Instituto de Investigaciones de la Amazonía Peruana
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Use of Therapeutants in Aquaculture

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INTRODUCTION

Mortality in aquaculture can be attributed to many causes, such as oxygen depletions, toxicants, predation, poaching, and nutritional imbalance, but losses due to disease and parasitism are by far the most significant factors. Although disease and parasite epizootics do not affect every culturist every year, most culturists do suffer losses to these causes at various times. The worst case scenario is that an aquaculturist would be driven to declare bankruptcy due to disease problems, but only about five percent of commercial fish farmers working in the United States have severe disease problems in any given year (Stickney 1979). Even still, each year the aquaculture industry reports significant losses of fish and money. In the first half of 1989, 115 million catfish with a value of $11.6 million were lost to disease (Meyer 1991). In 1988, the catfish industry reported that 39 million catfish were killed by disease (Meyer 1991). Also in 1988, the trout industry reported losses of fish due to disease at 10.4 million fish with a value of $2.5 million (Meyer 1991). These numbers indicate that diseases and parasites can present devastating problems to fish farmers.

The importance of therapeutants to aquaculture cannot be overstated. Even under the care of the most meticulous culturist who employs best management practices, outbreaks of disease are going to occur. As a result, anesthetics are needed to reduce stress of fish during handling, disinfectants are called upon to remove pathogens from equipment and facilities, and chemicals are needed to improve water quality and to control vegetation. Also, parasiticides, antibacterials, and fungicides are used to control specific organisms. It is inevitable that diseases and parasitism will continue to be a limiting factor in the aquaculture industry. Presently there are only five registered chemicals approved for therapeutic use in food fish aquaculture. If the aquaculture industry is going to progress and reach its full potential, then chemicals which are safe and effective must be registered and made available for use by aquaculturists.

HISTORY

The current status of the use of animal drugs in aquaculture is in a drastic state of change. To gain a better understanding of what is happening now, a review of the events that happened in this area over the past thirty years is necessary. During the 1950's and 1960's, workers in fish culture and fish management used chemicals which were developed and registered for completely different purposes (Lennon 1967). Those in the field of fisheries were scavengers, borrowing chemicals from other disciplines and using them in a careless manner, not knowing what possible effects a drug might have (Lennon 1967). At this time, only eight chemicals were registered and approved for aquatic use (Lennon 1967).

Congress charged the U.S. Environmental Protection Agency (EPA) with the control of the use of pesticides and the U.S. Food and Drug Administration (FDA) with the control of the use of drugs (Meyer 1976). In 1972, the Federal Environmental Pesticide Control Act (FEPCA) was enacted, which required that all chemical uses be covered by approved registrations granted by the EPA or FDA (Meyer 1976). Most chemicals used in fisheries lacked proper registrations and were being used in violation of this act (Meyer et al. 1976). FEPCA also required that existing registrations of pesticides be reviewed by the EPA (Meyer et al. 1976). Investigation of existing registrations showed that many registrations were too limited or inadequate to cover the many uses employed in aquaculture and fisheries management (Meyer et al. 1976). So, in order to support claims of human and environmental safety, most registrations needed additional data (Meyer et al. 1976). Only two chemicals were exempt from re-registration: Terramycin and sulfamerazine (Meyer et al. 1976). All other chemicals needed additional research; even such ancient remedies of salt and formalin, which had been used for over 100 years were not exempt from these requirements (Meyer et al. 1976). To spearhead this registration process, the U.S. Fish and Wildlife Service (USFWS) formulated a list, from surveys of fishery professionals, of the most needed chemicals for use in

There is a recognizable separation which exists in the available literature and enforcement of regulations for therapeutic drugs which are used in aquaculture. This division occurs among those species which are considered as "food" or "non-food" species. "A species or population will be considered 'food' if it is reasonably likely to be consumed for food either by humans or food producing animals" (Stefan 1992). The FDA, as established in its mission, is concerned with the use of therapeutants with food fish species. The FDA recognizes three categories of commonly cultured fish: baitfish and ornamental/aquaria fish, both of which are viewed as non-food species, and broodfish, which are all regarded as food species. The use of drugs during the different life stages of broodfish has become a highly controversial issue. The FDA's current stand on food fish is that they will be "considered food at all life stages" (Stefan 1992).

"Enforcement policies for food and non-food species and populations are likely to differ in important ways because the public health considerations for the two groups are different." (Stefan 1992). Those drugs which are approved for use with food species have changed frequently during the past several years, and are found often throughout the literature. There appears, however, to be a lack of current information available for those species which are non-food. The FDA is considerably less concerned with drugs used for non-food species due to the elimination of the threat to human food safety. Certain drugs are approved for use with food species, while numerous others, which by their historical uses have proven innocuous to the target fish, the environment, and the applicator, are allowed for use with non-food species.

The FFDCA provides three methods by which a drug may legally be used to treat aquaculture species. A drug may legally be used if it meets any one of the following criteria: 1) if it is GRAS and GRAE; 2) if it is an approved new animal drug application (NADA); or 3) if it has been granted an INAD exemption. A drug which is GRAS and GRAE or that possesses an NADA, is considered an approved drug. An INAD exemption is the legal method for use

fisheries and designated the Fish Control Laboratory at LaCrosse, Wisconsin as the lead facility to coordinate the registration effort (Meyer et al. 1976). Also, because of the overwhelming needs for research, industry was called upon to assist in the process (Meyer et al. 1976).

By 1976, eighteen chemicals were registered for specified uses in fisheries, and ten of these were for use on fish used as human food (Meyer et al. 1976). Some new chemicals had been registered by 1985 which brought the total up to over thirty (Schnick et al. 1985). By 1989, the number of drugs and chemicals approved for treating diseases of fish and shellfish was thirty-nine (Schnick et al. 1989). But even though new chemicals were being registered, some chemicals were being lost. One way that this happened was that in order to save money, sometimes fish culturists purchased generic products rather than registered chemicals (Schnick et al. 1985). This prevented sponsors from recovering the cost of registering their product and the manufacturer suspended or canceled production and sale of the product (Schnick et al. 1985). Two examples of loss of a fishery chemical are Masoten and sulfamerazine (Meyer 1989). Another way therapeutic chemicals can be lost is if they are used so much that fish pathogens develop resistant strains (Meyer 1989). Some strains of Aeromonas and Pseudomonas have become increasingly resistant of Terramycin (Meyer 1989).

