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Environmental aspects of drug and chemical use in aquaculture: An overview

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Abstract. A variety of chemicals and drugs are used in aquaculture for purposes such as sediment and water management, enhancement of natural aquatic productivity, transport of live organisms, feed formulation, manipulation and enhancement of reproduction, growth promotion, health management, processing and adding value to the final product. The reliance on these chemicals and drugs has become greater with the intensification of production, and their environmental toxicity has to be questioned. In this document, we give an overview of environmental aspects concerning drug and chemical use in aquaculture.

Keywords. Aquaculture – Chemicals – Drugs – Environment – Toxicity.

Aspects environnementaux de l'utilisation de médicaments et produits chimiques en aquaculture : Vue d'ensemble

Résumé. De nombreux produits chimiques et pharmaceutiques sont utilisés en aquaculture à des fins diverses, comme le traitement de l'eau et des sédiments, l'amélioration de la productivité naturelle du milieu aquatique, le transport d'animaux vivants, la manipulation et l'amélioration de la reproduction, la stimulation de la croissance, la gestion sanitaire et la valorisation du produit fini. La dépendance de ces produits chimiques et pharmaceutiques s'est accentuée avec l'intensification de la production, et la question de leur toxicité environnementale doit être posée. Le but de ce document est de donner une vue générale des aspects environnementaux concernant l'utilisation de ces produits en aquaculture.

Mots-clés. Aquaculture – Produits chimiques – Produits pharmaceutiques – Environnement – Toxicité.

I – Introduction

A variety of chemicals are used in aquaculture for purposes such as sediment and water management, enhancement of natural aquatic productivity, transport of live organisms, feed formulation, manipulation and enhancement of reproduction, growth promotion, health management, processing and adding value to the final product (Table 1). Chemical needs are minimal in extensive and semi-intensive culture methods, but intensification of production is often accompanied by a greater reliance on chemicals (GESAMP, 1997).

The aim of this document is to give an overview of the environmental aspects concerning drug and chemical use in aquaculture. Some other authors have made even wider syntheses of great interest on particular substances, and their articles should be consulted for a clear detailed state of the knowledge on these substances. The elements given here are examples, since the objective is to give an order of importance of toxicity for several marine species, to highlight the main effects that could be observed or that could lead to a particular reflection (for example, a slight gill lesion is only a histological observation but can also indicate chronic anoxia and decrease of performance).

Table 1. Chemicals used in aquaculture in the world (GESAMP, 1997)

Structural materials and substances for maintenance		
Chemicals associated with structural materials	Antioxidants, fungicides, plasticizers, flame retardants...	
Soil and water treatment	Lime	EDTA
	Chlorine	Gypsum
	Alum	Zeolite
Disinfectants	Quaternary ammonium	Chloramine T and chlorinated compounds
	Iodine	Formalin
	Ozone	
Pesticides	Organotin Compounds	Rotenone
	Tributyltin	Ammonia
	Dibutyltin	Saponin
Herbicides, algacides	Copper	
	Simazine	
Substances connected with zootechnical practices		
Fertilisers	Manure, ammonium phosphate, urea...	
Feed additives	Vitamins	Pigments
	Butylated hydroxytoluene	Attractants
	Heavy metals	Immunostimulants
Heavy metals	Copper, cadmium, manganese...	
Anaesthetics	Benzocaine	2-phenoxyethanol
	Carbon dioxide	Tricaine
	Metomidate	Eugenol
Hormones	17a-methyltestosterone, growth hormone...	
Therapeutants		
Antibacterial agents	Penicillins	Quinolones
	Nitrofurans	Sulphonamides
	Macrolides	Tetracyclines
	Phenicol	
Pesticides	Organophosphorus	Carbamates
	Azinphos ethyl	Carbaryl
	Azamethiphos	Pyrethrins
	Fenitrothion	Avermectins
	Diazinon	Ivermectin
	Chlorpyrifos	Emamectin
	Trichlorphon	
	Dichlorvos and di-n-butylphthalate	Diflubenzuron
		Hydrogen peroxide
	Organochlorines	
	Endosulfan	
	Endrin	
	Lindane	
Other therapeutants	Acriflavine	Trifluralin
	Copper compounds	Dimetridazole, metronidazole
	Formalin	Glutaraldehyde
	Levamisole	Methylene blue
	Malachite green	Niclosamide
	Potassium permanganate	

We have taken into account three characteristics and some relative parameters often studied by scientists:

- (i) Acute and chronic toxicity : effective dose, lethal dose...

(ii) Accumulation of substances in organisms: bioconcentration factor, octanol-water partition coefficient...

(iii) Persistence and bioavailability of the substances: half-life, hydrolysis...

Even if the mode of action of the toxicant is known, the assessment of its toxicity remains difficult. A well-known problem for scientists is the difference between the results of a laboratory experiment and those of field observations. The complexity of natural ecosystems makes it difficult to reconstitute representative laboratory conditions. Besides, it is always complicated to compare several experiments: were the methods used similar? Are essential parameters specified (pH, temperature, salinity...)?

The following substances have been found in the literature to have a toxic effect on biota. However, this list is far from exhaustive and complete, since many chemicals have unknown toxic activity, so as a "precautionary principle", they should be regarded as potentially harmful.

II – Undesirable and toxic effects of chemicals

1. Chemicals associated with structural materials

Large synthetic polymers are generally inert (Horowitz *et al.*, 2001) but construction materials often contain a wide range of additives, with high loads for some of them (GESAMP, 1997). Plastics can contain stabilisers, pigments, antioxidants, plasticizers, UV absorbers, flame retardants, fungicides, disinfectants, anti-ozonants, curing agents (peroxide), lubricants, and antistatics. Floats made of polystyrene can contain flame retardant halogenated compounds, and paints and wood can contain heavy metals.

The situation has become worse as a result of the recycling of plastics, and it is difficult to obtain information about any specific additives (Zitko, 1994).

The polymers have very different behaviours and, in general, the kinetics of leaching is unknown, and so are any sub-lethal effects, above all in open-water conditions. The rate of leaching is determined primarily by the type, size and amount of additive molecules, density of the polymer and temperature. Other factors, such as pH (Horowitz *et al.*, 2001), can also interfere.

