the beginning of 2002 by extrapolating the MRLs for major species of twelve antibiotics to all food producing animal species (Table 2), thus making them available for off-label treating of fish, under the veterinary "cascade" system. However, it is very difficult to put the cascade system into practice because it sets potentially dangerous regulatory and liability problems, and fully registered antibiotics are still very scarce. Furthermore, despite the theoretical regulatory possibilities of harmonisation, there is still absolutely no common market at this level and the list of pharmaceuticals licensed for fish varies a lot among European countries (Table 3): one in Spain, four in France, twelve in the UK.

On the other hand, of particular concern for sea farming is the reduced bioavailability of antibiotics in seawater compared to freshwater (Rigos and Troisi, 2005). Quinolones and tetracyclines are known to bind with seawater-borne metal ions, notably the divalent cations Mg^{2+} and Ca^{2+} (Lunestad and Goksøyr, 1990; Pye-MacSwain *et al.*, 1992; Barnes *et al.*, 1995; Smith *et al.*, 1996). Normal seawater concentrations of Mg^{2+} have a marked effect on both antibacterial activity and uptake by fish of various antibiotics. Barnes *et al.* (1995) found the minimum inhibitory concentration (MIC) of oxolinic acid against *Aeromonas salmonicida* was increased 40- to 60-fold with Mg^{2+} in seawater.

Such an effect is particularly important when the antibiotic is distributed by oil coating on the surface of the pellets, which is a method that also leads to product loss by leaching and depressed palatability. This latter problem is of particular importance for fussy flatfish species or Mediterranean species such as sea bass (*Dicentrarchus labrax*) (Rigos *et al.*, 1999).

IV – Sea lice therapies

Sea lice therapies are perhaps the field where most advances have occurred in recent years. Although crustacean parasites are not among the most feared pathogens in Mediterranean aquaculture, the treatment of these parasites can benefit from major developments in the salmonid industry, where sea lice have always been a major problem. The original treatments, trichlorfon (syn. metrifonate, Neguvon®) and dichlorvos (Aquagard®) and other organophosphates, were effective but difficult to use and highly hazardous both for the users and for the environment. A more effective but still toxic organophosphate, azamethiphos (Salmosan®) was introduced and is still licensed in the UK.

Hydrogen peroxide was found to be an effective and more environmentally friendly alternative to organophosphates. Synthetic pyrethroids appeared as other alternatives in the late 1990's: cypermethrin (Excis®), followed by deltamethrin (Alphamax®) in Norway. All these compounds are used as bath treatments and thus present practical difficulties, such as requiring the use of enclosing skirts for sea cage treatments. Cypermethrin is classified in Annex III of the MRL regulation and only had a provisional MRL that expired in July 2003.

Table 2. Main compounds having a fixed MRL (Annexes I or III) or listed in Annex II of the MRL regulation as of February 2nd, 2008.

Compound	Annex	Species†	Tissue††	MRL	Comments
Antibacterials					
amoxicillin	I	All FPS	Muscle	50 µg/kg	
ampicillin	I	All FPS	Muscle	50 µg/kg	
benzylpenicillin	I	All FPS	Muscle	50 µg/kg	
chlortetracycline	I	All FPS	Muscle	100 µg/kg	
cloxacillin	I	All FPS	Muscle	300 µg/kg	
colistine	I	All FPS	Muscle	150 µg/kg	
danofloxacin	I	All FPS	Muscle	100 µg/kg	
dicloxacillin	I	All FPS	Muscle	300 µg/kg	
difloxacin	I	All FPS	Muscle	300 µg/kg	
enrofloxacin	I	All FPS	Muscle	100 µg/kg	Sum (enro+ciprofloxacin)
erythromycin	I	All FPS	Muscle	200 µg/kg	Erythromycin A
florfenicol (fish)	I	Fish	Muscle+skin	1000 µg/kg	
flumequine	I	Fish	Muscle+skin	600 µg/kg	
lincomycin	I	All FPS	Muscle	100 µg/kg	
neomycin (incl. framycetin)	I	All FPS	Muscle	500 µg/kg	Neomycin B
oxacillin	I	All FPS	Muscle	300 µg/kg	
oxolinic acid	I	Fish	Muscle+skin	100 µg/kg	
oxytetracycline	I	All FPS	Muscle	100 µg/kg	
paromomycin	I	All FPS	Muscle	500 µg/kg	
sarafloxacin (fish & poultry)	I	Salmonids	Muscle+skin	30 µg/kg	
spectinomycin	I	All FPS	Muscle	300 µg/kg	
sulfonamides (all)	I	All FPS	Muscle	100 µg/kg	
tetracycline	I	All FPS	Muscle	100 µg/kg	
thiamphenicol	I	All FPS	Muscle	50 µg/kg	
tilmicosine	I	All FPS	Muscle	50 µg/kg	
trimethoprim	I	All FPS	Muscle	50 µg/kg	
tylosine	I	All FPS	Muscle	100 µg/kg	
Sea lice treatments					
azamethiphos	II	Salmonids			
cypermethrin (fish extension)	I	Salmonids	Muscle+skin	50 µg/kg	
deltamethrin (fish extension)	I	Fish	Muscle+skin	10 µg/kg	
emamectin	I	Salmonids	Muscle+skin	100 µg/kg	
diflubenzuron	I	Salmonids	Muscle+skin	1000 µg/kg	
teflubenzuron	I	Salmonids	Muscle+skin	500 µg/kg	
Other antiparasitics & microbicides					
acetic acid	II	All FPS			
peracetic acid	II	All FPS			
bronopol	II	Salmonids			Only fish fecunded eggs
bronopol (fish extension)	II	Fish			Only fish fecunded eggs
chloramine T	II	Fish			For water treatment only
copper sulfate	II	All FPS			
formalin	II	All FPS			
glutaraldehyde	II	All FPS			
hydrogen peroxyde	II	All FPS			
iodophors	II	Fish			
salt (sodium chloride)	II	All FPS			
Anaesthetics					
benzocaine	II	All FPS			For use as anesthetic only
tricaine methane sulfonate	II	Fish			For water treatment only

†All FPS: all food producing species (with some exclusions, depending on each compound).
††For all fish MRLs, the target tissues "muscle" or "muscle and skin" shall be understood as "muscle and skin in natural proportions".