To help register or approve drugs for use in fisheries, the FDA can issue an investigational new animal drug (INAD) exemption (Stefan 1992). With this INAD exemption a culturist has the right to use an unapproved compound, but would have to submit data back to the FDA to support the drugs formal approval (Stefan 1992). In the past, the FDA was very lenient in granting INAD's for aquaculture, and in return very little data was being developed to support the formal approval of the drug (Stefan 1992). This process was not effective; the subsequent failure to develop data was not putting the aquaculture industry in a position to use various compounds legally (Stefan 1992). Therefore, many of the registrations of drugs were revoked, leaving only five drugs approved for use with aquaculture species

(Stefan 1992). The INAD process still exists today, but the integrity of this process was restored by making it difficult to get INAD's and the FDA's demanding the development of data on unapproved drugs (Stefan 1992).

CURRENT STATUS

The FDA's basic mission is to:

> assure that foods are pure and wholesome, safe to eat and produced under sanitary conditions; that drugs and medical devices are safe and made effective for their intended uses; that cosmetics are safe and made from appropriate ingredients; and that labeling and packaging for all these products is truthful, informative, and not deceptive (Beaulieu 1992; CVM, USFDA 1992; Stefan 1992).

The Federal Food, Drug and Cosmetic Act (FFDCA) and its accompanying regulations provide guidance for the FDA by clearly defining what is required and/or permitted regarding products that fall under the FDA's jurisdiction. Among the products which are under the FDA's jurisdiction are animal feeds, animal feed additives, and animal drugs.

> The claims made for a product (or the way in which it is actually used) determine whether it is a drug. By definition, a drug is an article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and articles (other than food) intended to affect the structure or any function of the body of man or other animals. A drug intended for use in animals that is not generally recognized by experts qualified by scientific training and experience to evaluate the safety and effectiveness of new animal drugs as safe and effective is a "new animal drug" (Beaulieu 1992; CVM, USFDA 1992; Stefan 1992).

A drug that is generally recognized as safe (GRAS), is a drug that is safe for the animal, the person administering the drug, persons eating food products derived from the animal, and the environment. A drug that is generally recognized as effective (GRAE), is a product that will do what it is claimed to do, consistently and uniformly. Recognition of a drug as safe or effective is determined from data obtained from controlled studies, which have published results in professional scientific literature.

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of a new animal drug or for an unapproved use of an approved drug.

The estimated expense of meeting the necessary data requirements for the approval of a drug is estimated at $5.2 million (Schnick (undated)). Chemical manufacturing companies generally provide funding for the necessary research for approval, or only those drugs which the company is assured will be able to recover the research and development costs, and additionally, will provide a profit for their company. For this reason, very few drugs receive funding for research towards their approval from actual drug manufacturing companies. In addition to the expense involved, all of the data is required to be published in the scientific literature for a drug to be recognized as GRAS and GRAE. To be approved as a NADA the data requirements are essentially the same as those to be GRAS and GRAE, however, there is no obligation for the data to be published in professional literature. Because of this obligation of data to be published in professional literature, drugs can actually be more likely to be approved under the NADA process. There are no drugs being used in aquaculture that have met the requirements to be GRAS and GRAE, consequently, all approved drugs currently being used in aquaculture are NADA's.

A drug granted a NADA is limited to a drug manufactured by a specific sponsor, for use with a specific species, for specified indications, at specific doses, and with specified limitations, including any withdrawal time between treatment of the product and the availability of the treated product to the public. To date, five drugs have been approved for (limited) use as therapeutants with food species in aquaculture (Table 1), however, only four of these drugs are commercially available It is important to understand that these are limited approvals of the drugs, and that these drugs may not be used for all purposes, nor with all species.

An INAD exemption is legally required for the use of any unapproved drug, and therefore, is the first step in obtaining a NADA for any new animal drug. An INAD exemption allows for legal interstate shipment of unapproved drugs, as well as, the

authorization for treated animals intended to be released for human consumption or slaughtered. Most importantly, the INAD exemption allows (and is necessary) for the unapproved use of a new animal drug for the purpose of conducting research for its future approval as a NADA.

INAD's are granted by the Center for Veterinary Medicine (CVM). Approval or rejection of a requested INAD exemption generally takes approximately 180 days. An INAD is valid for a one year period, therefore, it must be renewed annually. A request for an INAD exemption must include: the name of the specific drug of interest (from the specific manufacturer), available data on human food safety, environmental safety, target animal safety, efficacy information (especially dose), design of study protocols, and a sponsor, a monitor, and an investigator must be identified (CVM, USFDA 1992).

The sponsor is the actual holder of the INAD exemption and is ultimately responsible for the use of the drug as authorized by the exemption. A sponsor is responsible for establishing protocols, appointing a monitor, and keeping all necessary contacts with the CVM. The CVM also holds the sponsor liable for ensuring that the studies and distribution of the drug are not unduly prolonged (Beaulieu 1992; CVM, USFDA 1992). Sponsors gather all of the various data generated under the INAD and submit them to the CVM, who then places the data in a Public Master File (PMF), which can then be accessed, referenced, and used as support by the manufacturing company seeking the NADA.

The monitor, who is established by and works for the sponsor, is to ensure that the "investigator understands and accepts his responsibilities as an investigator and has adequate facilities, resources, animals, and time to conduct the proposed study" (Beaulieu 1992; CVM, USFDA 1992). Monitors are expected to keep personal contacts with and visit the facilities of each investigator over the course of the entire study, and without directly participating in the collection of data, ensure that data are being generated and properly recorded. They should keep a complete record of all contacts with investigators and finally certify that the results reported by the investigator

completely and accurately reflect the results of the study" (Beaulieu 1992; CVM, USFDA 1992).

The investigator keeps a copy of the protocols, signed by both the sponsor and the monitor, and is responsible for carrying out research as established by these protocols. It is important that the investigator maintain thorough and accurate records throughout the study, as well as, collect and submit all data that is generated. The investigator is responsible for the implementation of the drug study, control of the drug, the test animals, and of their proper disposal. Upon completion of the study, the investigator must submit all data and records to the sponsor, along with a certification as to the completeness and accuracy of these materials.

The amount of work and time which are required to gather all the data necessary for a drug's approval are extensive. This, coupled with the high costs associated with testing, keeps many chemical manufacturers from seeking INAD sponsorship. This can be a major problem for the producer because he/she generally does not have the experience, facilities, or the capital, necessary for receiving an INAD sponsorship. The FDA has provided an intentional loop-hole by which producers may obtain short-term INAD exemptions if they are able to meet specific conditions and requirements.