Toxicity is observed in the water surrounding open systems, but toxicity is enhanced in semi-closed or closed systems. Some components can accumulate in animals (for example, heavy metals, the plasticizer triphenyl phosphate) (Horowitz *et al.*, 2001).

Few examples of poisoning by additives of materials are reported in the literature. Zitko *et al.* (1985) reported mortalities of Atlantic salmon, probably caused by the presence of OBPA (10,10'-oxybis-1OH-phenoxarsine), a fungicide, in a plastic liner.

Stress and mortalities associated with chronic toxicity were observed in the shrimp *Litopenaeus setiferus* in a recirculating superintensive system and the chemicals involved were part of the rubber polymer liner. The substances were also toxic for nitrifying bacteria, which favoured ammonia accumulation.

In some cases, simple washes of the polymers remove the toxic effect, but not always, and washing methods have to be specific for each type of polymer. Routine tests do not exist to assess the toxicity of construction materials. Some possibilities are described by Zitko *et al.* (1985).

2. Fertilisers

Organic and inorganic fertilisers are used in increasing amounts with intensification of

production. As for numerous other substances, they are not toxic when correctly applied, according to the structure and the needs of the system (Pillay, 1992; GESAMP, 1997). However, if they are toxic, there are risks of algal blooms, excessive sedimentation at the bottom of the ponds, and ammonia reaching a high toxicity level. Manure can be contaminated with heavy metals (Boyd and Massaut, 1999).

3. Soil and water treatments

A. Lime

Again, risks are associated with excessive use. Lime is practised to increase alkalinity in soil and water, and also to kill pests and predators. The quantities applied are more important when preparing the pond than during rearing. At a high dose, the pH is greatly modified although it rapidly decreases again (Boyd and Massaut, 1999).

B. Chlorine

Prolonged contact can rapidly corrode metal and damage many plastics. Also see under "Chloramine T".

4. Disinfectants

A. Quaternary ammonium

Those substances are very toxic but tend to get absorbed onto particulates (Zitko, 1994). Death of marine diatoms has been reported with benzalkonium chloride used at 10 mg/l for 7 days (Beveridge *et al.*, 1998).

B. Iodine

Free iodine is highly toxic to fish (irritation of skin and mucous membranes) but it can be complexed with quaternary ammonium (Meyer and Schnick, 1989). When used, iodine can form stable foam, and degradation products may be toxic and degrade slowly (Zitko, 1994).

C. Ozone

Ozone is rapidly degraded, but because of its highly reactive nature, O₃ residues are highly toxic. Ozone reacts with plastics such as PVC, and accelerates the corrosion process for unprotected steel (Gill, 2000).

D. Chloramine T and chlorinated compounds

In aqueous solution, chloramine T dissociates into hypochlorite (ClO⁻), the active component, and paratoluene sulphonamide (PTS). The quantity of ClO⁻ increases with decrease of pH (Massuyeau, 1990). Chloramine T is degraded by sunlight and oxidation (GESAMP, 1997) and chemical reactions with organic substances result in significant concentrations of halogenated hydrocarbons. It also reacts with bromine and ammoniac, to form HBrO and NH₂Cl, respectively.

ClO⁻ is highly toxic to aquatic life. PTS is suspected to accumulate in organisms and its toxicity is unknown. HBrO and NH₂Cl are toxic for fish (Massuyeau, 1990).

Toxicity increases with temperature and is time-dependent. Crustaceans should be more sensitive during moulting, when uptake of water is high (Scarano and Saroglia, 1981). The known 96 h LC 50 (50% lethal concentration) of ClO⁻ ranges from 0.04 to 0.15 mg/l for numerous organisms, including phytoplankton (GESAMP, 1997). The 48 h LC 50 for fishes is

about several tens of mg/l. Chlorine dioxide kills *Artemia* when used at 0.3-2.9 mg/l (Puente *et al.*, 1992).

Chloramine T inhibits bacterial nitrification, so that ammoniac in ponds may increase up to toxic concentrations (Nimenya *et al.*, 1999).

Before release, chlorinated water should be treated with sodium thiosulphate. All oxidising agents can create oxidant potentially toxic by-products, and even if the parent compound disappears within a few hours, by-products can persist for much longer (Massuyeau, 1990).

E. Formalin (37-40% formaldehyde)

Formaldehyde is a product of normal metabolism. It has been detected in shellfish and teleosts at about 0.1-31.8 µg/g. Exogenous formaldehyde has not been shown to accumulate, and exposition dose or duration do not affect it. Half-life is about 2-3 days (longer in non-aerated water). So, it is not related with chronic toxicity (Jung *et al.*, 2001).

Formalin is not usually recommended for treating fish ponds because each 5 mg/l of formalin removes 1 mg/l of dissolved oxygen from the water.

Formalin is toxic to aquatic life at low concentrations, with 96 h LC 50 of 1 to 1000 µl/l (GESAMP, 1997). Some fish are sensitive to it, so a bioassay is recommended before use (Noga, 1996).

The 96 h LC 50 is 0.141 mg/l for *Paralichthys olivaceus* fingerlings (olive flounder) and 10.8 mg/l for *Morone saxatilis* fingerlings in brackish water (15‰) (Jung and Kim, 1998). Severe gill pathology has been reported for *Epinephelus coiodes* held for more than 96 h at 300 ppm or more (Yusoff and Mustafa, 1998).

Inhibition of growth and mortality have been reported for phytoplankton and macrophytes (aquarium plants). Its algicidal property can further reduce oxygen.

Toxicity is more important in acid water and at high temperature. When stored at room temperature, formalin can develop a white precipitate of paraformaldehyde, which is more toxic than pure formalin. To avoid it formalin can be mixed with 12-15% methanol (Howe *et al.*, 1995).

5. Pesticides for maintenance

A. Organotin compounds: Tributyltin (TBT) and dibutyltin (DBT)

Organotin compounds are lipophilic substances (Log Kow = 3.8 and 1.49 for TBT and DBT) (Donkin *et al.*, 1997) and they tend to accumulate in sediments and marine biota. Degradation time in sediments is 120-160 days and rapid photodegradation occurs in water (Alzieu *et al.*, 1980; GESAMP, 1989).