Table 3. Licensed aquaculture pharmaceuticals in some European countries

Compound	Spain	France	UK
Antibacterials			
flumequine	X	X	
oxytetracycline		X	X
sulfadiazine-trimethoprim		X	X
oxolinic acid		X	
florfenicol			X
sarafloxacin			X
amoxicillin			X
Antiparasitics			
bronopol			X
hydrogen peroxide			X
emamectin benzoate			X
teflubenzuron			X
cypermethrin			X
azamethiphos			X
Anaesthetics			
tricaine methane sulfonate			X

Diflubenzuron (Lepsidon®) and teflubenzuron (Ektobann®, Calicide®) represent another valued family of compounds that act as chitin synthesis inhibitors, which can also be used as oral treatments, but they bear the problem of binding to marine sediments, thus creating potential environmental concerns.

V – Other antiparasitic and antimicrobial agents

Most ectoparasites have always been and are still commonly treated with a range of unlicensed chemicals and disinfectants. In Europe, however, the definition of veterinary medical products is wide and in practice encompasses all the health and hygiene products that are used on animals. All the very old and well known compounds had thus to be reviewed, at least for the MRL assessment (Schnick *et al.*, 1997). By now most of these compounds have been classified in Annex II, which means that they are, to use the American designation, "generally recognised as safe" and they don't need an MLR. Formally speaking, all these compounds, if used as fish treatments, would need to go through a full marketing authorisation process. In practice, however, one can consider that those compounds listed in Annex II can be used in aquaculture: acetic acid, peracetic acid, bronopol (Pyceze®), chloramine T, copper sulphate, formalin, glutaraldehyde, hydrogen peroxide, iodophors and salt (sodium chloride). Similarly, the US Federal Drug Administration (FDA) has drawn up a list of 17 unapproved drugs of "low regulatory priority" for which the FDA "is unlikely to object at present to their use", provided they are utilised according to good management practices.

Note that benzalkonium chloride, a disinfectant used in some countries such as Spain and the UK (Planas, 2001; Chris Gould, pers. comm.), has an MRL but for use only as an excipient, at a concentration of up to 0.05%. There is no MRL for using it as a treatment. This is also the case for other quaternary ammonium compounds and for potassium permanganate.

VI – Anaesthetics

Currently, in many European countries, there is no fish anaesthetic registered. The only compounds that have fixed MRLs are benzocaine and tricaine methane sulfonate (TMS, MS 222, Finquel®), an analogue of benzocaine with superior water solubility. In the UK and in North America, the only registered anaesthetic is TMS. It has a withdrawal time of 5 days in Canada

and 21 days in the USA (Stoskopf, 1993). Apart from being much less water soluble than TMS, benzocaine also has a narrower safety margin. Norway, for instance, requires a 21 day withdrawal time for benzocaine (Shao, 2001). Other anaesthetics, such as 2-phenoxyethanol, quinaldine sulphate and metomidate have no set MRL.

Clove essential oil, which contains approximately 82 to 87% of the active ingredient eugenol, has been described as a very promising anaesthetic agent in terms of safety, environmental friendliness and efficacy in various salmonids (Keene *et al.*, 1998; Taylor and Roberts, 1999; Cho and Heath, 2000) and other marine species (Sladky *et al.*, 2001). An inconvenience of clove oil or eugenol itself is that they are not readily water soluble and need to be mixed in an alcohol solution prior to use. A commercial drug containing eugenol, AQUI-S®, has gained approval in New Zealand, Australia and Chile. As opposed to clove oil or eugenol, it is a water dispersible liquid that can be directly used as an anaesthetic for fish, crustaceans and shellfish (Stehly and Gingerich, 1999; Kuhlmann *et al.*, 2000). In the countries where AQUI-S is currently registered it has a nil withdrawal time, thus permitting its use for fish harvesting.

VII – Conclusion

Although the MRL regulation adopted in 1990 raised a lot of anxiety as to whether the few veterinary medicines licensed for fish would remain authorized, it appears almost twenty years later that a relatively large range of compounds is still legally available for treating aquacultured fish. Though most of such compounds are not fully licensed for use in fish species, they can still be utilized on an off-label basis, under the veterinary "cascade" scheme.

Quite logically, antibiotics are by far the largest group of chemotherapeutants legally available for fish. However, next to the legal requirements, but of particular concern in seawater, is the bio-availability of the drugs, which is often seriously reduced as compared to freshwater.

Apart from antibiotics, sea lice oral treatments have represented a field of intensive research and innovation in recent years. Perhaps more worrying though are the classical antiparasitic external treatments and the anaesthetics, for which a variety of unlicensed chemicals are still commonly utilized, since very poor progress has been made in replacing the old efficient compounds that have been banned from the classical aquaculture treatment armoury.

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