The "Compassionate INAD Policy" allows for a compassionate INAD to be granted if: 1) animals' lives are threatened and subject to suffering or death if not treated; 2) there is sufficient scientific evidence supporting the use of the drug for the condition to be treated; and 3) there is no existing approved drug or treatment for the condition, or, if the proposed treatment offers some significant advantage over existing approved treatments (Stefan 1988). An emergency INAD exemption may be granted for a one-time emergency situation in which the aforementioned conditions and requirements have been met. The agency will not tolerate the use of an emergency INAD in cases where producers simply have not planned for recurring or seasonal problems. These INAD exemptions do not exempt the holder from the responsibility of collecting data, they are simply additional

methods by which an INAD exemption may be obtained.

Producers can provide a major part of the data which will be necessary for a new animal drug's future approval by obtaining INAD exemptions as allowed under the compassionate INAD process. By initiating the data collection for new animal drugs, producers lessen the time and costs involved for manufacturers who can get the drugs produced, labeled, and marketed, to obtain a NADA. The producers' initial efforts towards a particular drug's approval may encourage a university, a Federal or state group, an aquaculture producer group, or the CVM's IR-4 program, to acquire sponsorship of the drug. Through the use of the compassionate INAD policy, producers can benefit themselves by becoming involved in, and actually becoming apart of, the NADA process for a new animal drug.

The agency allows the use of certain new animal drugs under specific conditions by two methods in addition to an INAD exemption. Low enforcement priority is a decision by the agency to use "regulatory discretion" in the enforcement of the use of some drugs which have been historically used in aquaculture. Seven drugs (Table 2) are recognized as having low regulatory priority. These drugs and their accepted uses, from their historical use in aquaculture, have been sufficiently documented in scientific literature for the agency to make its decision "based on the nature of the drug, information regarding its metabolism and residues in the treated animal, the toxicity of the drug residues to man, and other factors including the manner in which it is generally used in practice" (USFDA 1992).

Extra-label use is an exemption available only to veterinarians. It allows veterinarians to use an unapproved drug or an approved drug for uses other than its specified use in the treatment of a disease; this does not allow unapproved use for the prevention of disease. The agency does not allow extra-label use of chloramphenicol nor nitrofurans. When a veterinarian exercises extra-label use, he/she is accepting responsibility for safety and efficacy of treatment, as well as, the absence of harmful residues if used in food species.

Data needed for the approval of a drug include: efficacy,

safety of target species, human food safety, environmental safety and affects, and manufacturing and control information. INAD's generally only provide data which can be obtained from field studies. Many data must be obtained from well controlled studies which cannot be performed in the field. These data are usually generated by the supporting manufacturing companies, additional testing companies, and by the CVM's IR-4 Project studies. The use of INAD's to generate as much data as possible benefits the chances of new animal drugs being approved and granted an NADA's.

NADA TESTING REQUIREMENTS

Safety and effectiveness data are required to be submitted to the FDA as part of the NADA. Safety studies are designed to show that the drug, or test article, is safe for the target animal, or the animal being treated, safe for humans, and safe for the environment. Effectiveness studies must show that the drug is effective for its label claim, or intended use. The FDA defines safety and effectiveness data as "all studies and tests of an animal drug on animals and all studies and tests on the animal drug for identity, stability, purity, potency, and bioavailability" (21 CFR 514.11.h). Studies and tests on the new animal drug may include testing the active ingredient of the drug formulation, the inert ingredients, impurities, metabolites, or degradation products of the drug. For simplification, an FDA term, "test article", is used to describe the chemical or mixture under study. The purpose of this section is to highlight the numerous studies which may be required for registration of an aquaculture therapeutant and to briefly describe the nature of selected studies.

Information on the product identity and composition is required to assess the need for studies to evaluate the safety and effectiveness of all components of the product (21 CFR 514 and 40 CFR 158). Product identity and composition data must include a description of the product identity and disclosure of ingredients, description of beginning materials and manufacturing processes, and a discussion of formation of impurities. All impurities present in concentrations equal to or greater than 0.1

weight percent must be identified and quantified. An applicant's product identity and composition will be compared by the agency with currently registered products to determine if the compositions are identical or substantially different. Also, knowing the product's composition will lead to proper labeling, packaging and possible use restriction requirements.

Certified limits, or the outer range of concentration of all the product's ingredients, as well as the nominal concentration, or the normally expected concentration, of the active ingredient must be supplied to the agency. The certified limits and nominal concentration are enforceable and legally binding for registered products from the date of production to the date of use, unless the stability of the product requires the assignment of an expiration date. The applicant must supply the agency with samples of the product, samples of analytical reference standards, and the analytical methods used to determine the product's active ingredients, inert ingredients, and impurities of toxicological significance. If samples of commercial product are tested by the agency and the certified limits are found outside the claimed values, regulatory action may result, which may involve product recall.

Physical and chemical characteristics listed in Table 3 are required data for the active ingredient and any inert ingredients or impurities of toxicological significance. The requirement of certain data may be waived depending on the physical state or chemical class of the test article. For example, the melting point study is required for those test articles that are solids at room temperature and therefore, the boiling point study would not be required. Likewise, the octanol/water partition coefficient study is required only if the test article is organic and non-polar.

Physical and chemical characteristics are used in confirmation or support of identification of the product, its active and inert ingredients, and impurities. Color, physical state, and odor are used in emergency situations for response to spills and accidents or in poisoning cases where minimal information is known about the test article. Melting or boiling

points, vapor pressure, and pH are important for worker safety. Octanol/water partition coefficient is necessary to determine the need for further fish and wildlife accumulation studies. Storage stability studies determine the need for toxicity studies of the degradation products if the test article composition changes due to the storage conditions. Corrosion characteristics studies are designed to determine the chemical compatibility with its container, lid, liners, and seams of the container. Stability, oxidizing or reducing action, flammability, explodability, storage stability, corrosion characteristics, and dielectric breakdown voltage are used directly in hazards assessment.

Further data requirements may be placed in the categories of residue chemistry, environmental fate, toxicology, reentry protection, spray drift, wildlife and aquatic organisms, nontarget insect, and efficacy studies. Each category has required and conditionally required data which reflects the general use pattern of the drug to be registered; in this case the general use pattern of the new animal drug is use on an aquatic food crop.