Accumulation in chinook salmon, *Oncorhynchus tshawytscha*, is time and dose dependent (Short and Thrower, 1987). *Crassostrea gigas* accumulates TBT more and eliminates it more slowly than *Ostrea edulis*, whereas depuration is long, more than 20 days in oysters (Waldock *et al.*, 1983), half life is 11-36 days in *Ruditapes decussatus* (Gómez-Ariza *et al.*, 1999). Salmon rapidly accumulate high concentrations (GESAMP, 1997).

BCFs (Bioconcentration Factors) ranges from 500 to 4400 for crustaceans, and from 2000 to 90,000 for bivalves (higher for the clam *Ruditapes decussatus* than oysters and mussels) (Meador, 1997; Jacobson and Willingham, 2000). BCF is 2000-10000 for TBT and 110 for DBT in *Mytilus edulis*. Maximum accumulation occurs in liver for salmon (BCF = 4300) (Huang and Wang, 1995; Donkin *et al.*, 1997).

The minimum reported sub lethal response in biota is 0.01 mg/ml (Meador, 2000), and 0.1 µg/ml for mortality (GESAMP, 1997). Acute toxicity is effective at concentrations in the order of a few

µg/l for many species. Some reported 96 h LC 50 are 1.5, 41 and 200-300 µg/l, respectively, for juveniles of Chinook salmon, shrimps and oysters (Hall and Pinkey, 1985; Short and Thrower, 1987). LR 50 (lethal residues) range from 30 to 115 µg/g for most marine species (Meador, 2000).

A well known example of chronic toxicity is imposex, for *Nucella lapillus* at 1 ng/l (GESAMP, 1989). Damages have been observed in mitochondria of *Mytilus edulis* after chronic exposure (Huang and Wang, 1995).

The impact on oysters has been extensively studied. At 0.06 µg/l, histological changes are observed in digestive gland and gills and at 0.1 µg/l growth can be affected and mortality occurs. Concentrations of 0.15-1 µg/l provoke chambering, gel secretion, and shell thickening, whereas at 100 µg/l reproduction is affected and the sex ratio is modified in the population (predominance of males). Organotins also disturb the metabolism of metals, and immunity (Kennedy *et al.*, 1996).

Organotins affect growth and immunity in fish as well (Short and Thrower, 1987). In penaeids, they seem to mimic growth hormones, disturbing the endocrine system (Bainy, 2000). They are algistatic at 0.01-0.08 mg/l (Hall and Pinkey, 1985). In coastal heavily contaminated "hot spots" (about 1 µg/l), a severe decrease of population density is commonly noted (GESAMP, 1989).

B. Rotenone

1 ppm for 8 days causes acute toxicity for *Ostrea edulis*. Rotenone is rapidly degraded in seawater (Samuelsen *et al.*, 1988).

C. Saponin (tea seed cake = 5.2 - 7.2% saponin)

Fish are more susceptible than shrimp. Saponin can destroy erythrocytes and damages the respiratory epithelium, and a similar reaction may occur with penaeids containing haemocyanin (Minsalan and Chiu, 1986). A dose of 15 mg/l of tea seed cake kills fishes within 6 hours (Chen *et al.*, 1996). The 48 h LC 50 is 21 mg/l saponin for *Penaeus japonicus* juveniles, and a decrease in feeding, growth and moulting frequency are reported at 0.5 mg/l (Gräslund and Bengtsson, 2001). *Penaeus monodon* stop feeding at 15 mg/l (Chen *et al.*, 1996).

6. Antibiotics

A. Fate

If an antibiotic is given through food to a fish part of it will be ingested, and only another smaller part will be absorbed in the digestive tract. The lost fraction can exert its action on another species, in another pond, if it is not degraded first. Therefore, two points should be determined for antibiotics: how much is released into the environment, and what is the degradation rate of this antibiotic in water and sediments? This may be important when water is recirculated. Once in the organism, can the antibiotic accumulate, and where (is it available, or neutralized?), and for how long (chronic action and secondary poisoning)?

Few antibiotics have really been studied, and even fewer have a marketing authorization for fishes or aquacultural animals.

B. Bioavailability in reared species

Bioavailability is dependent on the way of administrating the antibiotic. Injection is the best way to be sure of the given dose, but there is always a non-available fraction for the organism. Bioavailability for *Penaeus vannamei* is 30 and 32% for sulphadimethoxine and ormetoprim, respectively, after intra-sinus injection (Park *et al.*, 1995). When given orally to sea bass or sea bream, it is generally accepted that a maximum of 80% of oxytetracycline (OTC) and oxolinic acid (OA) is ingested (depending on fish appetite and access to food). Once in the gut, 10 and

30% of OTC and OA, respectively, are absorbed (Videau and Merceron, 1992; Pouliquen, 1994). A particularity of OTC and flumequine is that they bind to calcium and magnesium and other divalent cations in water, in sediment and in food, which greatly reduces the available active fraction. (Samuelsen, 1994; Barnes *et al.*, 1995; Malvisi *et al.*, 1997).

Up to 45% of the given dose of flumequine is absorbed by salmon (Malvisi *et al.*, 1997). Enrofloxacin is not well absorbed in seabass (concentration peak in muscle and skin at 10 h after an oral treatment of 5 mg/kg) (Intorre *et al.*, 2000) but, on the contrary, 99% of ingested chloramphenicol passes through the intestinal barrier (Videau and Merceron, 1992).

C. Fate in the environment

Antibiotics can be degraded through hydrolysis, photodegradation, microbial degradation and chemical degradation (oxidation). Kinetics of degradation depend on the conditions encountered and on the mechanisms involved. For example, OTC and sarafloxacin, which are photodegraded, should be less persistent than ones being degraded more slowly by bacteria, but some antibiotics can be degraded by the two processes (e.g. furazolidone). Both OTC and OA can be degraded by high temperature and sunlight but OTC is much more sensitive to their action and is more rapidly degraded (Pouliquen, 1994).

When half-lives are measured in laboratory experiments, it is important to be perfectly aware of the conditions employed, particularly whether degradation is measured in water or in water and sediment? This type of difference can give absolutely different observations, each one having a special significance, and the two can not be compared. However, field observations are not any more simple either, since one should consider the water dynamics, the depth of water and the light available, etc.

According to different studies, the half life of OTC in marine sediment varies from 5 to 419 days (Pouliquen, 1994).

D. Accumulation and excretion in aquatic organisms

Antibiotics can accumulate in all organs, with preferences due to physical and pharmacodynamic properties. As a major organ of detoxification and because of a high lipid content, the liver of vertebrates often has a heavy load of antibiotics or metabolites. OTC tightly binds to muscles because of their calcium and magnesium content (Pouliquen, 1994).

Crustaceans have original ways of detoxification, and antibiotics reach high concentrations in shell; the substances are then not available for direct toxicity, but are released in the environment during ecdysis (Endo, 1991).

Excretion kinetics is highly variable between antibiotics. For example, in molluscs, OA is excreted more rapidly (a half-life of less than two days) by oysters and mussels than OTC. Of course the detoxification process and the intensity of metabolism vary between species, and even from one bivalve to another with antibiotics generally having shorter half-lives in mussels than in oysters (e.g. OTC has a half-life of 6-7 days in blue mussels and 10 days in Japanese oysters) (Pouliquen *et al.*, 1996). However, as mussels seem to have a more intense water filtration rate whilst feeding than oysters, it is not easy to predict which one will have the highest final residue.

Excretion poses a special problem when antibiotics are emitted in an unchanged, active form, as is the case for OTC and a proportion of OA for fish (Pouliquen, 1994).

E. Toxicity

a] Bivalves

Neither mortality nor morphological abnormalities were observed in *C. virginica*, *M. mercenaria* and *M. edulis* after using 200 mg/kg of tetracycline but there is no information for oxytetracycline (Pouliquen, 1994).

b] Fishes

There are very few details regarding toxicity for fish:

Chloramphenicol provokes growth retardation for salmon after prolonged treatment (Michel, 1986) and nitrofurazone can prevent reproduction in treated fish (Nimenya *et al.*, 1999) (doses not specified).

Nephrotoxicity is reported for tobramycin in coho salmon. Kidney damage was observed using 7.5 mg/kg every other day for five days in *Oncorhynchus kisutch*. In a group receiving 5 mg/kg day⁻¹, a cumulative mortality of 100% was observed in 21 days (Schneider *et al.*, 1980).

Erythromycin has acute toxicity for yellowtail (*Seriola quinqueradiata*) at more than 2 g/kg (Alderman *et al.*, 1994).

If given at up to 20 times the normal dose, oxolinic acid (120 mg/kg/d for 7 days) and nalidixic acid (400 mg/kg/d for 7 days) have side effects, such as loss of appetite, body discolouration, macrocytic anaemia, fat reduction of hepatocytes, and nephrosis (Miyazaki *et al.*, 1984).

Oxytetracycline injected twice a year for 3 years produced no toxicity to adult chinook salmon but it was teratogenic to 0-15% of the progeny.

Quinolones, nitrofurans, and oxytetracycline are reported to have low toxicity for fish, but oxolinic acid has sub-acute toxicity for ayu (*Plecoglossus altivelis*) (Michel, 1986). Some sulphonamides could be toxic for fish but side effects are rare. Logically, toxicity is more likely to appear when antibiotics are employed by injection. Some examples are known for freshwater fishes (Moutou *et al.*, 1997).

When antibiotics are used as an extra-label application, it is best to use preparations that do not have carriers, since they may be toxic to fish (Noga, 1996).

c] Algae

Toxicity is pronounced and induces inhibition of growth (Holten-Lutzhoft *et al.*, 1999).

d] Others

Oxytetracycline reduces gonadal growth in the green sea urchin *Psammechinus miliaris*, but this observation could be related to the stress of treatment. It also reduces sub-cuticular bacteria, which could affect growth or survival since these bacteria play a role in the nitrogen budget of the host (Campbell *et al.*, 2001).

As *Artemia* are usually used for laboratory tests, toxicity is well documented (Table 2).

Of course, generally speaking, antibiotics have a marked influence on bacterial populations, and consequently they can damage biological filters in recirculating systems. Antibiotics such as enrofloxacin, ampicillin, erythromycin and polymyxin B can influence the growth of nitrifying bacteria populations (Nimenya *et al.*, 1999).

Table 2. LC 50 (mg/l) of antibiotics for *Artemia* (Migliore *et al.*, 1997; Halling-Sorensen *et al.*, 1998)

	24 h LC 50	48 h LC 50	72 h LC 50	96 h LC 50
Aminoside	-	2220	846.5	-
Bacitracin	34.06	21.82	-	-
Flumequine†	476.8	307.7	96.35	-
Lincomycin	-	-	283.1	-
Sulfadimethoxine	1800	900	500	19

†Also causes *Artemia nauplii* pigmentation problems.

In conclusion, antibiotics are generally considered to be relatively safe in terms of toxicity. As they should be used punctually, they do not seem ideal candidates for cumulative chronic toxicity because their half-lives are relatively short compared to the interval between two treatments. Acute toxicity is especially poorly studied, but it seems weak for fish and shellfish. Nevertheless, more information would be welcome.

F. Effects on aquatic microflora: Resistance to antibacterials

Bacterial resistance is a crucial problem in all types of animal husbandry, including aquaculture. From a microbiological point of view, resistance to an antibiotic is the decrease or absence of sensitivity of bacteria towards the antibiotic. Clinically speaking, bacteria can withstand concentrations of antibiotic higher than those that can be reached in the host without toxicological effects.

Bacterial resistance can be intrinsic or acquired. Some species are intrinsically resistant to one or to several antibiotics, because of structural properties (lack of the intracellular target, impermeable cell wall), which means that all the strains of the species are resistant. Acquired resistance is a particular property of a strain of a normally (=intrinsically) susceptible species. It means the strain can withstand concentrations higher than the usual minimum inhibitory concentration (MIC) of the antibiotic for the species. Resistance can be due to a chromosomal property (mutation of a target gene), or it can also be plasmid-mediated. In the latter case, transmission between bacteria occurs mainly through conjugation. Resistant bacteria resulting from these rare mutations or plasmid gains remain infrequent in an unselective environment, but are selected for under the "selective pressure" of antibiotics. As a matter of fact, numerous observations show that emergence of resistant strains within a susceptible species is related to the use of antibiotics for therapy (Samuelsen *et al.*, 1992; Ervik *et al.*, 1994). Thus, the antibiotic does not *create* resistance, but selects resistant bacteria, thus transforming the structure of the initial population.