Residue chemistry data requirements listed in Table 4 are used by the agency to estimate the exposure to residues in food or water consumed by humans or animals. This data is used in setting tolerances in or on food or feed. Nature of the residue studies typically use radiolabelled test articles and are designed to determine the degree of uptake of the test article by the treated animal, the most potentially affected organs (target organs), and identification of the metabolites of the test article. Magnitude of the residue studies are required to show the concentration of the residues in a disappearance curve for assignment of a withdrawal time. Withdrawal time is the amount of time that must lapse after the last application of the new animal drug to achieve the legally assigned tolerance level of residues allowed in the edible products of the treated animal. The best drug candidates for new animal drugs would be those compounds with short withdrawal times and very negligible residues.

Environmental fate data requirements listed in Table 5 aid in establishing label restrictions and in protection of threatened or endangered species or wildlife populations at risk. Degradation studies determine the rate of degradation of the test article when exposed to the environment. If the test article persists in the environment, the need for nontarget organism testing is identified and adverse effects must be determined. Metabolism studies are designed to determine the availability of the test article to rotational crops as well as its persistence in soil and aquatic environments. Mobility studies determine the mode of transport and eventual destination of the test article in the environment. Potential environmental hazards are assessed for contamination of human and animal food, loss of usable land and water resources due to contamination, and habitat loss due to transport in the environment. Dissipation studies are designed to determine the hazards of reentry in treated areas, residues in rotational crops and other food sources, loss of land, surface, and ground water to contamination. Accumulation studies determine residue levels in food supplies from wild sources or rotational crops and the residues in aquatic animals eaten by humans.

Toxicology data requirements listed in Table 6 are necessary to determine the short-term and long-term risks of exposure to the test article. Acute toxicity testing evaluates the toxic characteristics and health hazards due to short-term exposure to the test article. Initial information is determined for drug classification, cautionary labeling, and the need for child resistant packaging. Acute studies assess the initial mode of toxic action of the test article and also provide dose levels for subchronic testing. Subchronic testing determines health hazards resulting from repeated exposure over limited time periods. Target organs, the accumulation potential of the test article, and dose levels for chronic studies are also determined in subchronic studies.

Chronic toxicology studies are designed to determine the cumulative effects due to prolonged and repeated exposure to a

test article. In oncogenicity studies, animals are observed over most of their life span for development of neoplastic lesions. Teratogenicity studies determine if structural or other abnormalities are induced to the fetus after the mother is exposed to the test article during pregnancy. Reproduction studies determine the effects on gonadal functions, estrus cycles, mating behavior, conception, parturition, lactation, weaning, growth and development of offspring, as well as neonatal morbidity and mortality. Mutagenicity testing evaluates the test article's potential affects on mammalian cellular genetic components.

Reentry protection and spray drift studies listed in Table 7 determine the need for precautionary labeling to minimize the effect to nontarget organisms, especially humans. Wildlife and aquatic organism studies listed in Table 8 and nontarget organism studies listed in Table 9 determine the need for precautionary labeling to minimize the effect to nontarget organisms. Efficacy studies listed in Table 10 (Schnick 1992) are required by the FDA regardless of the method of administration of the new animal drug.

Many studies are conditionally required depending on the nature of the test article. For instance, registration of a test article would not require the full battery of chronic toxicity studies if the acute and subchronic toxicity studies showed it to be relatively non-toxic. The data requirements for a particular test article are determined by the FDA on a case-by-case basis. The study design and protocol for the study should be reviewed with the FDA prior to scheduling the study. According to Beleau (1991) there are nine steps involved in successfully completing a study. Each of the following steps may

involve the sponsor, testing laboratory personnel, and representatives of the FDA: identify laboratory, develop protocol, method development, method validation, Good Laboratory Practices, approve protocol, conduct study, monitor study, and final report.

Identifying the laboratory to conduct the study would include considerations of the scientific and managerial expertise available, and the species and facilities required. A protocol which details the objectives and all methods for the conduct of the study is developed usually by the sponsor and testing facility personnel. All techniques and analytical methods are developed and further validated prior to use in the study.

Good Laboratory Practice (GLP) regulations (21 CFR 58) are the minimum requirements for conducting a study to assure the quality and integrity of the data. Briefly, the GLP regulations require that each and every procedure is fully documented, the use and distribution of the test article is controlled and documented, all facilities and equipment have written procedures and maintenance documented, the procedures are monitored by quality assurance personnel, and that personnel are properly trained for their assigned duties and responsibilities. Documentation of all procedures followed, the protocol, all correspondence pertaining to a study, quality assurance audit reports and findings, and the final report of a study, as well as samples of the test article and treated animals, must be kept in a designated, separate archive area for a minimum of two years and usually for the duration of the new animal drug registration. After the sponsor is assured that the identified laboratory can

perform the study in compliance with the GLP regulations, the protocol is reviewed by the FDA. The sponsor has a great deal of work invested in the study even before the testing actually begins. The study is conducted by aquacultural scientists and monitored by the testing facility quality assurance personnel. The sponsor also monitors the study for compliance with the GLP regulations and adherence to the written, approved protocol. Finally, a final report is written by the testing facility personnel, reviewed by the sponsor, and submitted to the FDA as a part of the registration package.

Discussions with the FDA throughout the nine step process is highly desirable for the maximum exchange of information and coordination of efforts to broaden the scope of the studies whenever possible (Beleau 1991). In order to achieve a worldwide market for the new animal drug, the sponsor must provide data for registration of the drug to each country's governing agency. For example, if it was desirable to market the new animal drug in the United Kingdom, much of the required data for registration of the drug by the United States FDA would also be required by the Ministry of Agriculture, Fisheries, and Food (MAFF); therefore, officials of MAFF should be included in initial discussions of the studies required to be performed and the protocol requirements.

COMMON FISH DISEASES AND PARASITES

When fish and other aquatic animals are cultured intensively the chance for diseases to occur increases appreciably. Parasites, fungi, and bacteria are the main groups of pathogenic

organisms that can be treated with the use of specific therapeutants. Viruses may also affect fish; however, as there are no effective therapeutants for these viruses, they will not be discussed. Since it is beyond the scope of this paper to detail the multitudes of parasitic, fungal, and bacterial infections that affect fish, only a brief summary of the common pathogens, the associated clinical signs, and therapeutic treatments for representative diseases will be noted.

PARASITES

Parasite infection is second only to bacterial infection as the major cause of disease among cultured fish. Young fish are especially vulnerable to parasite infection because their protective layers of skin and mucus are much thinner in comparison to adult fish. There are several major classes of parasites which infect fish, each of which are represented by numerous species. Clinical signs of parasitic infection which are similar to other fish diseases are sluggishness, lethargy, excessive mucus, lack of appetite, fin erosion, and emaciation. Flashing is a sign which tends to be more unique to parasite infestation.