In a natural environment, there are few antibacterial selective pressures, thus low emergence of new resistant strains. Pressure is effective in farms, and in marine environments receiving sewage charged with antibiotics (residues from fresh water, veterinary use in terrestrial environment, hospital residues). However, there is no proportional relationship between the quantity of antibiotic present in the environment and the frequency of new resistance (Weston, 1996). Smith (1991) states that exposure of bacteria to an antibiotic concentration close to the values needed to inhibit them is the optimum method of selecting for resistant variants. Indeed, a population is modified when susceptible cells are inhibited, but also if their growth is just retarded (Smith, 1995).

At first sight, a solution for avoid resistance would be to use different antibiotics for successive treatments, to limit selective pressure. However, in fact, rotating the use of several antibiotics often contributes to the occurrence of multiple drug resistance patterns (Debernardi, 1992; Honculada-Primavera *et al.*, 1993). When multiple resistance is determined by the presence of a single plasmid carrying several resistance genes, the use of only one antibiotic selects for all the resistance genes at one time. Moreover, in aquaculture, rotating use is complicated by the fact that few antibiotics have a marketing authorization.

Cross-resistance also results in resistance to several antibiotics, but should be distinguished from plasmid-mediated multi-resistance. It is obtained when biochemical mechanisms responsible for the resistance are common to two antibiotics. This is most often the case for antibiotics of the same family, for instance, oxolinic acid and flumequine, which are both quinolones. Cross resistance to structurally different compounds is also sometimes due to membrane alterations causing decreased permeability (Smith, 1995).

The major consequence of resistance is failure of chemotherapy. In this case, increasing dosage is absolutely useless, since it reinforces the process of resistance and the risk of elevated residues in water and product flesh.

Bacterial populations are selected wherever they are in contact with antibiotics – in the animal gut, in biological filters, in sediments. In sediments, functional properties can be modified (e.g. sulphate reduction rates) in parallel. In addition, reduction in total microbial density can also be observed, which is of importance as sediment microbes are food for meio- and macrofaunal invertebrates. Finally, the whole ecosystem function can be altered (Weston, 1996).

It is difficult to assess how long bacteria maintain their antibiotic resistance (Smith, 1995). Persistent drug residues in sediments can exert a continued selective pressure, favouring resistance maintenance. In the absence of antibiotics, some studies report a rapid decline of resistance frequencies in the case of effluents from culture systems, but this may be the consequence of a dilution of the resistant population with non-resistant microbes. Fish and bivalve flora reflect the flora present in the water, so if the antibiotic concentration decreases, the resistance frequency of their flora may decrease too. This high variability in natural flora is an advantage against resistances, but its counterpart is that antibiotics can not be easily used as growth promoters (Samuelsen *et al.*, 1992; MacMillan, 2001).

Antibiotics are widely used in aquaculture, and their efficacy has been proved, but use is often systematic, even anarchic, with no medical justification. Consequently, antibiotics that are not subject to resistance are the exception rather than the rule. Even if resistance frequencies have not significantly increased, apart from oxytetracycline, from 1982 to 1994, Richards *et al.* (1991) found that multiple resistances were of great concern.

As always, scientific experiments can be misinterpreted when conditions are not specified clearly enough. On the one hand, very weak levels of contamination may have a significant selective impact when applied to very large bacterial microflora and over large time scales. On the other hand, high levels of contamination are not necessarily significant in terms of resistance selection, depending on the context. As an example, we have seen that oxytetracycline is chelated with divalent cations in seawater, which considerably decreases its activity on bacteria. Smith *et al.* (1994) published an outstanding study on evaluation of methods and meaning of bacterial resistance, which should be consulted.

Another difficulty is that factors other than antibiotherapy can enhance resistance to antibiotics. Kerry *et al.* (1997) have underlined the existence of resistant bacteria in fish feed, but their influence on salmon flora seemed negligible. In sediments, the resistance observed can decrease simply because of dilution of the resistant population with other bacteria coming from the surroundings. Live feed, like *Artemia*, can also be a vector either for antibiotic residues, or for resistant bacteria (Sahul Hameed and Balasubramanian, 2000). Weston (1996) reports that exposure to heavy metals and other toxicants can confer resistance to some antibacterials as well.

7. Therapeutants other than antibacterials

A. Acriflavine

Acriflavine is highly soluble in water. It seems well tolerated by lobsters up to 10-20 ppm (Abrahams and Brown, 1977).

B. Copper compounds

See below heavy metals.

C. Formalin

See below disinfectants.

D. Hydrogen peroxide

Damage restricted to gills of salmon has been reported at 3.7 g/l (30 min for 6°C) (Roth *et al.*, 1993). This substance is not persistent in the environment.

E. Levamisole

No information is available but waste beneath cages could affect local fauna if in significant amounts (Alderman *et al.*, 1994). It could inhibit nitrification process and enhance ammonia toxicity (Nimenya *et al.*, 1999).

F. Malachite green

Malachite green is not very soluble in water, and it binds to sediments (Bergheim and Asgard, 1996). It accumulates in biota but above all in simple organisms. In fish, malachite green can be found in all organs in great quantities, above all in kidney (Alderman *et al.*, 1994). Residues of 2,400 µg/g have been found in fish, and they are persistent (Zitko, 1994).

Malachite green is a respiratory enzyme poison (Gräslund and Bengtsson, 2001). Its toxicity is time, temperature and pH dependent. Toxicity concerns nitrifying bacteria, algae and zooplankton (at concentrations below treatment levels) (Bergheim and Asgard, 1996), and fishes are more sensitive than crustaceans and bivalves. The 96 h LC 50 is 122 mg/l for *Corbicula leana* and about 0.2-0.3 mg/l for *Oncorhynchus* sp. *Homarus americanus* can be treated with 700-800 mg/l without problem, probably because the exoskeleton constitutes protection.

In fish, numerous haematological, biochemical and histological effects have been observed, but some of them could also be due to stress (Gouranchat, 2000).

G. Potassium permanganate

In water, potassium permanganate is quickly transformed into non-toxic manganese dioxide, which precipitates out (Boyd and Massaut, 1999). It is toxic for phytoplankton and slightly to moderately toxic to marine fish (96 h LC 50 = 1.48 mg/l for juvenile sea-bass *Morone saxatilis* at 15‰ salinity) (Reardon and Harrell, 1994; Gräslund and Bengtsson, 2001). Ethanolamine salt is a molluscicide (Alderman *et al.*, 1994).