Protozoan

Protozoans are classified into four major groups: the flagellates, which move by one or more whiplike structures, the ciliates, which move by many short hairlike projections, the sporozoans, a parasitic group with sporelike infective stages, and the Sarcodina, which move by pseudopods. These protozoa are

more easily distinguished when divided into two groups, the external and the internal. Although it is recognized that some species of protozoans will infest fish both externally and internally alike.

External parasites attach themselves to the skin, gill tissue, or fins where they may cause physical damage as well as depletion of the vital components of the blood and other tissues. Some parasites, such as Trichophrya, which do not feed directly upon the fish, are parasitic in nature in that they benefit from this symbiotic relationship while causing irritation and harm to the host. Other external protozoans feed directly upon the fish causing irritation and weakening of the fish, which could lead to secondary infection and possible death. Most of the external protozoa cannot be seen with the naked eye with the exception of the organism Ichthyophthirius, commonly referred to as "Ich". Ich are found under the epithelium of the skin, fins, and gills. It is one of the more serious parasites with which aquaculturists must be concerned since it can decimate an infected fish population (Warren 1981).

Protozoans, such as Trichophrya or Ambiphrya, may cause death indirectly by building up to such a degree on the gills that suffocation follows. Ichtyobodo, commonly referred to as Costia, causes irritation which results in respiratory difficulties, as well as loss of salts and fluids through small breaks in the epithelium.

Treatment for external protozoa may take several forms. Formalin is effectively used for treatment of most external parasites. It is used at a concentration of 167-250 ppm for one

hour in tanks, troughs, and concrete ponds, and 15-25 ppm in ponds, repeating 3-5 days later, if necessary (Moore 1984). Other treatments include potassium permanganate, copper sulfate, and malachite green oxalate. Some of these treatments may be combined to obtain better results. For example, in the treatment of Costia, copper sulfate is used followed by potassium permanganate 24 hours later (Untergasser 1989). An acetic acid dip at a ratio of 1:500 may help in the case of Ichtyobodo (Moore 1984). Sometimes a 3% salt solution dip, or 0.5-1.0% indefinite period treatment, can help the fish rid themselves of some external parasites; if the infestation is relatively light this may be all that is needed (Warren 1981).

Internal parasitic protozoa infect the intestine, the kidney, or any other organ with a good blood supply. Some infect the ovaries rendering the eggs or offspring unviable. For many of these there is no known effective therapeutant. One organism for which there is known to be an effective therapeutant is Hexamita. Hexamita may become abundant in fish fed meat diets causing irritation to the lining of the gut (Warren 1981). Problems caused by Hexamita have been reduced by the use of processed diets. It is treatable with magnesium sulfate at a rate of 3% of the ration for two to three days, repeating as nessary. Other internal protozoa such as Cryptobia, found in the bloodstream of fish are much more problematic and less treatable with current available therapeutants. A major disadvantage for the aquaculturist in treating protozoan infections is, with the exception of formalin and potassium permanganate, the therapeutants which are effective are not approved for use.

However, salt is listed on the low regulatory priority list.

Trematodes

The trematodes, or flatworms, are classified either as monogenic or digenic, depending on the number of hosts required to complete their life cycle. Monogenic trematodes utilize a single host. Species of the genus Gyrodactylidae are generally found on the body and fins but occasionally may be found on the gills. Species of the genus Dactylogyridae are commonly found on the gills (Warren 1981). Most trematodes are microscopic, however some species attain a large enough size to be seen with the naked eye. They can cause damage to the surface of the skin and gills, possibly resulting in death. Cleidodiscus is one common trematode which infects the gills of catfish and a wide variety of other warmwater species. When numerous, it causes respiratory problems by severely damaging gill tissue (Warren 1981).

Treatment for monogenic trematodes includes potassium permanganate at 2-4 ppm for one hour on three successive days (Untergasser 1989). Chloramine T, Masoten, and formalin are other chemicals which has proven useful as treatments. Of these chemicals, potassium permanganate and formalin have approval for use on food fish. A 3% saltwater bath has proven useful in many instances before administering therapeutants.

Digenic trematodes require at least two hosts, living part of its life cycle in fish and part of its life cycle in an intermediate host, such as snails or birds. They may live in the

infestations, as they will retard the growth of the fish. The larval forms however, may be much more harmful. The larval forms of these and other species migrate through the body cavity and internal organs causing damage as they migrate. Adhesions develop in the organs as a result of the migration. Severe adhesions may render the fish unable to digest or absorb nutrients adequately for optimum growth. In some cases, when the ovaries are infected, adhesions can impair the ability of the fish to produce eggs and spawn. In cases where the species Ligula intestinalis is found, the tapeworm grows to such a degree inside the body cavity that it eventually causes the belly to rupture and the fish to die. Although tapeworms will not often be a major cause of loss of fish, another concern for the fish farmer is the fact that tapeworms may be passed on to humans and animals through improperly cooked fish. As a result, infected fish are not marketable.

Treatment of tapeworms must be done through oral medication and some species are untreatable. One treatment that is effective against some cestodes is piperazine citrate mixed with feed at a rate of 600 grams per kilogram of feed, fed once morning and evening on days one and eight (Untergasser 1989). The second treatment (day eight) is important to kill the segments that may have detached and can develop into worms. Other treatments which have proven effective are: di-n-butyl tin oxide at 0.3% of the food fed at 1% of body weight for three days. Some studies have found dibutylin dilaurate to be more effective than di-n-butyl tin oxide when given at the same proportions. Felixan, fed at a rate of 60 milligrams per

kilogram of fish, along with phenothiazine, scolaban and yomesan have proven effective against tapeworms (Mitchell et al. 1980). None of the above therapeutants have been approved for food fish.

Nematodes

Nematodes are nonsegmented roundworms, which in the adult stage, occur in the intestinal tract (Warren 1981). Some species however, such as the Philonema and Philometra will infect almost any tissue of the fish host. Nematodes are often associated with underfed fish (Mitchell (undated)). A few of the nematodes, such as Anisakia and Contracaecum, cause serious damage to the liver of many marine fish. The larvae of nematodes are very common in fish. Larval anisakids, which are ingested by people eating infected fish, can invade the wall of the human digestive tract (Landau 1992).

Treatment methods include 10% Concurat at 10 grams per kilograms of feed given once daily for five days. Another treatment that has worked successfully to varying degrees is 5% flubendazol at 1 gram per kilogram of feed five times every other day. Flubendazol may also be added to the water to destroy eggs that may remain after the fish are treated (Untergasser 1989).

Leeches

Leeches are an occasional cause of concern for the aquaculturist. Leeches attach to and damage a fish by living on its blood. Damage is not limited to the loss of blood, but lesions caused by blood sucking often become an entry point for secondary infection. The leech may infect the fish with

protozoan parasites which the leech may carry internally. Treatment for leeches is often a 3% salt bath for a few minutes. Dipping the fish into a lime bath also is an effective way of treating infected fish.

Copepods

The vast majority of the copepods in fresh and salt water are an important part of the diet of fish. Anchor worms of the genus Lernaea destroy scales and cause hemorrhagic and ulcerated areas at the point of penetration. The major injury to the host results from the consumption of blood and from the presence of an open wound at the attachment site which allows the establishment of secondary infections. This damage reduces the salability of the fish.

Fish lice, of the genus Argulus, puncture the skin, inject a cytolytic toxin through the sting and feed on blood. Some species in this genus are serious pathogens often causing severe mortality of fish in ponds. Those of the genera Achtheres and Ergasilus are found on the gills and fins. The infected sites on the fish may become ulcerated and provide an access for secondary infection (Warren 1981). In general, copepods inhibit the growth and reproduction of fish being cultured when infestation occurs. Some species of copepods have no known therapeutic control. Others are controlled by a combination of 0.5 ppm copper sulfate and 0.2 ppm ferric sulfate for six to nine days. Formalin and potassium permanganate are also successful in treating some copepods. The treatment of choice however is Masoten. Two applications, of 0.25 ppm at a

one week interval, of Masoten have been found to be effective. In other cases, Masoten must be used weekly for five weeks since in some species it kills only the larval stage.

FUNGAL INFECTIONS

Fungal infections are responsible for significant losses in most fish production facilities each year (Moore 1984). Fungal infections may occur either externally or internally. Clinical signs of fungal infection include a fuzzy appearance on the surface of the fish, lethargy, skin lesions, and lack of appetite.

Some species attack externally and penetrate inside the skin to the fish organs by means of their mycelium. Normally, fungal spores do not attack the healthy skin of the fish (Meyer 1976). If however, the skin has been damaged by bites or other wounds, bacterial infection, or parasites, then the spores find a suitable location to penetrate and germinate. The external fungi, usually Saprolegnia, appears in the form of fuzzy grayish, whitish, or dirty brown patches on the body. The fungal lesions occur almost anywhere on the body of the host, when they occur on the gills the fish is usually lost.

Branchiomyces is an internal fungus which is responsible for one type of gill rot. It has been found in most species of pond fish. It has a rapid onset and can often be too late to treat once detected, resulting in large fish losses. Once a fungal infection has become severe enough that the hyphae filaments can be seen by the naked eye, it is very difficult to save these particular infected fish. However, it is an indication that

treatment is necessary for the pond, raceway, or tank. In addition, eggs may become infected and require treatment.

Treatment exists for some species, however they are usually ineffective once the mycelia have formed. Treatments, which will kill zoospores, include the use of formalin at 25-50 ppm in ponds, and copper sulfate applied at a rate of 0.75 ppm for every 100 ppm total alkalinity, used only when total alkalinity exceeds 40 ppm (Moore 1984). Copper sulfate should be used three times at three day intervals. A malachite green dip and griseofulvin have also been used with some success (Untergasser 1989).

BACTERIAL DISEASES

Bacteria are microscopic organisms that can create external or internal lesions, ulcers and sores. Many bacteria are normal inhabitants of the water and only cause infections when fish are in poor conditions (Getchell 1992).

EXTERNAL DISEASE BACTERIA

Columnaris

This disease is caused by Chondrococcus columnaris (Cytophaga columnaris is now classified as Flexibacter columnaris). It is found in salmonids and many warmwater fishes. The bacteria affect small fingerlings to the catchable fish. Although columnaris disease attacks practically all species of freshwater fish, catfish are particularly susceptible. Columnaris is usually associated with some kind of eroded conditions and handling. Under appropriate conditions columnaris may take an explosive course and cause catastrophic losses in one or two days after the first

appearance of the disease (Piper et al. 1982).

Clinical signs of columnaris include early signs of grayish, white areas on the body, head, fins or gills. Edges of these lesions may appear reddened and hemorrhaged lesions are invaded secondarily by fungus. Most workers consider this disease to be both internal and external in nature, though only external lesions are seen.

Treatment for columnaris is usually two-fold; copper sulfate flushes for one to three successive days for treatment of the external stage and Terramycin fed in a medicated feed at the rate of 36 grams of TM-50 or TM-500 per 100 pound of fish (4 grams of pure antibiotic per 100 pounds of fish) for seven to ten consecutive days for treatment internally. Sulfamerazine, although not legal for food fish, can be used at 8 grams per 100 pounds of fish. When water temperatures are high and fish are crowded, the disease will continue under such conditions because the treatment only controls the disease and does not eliminate the pathogen. Piper et al. (1982) recommend the following:

1.-Diquat at 8.4 to 16.8 part per million (2-4 ppm active action) for one hour daily for three or four days.
2.-Terramycin as a prolonged bath at 15 ppm active ingredient (0.57 grams per 10 gallons, 4.25 grams per 10 cubic feet per 24 hours).
3.-Copper sulfate at 0.5 ppm for pond treatments.
4.-Furanace. For trout and salmon as a bath at 1 ppm active ingredient (0.0038 grams per gallon; 0.0283 grs per ten cubic feet) for an indefinite period.

5.-Potassium permanganate is the most effective for pond treatment for external columnaris infection in warmwater fish at the rate of 2 ppm (5.4 pounds per acre-foot). If the color changes from pink to brown in less than 12 hours it may be necessary to repeat the treatment.

Bacterial Gill Disease

Bacterial gill disease is most common in California and causes more losses than any other disease. Rainbow trout as well salmon can be infected (Leitritz and Lewis, 1980). Mud and silt in water supplies, and dusty starter diets are important factors that contribute to out break of the disease. The bacteria attaches to young channel catfish, largemouth bass, blue gill or redear sunfish (Piper et al. 1982). The bacterium is an unidentified species related to Chondrococcus columnaris, which flourishes in water temperatures above 56°C and in crowded conditions. Etiology of the disease has not been proven conclusively because induction of the disease with flexibacter isolated from diseased fish has not been consistently achieved. Other common soil and water bacteria, such Aeromonas sp. also may cause bacterial gill disease. Sudden lack of appetite, orientation in rows against the water current, lethargy, and distribution of individuals equidistant from each other are typical signs of fish infected with bacterial gill disease. In early stages the gills may be swollen and clubbed with large amounts of mucus and in later stages the gills may be destroyed (Piper et al. 1982). Microscopic examination of affected gill

tissue reveals long, thin bacteria arranged in patches over the epithelium.