H. Trifluralin

BCFs for this lipophilic compound range from 400 to 15,500. The half-life is 30-138 minutes in water and 21-50 days in soil, and degradation is faster in loam than in sandy soil (Alderman *et al.*, 1994; Gräslund and Bengtsson, 2001).

Trifluralin is acutely toxic to fish and flora, and can be highly chronically toxic to fish, where it rapidly accumulates. The 96 h LC 50 begins at less than 0.05 mg/l, and can reach up to 0.12 mg/l for many marine fishes. Deformities of the vertebral column, convulsions and lateral stripes caused by haemorrhages have been reported (Koyama, 1996). Toxicity is inferior for crustaceans (Alderman *et al.*, 1994).

8. Pesticides for therapeutic use

A. Organophosphorous

There is great concern about the action of all organophosphates on non-target crustaceans, and their toxicity is far from negligible for fishes. Extensive research has been carried out by Roth *et al.* (1993) on chemotherapeutics used against sea lice and should be consulted.

a] Azinphos ethyl

In black tiger shrimp *Penaeus monodon*, azinphos ethyl provokes hyperplasia of gill epithelium, necrosis of hepatopancreas and shell softening at concentrations between 1.5 and 150 ppb. The 96 h LC 50 for juveniles is 120 ppb (Baticados and Tendencia, 1991).

b] Azamethiphos

Azamethiphos is moderately water-soluble, its half-life in water is 10.8 days at 20°C and pH 7 (Roth *et al.*, 1993). At 100 µg/l (10% of the recommended concentration), mortalities can be observed in adult *Homarus americanus*. The no observed effect concentration is 1.03 µg/l for 120 minutes (Burridge *et al.*, 2000). Mortalities of Atlantic salmon were observed at 1 mg/l but it does not seem to be cumulative (Roth *et al.*, 1993).

c] Fenitrothion

Fenitrothion inhibits acetylcholinesterase, and causes severe damage to the nervous system (Bainy, 2000). The 96 h LC 50 is 0.8 µg/l for juveniles of *Penaeus japonicus*. It decreases osmoregulatory capacity, with exposure of juveniles at lethal and sub-lethal concentrations causing, in particular, thrombosis and necrosis of gills, and breakage of the epipodite cuticle (Lignot *et al.*, 1997).

d] Diazinon

The half-life of diazinon in 20 ppt salinity water is 8-11 days at 4.5 to 15°C. The 96 h LC 50 is 8.5 µg/l for *Mysidopsis bahia* (25‰ at 25°C) (Gräslund and Bengtsson, 2001). Atlantic salmon parr exposed to 0.3-45 µg/l for 120 h showed reduced levels of reproductive hormones (Moore and Waring, 1996).

e] Chlorpyrifos

It has a high affinity for sediments, therefore degradation is slow. A BCF of 400 has been measured for *Mytilus galloprovincialis* (38‰ at 18°C). The 48 h LC 50 is 5.2-6.5 µg/l for *Gammarus palustris* (15‰ at 20°C) (Gräslund and Bengtsson, 2001).

Artemia parthenogenetica show an increasing sensitivity as they grow older, and adults are the most sensitive with a 24 h LC 50 of 0.08 mg/l (38‰ at 18°C) (Varo *et al.*, 2000).

f] Trichlorfon

Most organophosphates degrade to less toxic products, but trichlorfon is a notable exception, since it is unstable in water and degrades into dichlorvos, which occurs more rapidly as the temperature and pH increase. Trichlorfon does not persist in fish tissues after treatment.

Toxicity increases with the number of treatments. Unpredictable fish kills are observed when recommended doses are not scrupulously followed (recommended doses range from 300 mg/l at temperatures below 6°C to 15 mg/l between 14 and 18°C) (Roth *et al.*, 1993).

For *Palaemonetes* sp., the 96 h LC 50 is 6-11 µg/l and the BCF is 28-186 (17-27°C at 15-30‰ salinity) (Gräslund and Bengtsson, 2001).

g] Dichlorvos (see also di-n-butylphthalate)

Stratification in ponds can be observed when treated with dichlorvos because of its liposolubility. It rapidly degrades into hydrosoluble, non-toxic compounds, which occurs more rapidly as salinity decreases. The half-life in seawater is from 1.2 to 6.3 days (4.5-15°C) (Gräslund and Bengtsson, 2001).

Accumulation has not been observed in either fish or invertebrates (BCF = 1.1 for *Mytilus edulis*) (Donkin *et al.*, 1997).

Its toxicity against acetylcholinesterase is cumulative and concentration dependent (Table 3). Fish are more susceptible to treatment if they are not given sufficient recovery time. Bivalve recovery is not good after several treatments. A concentration of 1 ppm dichlorvos has an acute toxic effect on zooplankton and phytoplankton (significant mortalities) (Henery *et al.*, 1997).

Table 3. LC 50 (ppm) of dichlorvos for marine biota (Esnault, 1992)

	Artemia	Fish	Shrimp	Lobster	Bivalve
24 h LC 50	16,800	–	36	–	8,200
96 h LC 50	–	170-11,600 100-3,000	4-15	5.7	–

At sub-lethal concentrations, bivalves show foot relaxation and the phenomenon is more or less persistent (e.g. more for *Ruditapes philippinarum* than for *C. gigas*) (Le Bris *et al.*, 1995). Growth decrease is noticeable in *Crassostrea gigas* at 30 µg/l, and in *Ruditapes decussatus* at 330 µg/l (Thain *et al.*, 1990). Marine algae are relatively insensitive but growth is affected by concentrations in the order of 1 mg/l. Fish are also subject to growth decrease after chronic exposure (Roth *et al.*, 1993).

B. Di-n-butylphthalate (Burrige *et al.*, 1995)

DBP is used as a solvent in a pesticide formulation of dichlorvos, Aquagard®. This compound has slow water solubility and is slowly hydrolyzed, and it is also degraded by bacteria and a fungus. It is metabolised, by decreasing order of efficacy, by fish, shrimp and oyster. Chronic exposure of fish to more than 0.09 mg/l can be detrimental. The reported BCFs are 21.1-41.6 for bivalves, 2.9-5000 for shrimps, 11.7 for fish and 1,350-6,500 for lobsters.

C. Carbamates: Carbaryl (1-naphthyl N-methylcarbamate)

Carbaryl can accumulate in sediments (log Kow = 2.36). In seawater, it is rapidly hydrolyzed to 1-naphtol, and more rapidly as temperature and exposure to sunlight increase. Free 1-naphtol is adsorbed onto sediments and it has its own toxicity. Its degradation is also dependent on temperature and sunlight but the products of degradation precipitate and are then almost as toxic as 1-naphtol (Bruno *et al.*, 1990).

Carbaryl does not seem to accumulate in fish and shellfish. Its toxicity is dependent on temperature but not on salinity. Convulsions of salmonids have been observed at 1 mg/l, but then they were shown to recover. A concentration of 11 µg/l seems to have significant impact on the population density of estuarine invertebrates. Sensitivity of algae is highly dependent on species (Roth *et al.*, 1993).

Toxicity of carbaryl is higher for crustaceans than for molluscs and fishes, but the reverse is true for 1-naphtol (Table 4) (Donkin *et al.*, 1997).

Table 4. ECs and LC 50 (mg/l) of carbaryl and 1-naphtol for marine biota (Stewart *et al.*, 1967)

	1-naphtol N-methylcarbamate	1-naphtol
Shrimp	24 h EC 50 [†] = 0.13	24 h EC 50 [†] = 6.6
Crab	24 h EC 50 [†] = 0.3-0.7	24 h EC 50 [†] = 40-80
Bivalve	48 h LD 50 = 2.2	-
Fish	24 h LC 50 = 3.9-6.7 96 h LC 50 = 0.69-39	24 h LC 50 = 1.3-3.2

[†]EC 50: loss of equilibrium, paralysis, or death of 50% of the animals (20°C at 25‰ salinity).

D. Organochlorine

Endosulfan degradation increases with temperature and pH, and is higher in water than in soil. The half-life is 24 to 40 days at 29-39°C. It is highly toxic to aquatic fauna and red algae. The 96 h LC 50 is 0.4 - 0.5 µg/l for *Gammarus palustris* (15 ‰ at 20°C) (G räslund and Bengtsson, 2001).

Endrin and lindane are two other organochlorine compounds. Their respective BCFs measured for *Mytilus edulis* are 2,274 and 343 when immersed in 3.58 and 0.12 µmol/l for 7 days. Their respective log Kow are 3.61 and 4.56 (Donkin *et al.*, 1997).

E. Pyrethrins

Those compounds are sparingly water-soluble, bind tightly to organic matter and they are highly photolytic. Examples of 96 h LC 50 are given in Table 5.

Table 5. The 96 h LC 50 (µg/l) of pyrethrins for marine species (Mairesse, 1994)

	Permethrin	Cypermethrin	Fenvalerate
Salmon	12	2	1.2
Lobster	0.73	0.04	0.002
Shrimp	0.13	0.01	–

Disruption of reproductive functions has also been observed in *Salmo salar* exposed at 0.004 µg/l for 5 days.

F. Avermectins

a] Ivermectin

Ivermectin is highly photolytic, poorly soluble in water and resistant to hydrolysis and as it binds to sediment and does not degrade in anaerobic conditions, the risk for crustaceans is high (Collier and Pinn, 1998). Elimination is slow for *Salmo salar*, and non-metabolised compound can be found in faeces (77% of that excreted is unchanged drug after one day). The BCF is 54-74 so it is not expected to accumulate in fish but high levels have been observed after extended periods of treatment (Roth *et al.*, 1993). The BCF is 750 for *Mytilus edulis* and the excretion half-life is 235 d (Davies *et al.*, 1997).

There is little information on toxicity to fish but signs of toxicity and significant mortalities are reported for Atlantic salmon at about 0.4 mg/l. It is less toxic to molluscs than to crustaceans but toxicity to crustaceans is variable (Table 6).

Table 6. The 96 h LC 50 (µg/l) of ivermectin for some invertebrates (Roth *et al.*, 1993; Davies *et al.*, 1997)

Invertebrates species	µg/l
<i>Mysidopsis bahia</i>	0.022
<i>Penaeus duorarum</i>	1.6
<i>Neomysis integer</i>	0.07
<i>Callinectes sapidus</i>	153
<i>Crassostrea virginica</i>	430

Ivermectin would have no effect on fungi, bacteria and protozoa of sediments.

b] Enamectin

Signs of toxicity begin at 356 µg/kg in salmon (7.1 times the recommended dose), with darker colour, lethargy, and flank lesions. The 96 h LC 50 is 1.34 mg/l for *Cyprinodon variegatus* (Roy *et al.*, 2000).

G. Diflubenzuron

Diflubenzuron is stable to photolysis, it binds to organic matter, and the half-life is about 18 days. It is poorly absorbed by the salmon gut, and the parent compound can be found in excreta.

Diflubenzuron is extremely toxic to crustaceans (mortalities with concentrations as low as 0.5 µg/l have been reported). Examples of sub-lethal effects are disturbance of reproduction and abnormal swimming. (Roth *et al.*, 1993)

H. Hydrogen peroxide

See under "therapeutants other than antibacterials".

9. Herbicides, algaecides

A. Copper

See below heavy metals.

B. Simazine

This algaecide is extremely toxic to phytoplankton but not to fish at the same concentration rate. It is not known to be bioaccumulative and its half-life is about two weeks (bacterial degradation) (Boyd and Massaut, 1999).

10. Feed additives and contaminants

Vitamins A, D, E can inhibit growth of rotifers when given at too high a concentration (higher than 2, 0.2 and 1 µg/ml, respectively), whereas small doses favour their reproduction (Satuito and Hirayama, 1986).

Butylated hydroxytoluene has toxic effects when used as a pure compound at excessively high levels of exposure. Feed can be supplemented or contaminated with heavy metals.

11. Anaesthetics

Anaesthetics should not be used often. They have variable, sometimes narrow, safety margins (e.g. the therapeutic index of tricaine sulphate is 1.3-1.5) and dosage should be adapted to temperature, salinity, and species. Even if some of them are used for both fish and shellfish, the recommended doses are not the same. They are rapidly eliminated, and because low doses are generally used, toxicity should be limited.

12. Hormones

No information is available on the toxicity of hormones. The risk seems to be low because treated fish are generally not sold for consumption, or hormones are given to fry (so the treated biomass is small) and the fish are sold much later (GESAMP, 1997).