Bacterial gill disease is controlled by dipping or flushing with copper sulfate. Terramycin, 36 grams TM-50 or TM-500 per 100 pounds of fish, may be used. The most reliable and often used treatment for bacterial gill disease are Roccal, Hyamin 1622 (98.8 % active), and Hyamin 3500 (50% active), although these treatments are not registered by the FDA (Piper et al. 1982).

Peduncle disease

Peduncle disease, in fingerling rainbow trout and catchable size rainbow, can cause serious mortalities. Salmon are also susceptible. The organism responsible for this disease is Cytophaga psychrophila and is found at temperatures below 56°C as well as at higher temperatures and does not require crowded conditions to affect fish. The bacteria is water borne and can be transmitted from carrier fish through the water supply. The tissue of the caudal fin and peduncle is affected causing much tissue destruction. Advanced stages result in complete loss of the caudal fin and the posterior end of the peduncle leaving exposed muscle and bone of the vertebral column (Piper et al. 1982)

Combined treatment of copper sulfate and flushing with sulfamerazine (8 grams per 100 pounds of fish) or Terramycin (36 grams TM-50 or TM-500 per 100 pounds of fish) added to the diet will control the disease. Sometimes no treatment will avail (Leitritz 1980). The best treatment for peduncle disease is the oral administration of drugs with food. Sulfisoxazole (Gastrisin) and Sulfamethazine at 9 grams per 100 pounds of fish

per day, or Oxytetracycline (Terramycin) at 2.5 grams per 100 pounds of fish per day should be given for 10-14 days (Piper et al. 1982).

Fin Rot

Advanced cases of fin rot can resemble peduncle disease. Fin rot is caused by bacteria, crowded conditions, concrete ponds, or improper diets. In bacterial cases the early stages exhibit a white decoloration along the outer edge of the fins. The tissues including fin rays are destroyed. No specific type of bacteria is recognized as its cause. Signs may occur incidentally in the course of another bacterial disease, such as furunculosis. In typical fin rot, fins first become opaque at the margins and lesions move progressively toward the base. Common bacteria such as Aeromonas hydrophila and Pseudomonas sp. often are found in lesions of a fin rot. Most species of salmonids are susceptible to this disease.

The best treatments for fin rot infections are a soluble form of Terramycin added to water at 10 to 50 ppm for one hour. The control can also be achieved with Hyamin or Roccal at a concentration of 1 to 2 ppm for one hour. A 1:200 solution of copper sulfate for a 1-2 minute bath is effective in treating fin rot. Avoiding overcrowded conditions and providing a nutritionally balanced diet will help prevent fin rot.

INTERNAL BACTERIAL DISEASE

Furunculosis

Furunculosis disease is caused by Aeromonas salmonicida and is

limited to freshwater and anadromous fishes; it has caused severe mortalities in hatchery and wild trout (Zeigler; Leitritz and Lewis, 1980; Piper et al. 1982). Rainbow trout are resistant but can act as carriers of the disease and infect other fish. The disease is essentially a bacterial "blood poisoning". The bacteria may collect in clumps in the smaller blood vessels, then rupture the blood vessels and invade the surrounding tissues. The disease progresses and the spots of bacterial growth may fuse, destroying the tissue and enlarging into a definite swollen area; the gills may also show hemorrhaged areas. The kidney is usually badly diseased and may be converted to a semiliquid mass. In fingerlings frequently the only evident signs are dark irregular areas on the sides between the dorsal and pectoral fins.

Precautions and control of furunculosis in hatcheries would be facilitated by the development of methods that monitor the presence of A. salmonicida before the bacteria produces clinical signs of the disease (Ford 1992). Sulfamerazine (10 grams per 100 pounds of fish per day) is a good treatment; however, due to sulfa-resistent bacterial strains Terramycin is used (36 grams TM-50 or TM-500 per 100 pounds of fish per day for 10 days). Bacteria can be resistent to Terramycin also, so Furox 50 has been used successfully under experimental conditions.

Ulcer disease

Ulcer disease is caused by Hemophilus piscium (Piper et al. 1982; Zeigler Aquaculture; Leitritz and Lewis 1980) and is characterized by ulcers or sores on the surface of the fish.

These resemble furunculosis but are essentially different in that the "sores" begin on the outside and work through the skin, whereas in furunculosis they develop beneath the skin as blood filled boils and may eventually break open externally if the fish lives long enough. Positive diagnosis requires procedures usually available only in a well developed bacteriological laboratory. This disease has not been reported in Western North America.

Control is afforded by chloramphenicol or Terramycin in the food at the rate of 2.5 to 3.5 grams pure antibiotic activity per 100 pounds of fish per day until losses have dropped to an acceptable level.

Red-Mouth Disease

Red-mouth disease is caused by a specific enteric bacteria which has been isolated but not characterized by generic classification. It is also considered restricted to rainbow trout. Unfortunately, several other bacteria have been isolated from rainbow trout and other salmonids which produce similar or overlapping signs (Leitritz and Lewis 1992). One of the most frequently encountered organisms is Aeromonas liquefaciens. Signs of red-mouth include lethargy, darkness, reddened skin lining, hemorrhages on the operculum, isthmus, gills, and associated membranes as well as the base of the fins. Internally, inflammation and hemorrhages are often seen in the posterior intestine. The visceral fat, liver, and lining of the body cavity are often studded with small hemorrhages. These signs may not always be present and it may be necessary to

examine the blood microscopically and carry out appropriate laboratory procedures.

Red-mouth disease is controlled by standard Terramycin feeding of 36 grams of TM-50 (or TM-500) per 100 pounds of fish daily for 10 days. Sulfamerazine fed at 10 grams per 100 pounds of fish per day for 10 days has also been used with reasonable success.

Fish Tuberculosis

It is related to human tuberculosis bacterium (Leitnetz and Lewis 1980; Piper et al. 1982). In California the disease has been found in adult kind salmon, silver salmon and steelhead. The disease has been minimized in hatcheries by eliminating the practice of feeding with infected salmon viscera and carcasses. There are no drugs presently known that can be used to treat this disease among salmonids. In infected fish the diseases resembles melany tuberculosis in humans. The lesions are caseous or purulent tubercles scattered among the kidney, liver, spleen, and digestive tract. The bacteria in active cases may be found in virtually all tissues. The significance of this disease among the anadromous salmonid fishery is unknown.