13. Heavy metals

Some heavy metals are essential for metabolism (e.g. Cu, Zn), whereas others are not (e.g. Hg, Cd) (Lorteau, 1994). However, even the useful ones can have a toxic effect beyond a certain threshold, because as usual toxicity is a matter of dose. Cu, Zn, Cd and Pb are among the most interesting metals because of their occurrence and ability to accumulate. Fish feed heavy metal content is not negligible (e.g. Zn = 134 ppm, Cu = 6.7 ppm, Mn = 100 ppm) (Uotila, 1991).

Accidental water contamination by exogenous pollution is not considered here, since any metal could be taken into account in that case. However, mercury can be considered because of its toxicological importance and copper because of its use as a therapeutant as well as an algacide, a food contaminant, and a metal leached by structural materials.

As for other toxicants, heavy metals can bind to biological structures (such as membranes and enzymes) and disturb their functioning, but some metals like copper can also produce free radicals, causing peroxidation of membranes (Kennedy *et al.*, 1996). When considering a metal it is important to know what species of the metal is present and in what proportion, since metal toxicity is often due to the free metal in solution, although, for example, mercury is methylated by bacteria in sediments or in the gut of fish, and methyl-mercury is more toxic than inorganic mercury. For arsenic, inorganic ions are more toxic than organic acids or salts (Ackefors *et al.*, 1989).

Interactions complicate toxicity assessments. Lead is known to compete with essential metals (Mn, Cu), whereas Cr, Zn and Pb are synergistic for metal uptake in *Artemia salina*, and Fe, Cd and Mn are antagonistic, whereas Cd and Zn are synergistic for shrimp *Pandalus montagui*. (Chen and Liu, 1987).

Interactions can concern substances other than metals. Trimethyltin chloride and Cd have a synergistic effect on *Artemia franciscana* mortality (Hadjispyrou *et al.*, 2001), and 0.1 mg/l of Pb provokes a decrease in calcium content of kidney in *Mytilus edulis*, etc. (Cossa *et al.*, 1993).

Accumulation of metals among marine biota is variable (Table 7). Bioaccumulation and bioconcentration factors (BCFs) are often inversely related to exposure concentration for most metals and organisms (Adams *et al.*, 2000). Several detoxification processes regulate toxicity to a certain extent, such as binding to metallothioneins and storage in granules for invertebrates, or mucus excretion in gills, skin and digestive tract in fish (Amiard-Triquet *et al.*, 1993). Mucus forms a mucus-metal ion complex and in extreme conditions death may occur by suffocation but normally the mucus is continuously sloughed off and the metal complex is effectively lost (Saward *et al.*, 1975). Once again, all chemical forms do not have the same behaviour. Methyl-mercury is absorbed five times faster than inorganic forms in fish, and accumulation in fish concerns almost exclusively this compound. It is accumulated in muscle and can be biomagnified (Handy, 1996). On the contrary, only between 1 and 17% of the metal stored in invertebrates is under the methylated form. In sea urchins, Cu, Pb, Hg and Cd are reported to accumulate in the intestinal tract, and Cd and Hg in the reproductive tract, and these organs in this species are eaten (Augier *et al.*, 1987).

Table 7. Some BCFs of heavy metals for marine biota (Schmitz, 1996)

	Plant	Invertebrate	Fish
Hg	1,000	100,000	1,670
Pb	200	200	60
	1,200-80,000 (macrophytes)		
Cd	1,000	250,000	3,000
Se	800	400	400

When chronic toxicity occurs, especially through a nutritional disease, classical non-specific observations include reduced growth and poor food conversion, possibly with chronic mortality. English sole exposed for 4 weeks to 5 µg/g of cadmium showed liver necrosis and mortalities (Hendricks and Bailey, 1989). A concentration of 100-200 µg/l cadmium for 20 weeks caused emaciation, inhibition of shell growth and adverse effects on the cellular defence of *Crassostrea virginica*. (Kennedy *et al.*, 1996). The cadmium 96 h LC 50 is 220 ppm for *Skeletonema costatum* (Nassiri *et al.*, 1995). A pre-exposition at 200 µg/l Cd of *Mytilus edulis* enhanced the genotoxicity of another mutagen, hydrogen peroxide (Pruski and Dixon, 2002).

A. Copper

Copper is highly acutely toxic to aquatic life, and in particular it inhibits respiration and photosynthesis in algae (Boyd and Massaut, 1999). However, some algae seem more or less tolerant to copper. In fact, other explanations can moderate this hypothesis. In *Enteromorpha compressa*, copper is bound to mucilage and by bacteria producing mucilage, and, especially at high concentrations, copper precipitates. This reduces the available copper in water, and any apparent toxicity (Gräslund and Bengtsson, 2001).

Table 8. Copper (Cu²⁺) 96 h LC 50 (ppm) for marine biota (Martin *et al.*, 1977; Reardon and Harrell, 1990; Chen and Lin, 2001)

Species	Copper (Cu ²⁺) 96 h LC 50 (ppm)
<i>Morone saxatilis</i>	788 (15‰ salinity)
<i>Haliotis cracherodii</i>	50 (33‰ salinity)
<i>Haliotis rufescens</i>	65 (33‰ salinity)
<i>Penaeus monodon</i>	3130 (15‰ salinity)
<i>Penaeus japonicus</i>	7730 (25‰ salinity)
	2050 (37‰ salinity)

Fish and crustaceans have the ability to regulate their copper content to some degree, but accumulation has been reported after dietary exposure (Handy, 1996).

A 10 µg/l copper concentration inhibited the sporophyte growth of *Laminaria saccharina* at 10°C (Gräslund and Bengtsson, 2001), whereas 0.5 mg/l causes destruction of gills in *Mytilus edulis* and it can also affect byssus thread production and protein metabolism (Sobral and Widdows, 1997). At 0.9 mg/l for 30 days the growth of *Penaeus monodon* was significantly affected (Chen and Lin, 2001). After 20 days exposure to 10 ppm (20°C at 35.6‰ salinity), *Ruditapes decussatus* growth was affected and the measured BCF was 3840 (Chen and Lin, 2001), and although the animals partially recovered there was clear impairment of physiological functions (Sobral and Widdows, 1997). Copper also impairs immunity in bivalves (Lorteau, 1994).

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