Kidney Disease

Kidney disease has not been a problem of significance in California (Leitritz and Lewis 1980). Occasionally it is found among eastern brook trout, and juvenile silver and kind salmon have been found with the typical signs. Kidney disease has caused serious losses amoung trout in the eastern U.S. and in juvenile salmon reared in Pacific Northwest hatcheries. The

gross signs include popeye, skin hemorrhages, hemorrhages at the bases of the fins, and sometimes deep ulcers which can be seen externally and the abdomen may be greatly swollen. Internally the kidney, liver and spleen often exhibit "white boil-like" lesions similar to those seen in fish tuberculosis. The infection to wild population could be from water supply or in fertilized eggs.

Control with sulfonamides (sulfamerazine and Sulfamethazine) at approximately 10 grams per 100 pounds of fish per day per 10 days may reduce the loss rate. This level is considered therapeutic and if removed from the diet the loss will rise again. When a prophylactic level of 2 grams per 100 pounds of fish per day is fed continually, following the treatment, the losses are minimized until liberation of the fish. Erythromycin at 4.5 grams per 100 pound of fish per day for three weeks has been used successfully. Terramycin at its standard dose (36 grams TM-50 or TM-500 per 100 pounds of fish) has also been used. Under laboratory conditions erythromycin given orally at the rate of 4.5 grams per 100 pounds of fish per day for three weeks gave best control but was not completely effective (Leitritz and Lewis 1980).

Motile Aeromonas Septicemia (MAS)

Motile Aeromonas Septicemia is a disease of many freshwater fishes caused by gram negative motile bacterium belonging to the genera Aeromonas and Pseudomonas. Two species frequently isolated in out break are A. hydrophila and P. Florescence. A

definitive diagnosis can be made only if the causative agent is isolated and identified. The most common signs of MAS are superficial circular or regular grayish red ulcerations, with inflammation and erosion in and around the mouth (in enteric red mouth disease) (Piper et al.1982). Fish may have abdomen filled with slightly opaque or bloody fluid (dropsy) or exophthalmia. Minnows may have furuncules like those in furunculosis, which may erupt to the surface. The kidney may be swollen and soft and the liver may become pale or greenish. Motile aeromonas septicemia occasionally takes an acute form in warmwater fish and severe losses can occur even though fish show few, if any, clinical signs of the disease. Largemouth bass and channel catfish are susceptible particularly during spawning and during summer if stressed by handling, crowding or low oxygen concentration. Aquarium fish can develop the disease at any time of the year. The disease has been identified throughout the world and apparently infects any species of freshwater fish. Broodfish can be injected with 25 milligrams active Terramycin per pound of body weight or fed medicated feed before they are handled in the spring as a prophylactic measure. Outbreaks of MAS in channel catfish and other warmwater fish that will eat artificial food can be treated by feeding 2.5-3.5 grams active Terramycin per 100 pounds of fish daily for 7-10 days. Outbreaks in salmonids have been treated successfully by Terramycin fed at 3.6 grams TM-50 per 100 pounds of fish daily for 10 days. Sulfamerazine fed at 10 grams per pound of fish per day for 10 days also has been used with reasonable success. A combination of sulfamerazine and NF-100 has been very effective in western USA.

OTHER DISEASES

Edwardsiellosis (enteric septicemia)

Edwardsiellosis is caused by Edwardsiella ictaluri and is a major bacterial pathogen of channel catfish. A number of investigators have recognized that vaccination (both with a bath or oral) enhances survival to a bad challenge of virulent bacteria when compared to nonvaccinated control (Lingenfelser and Blazer 1992). The bacteria enters through the olfactory sac and degenerates the olfactory neuron (Morrison 1992). Buldwing and Newton (1992) found that Edwardsiella pass through the intestinal mucosa and produce histologic lesions and necrosis.

Mycobacterium marinum

Newton et al. (1992) found Mycobacteriosis in hybrid striped bass in intensive culture. The fish had low growth, poor nutrient conditions, linear pale streaks in the gill, enlarged granular spleen and skin ulcerations.

Pasteurella piscicida

Hawke et al. (1992) detected P. piscicida as causative agent of disease in population of intensive cultured hybrid striped bass in Louisiana coastal water in December 1990 and spring 1991. They report significant mortality at two commercial brackish water fisheries in cages, raceways and net pens. P. piscicida was sensitive to Romet (Sulfadimethoxine and Ormetoprim) and Terramycin (oxytetracycline) when first isolated; however strains

isolated subsequent to medicated feed treatment were resistant to both chemotherapeutants.

Botulism Syndrome

Walker and Ekland (1992), report loss of 240,000 of rainbow trout (95 %) Oncorhynchus mykiss, occurring from late summer through the fall of 1986 at Riffle Fall State Fish Hatchery Colorado. The signs were affected behavior in swimming due to descending paralysis from botulism toxins and viscuous feces due to toxin that induced constipation. They affirm that botulism syndrome can occur under the right series of circumstances including pond designs, fish culture practices or prolonged temperature and overcrowded fish.

CONSIDERATIONS

Before a culturist treats a group of diseased fish, there are several considerations the aquaculturist must contemplate beforehand.

Recognition of disease, accurate diagnosis and selecting a chemical

Culturing high densities of fish increases the chances of outbreaks of disease and parasites. The chances of successfully treating fish is dependent upon the stage at which the problem is detected. If the first sign of a disease or parasite epizootic is numerous dead animals, it is often too late to treat the remaining fish which are alive (Stickney 1979). Chances of

successfully treating diseased fish drastically increase as diseases are detected in their early stages.

There are some obvious behavioral changes as well as physical changes that indicate that there is a problem. Some behavioral changes which indicate a fish may be suffering from a disease include cessation of feeding, abnormal distribution in a tank, such as many fish swimming at the surface, flashing, or lethargy (Piper et al. 1983). Physical changes which occur may be hemorrhaging, eroding areas of the body or fins, or discoloration (Piper et al. 1983). It is important to stress that the culturist must have frequent visual contact with the animal, so that disease problems can be detected early.

If the fish farmer can identify that there is a problem and then accurately diagnose the disease, then a treatment can begin right away. However, in many cases the aquaculturist can not diagnose diseases accurately and therefore has to submit samples to a diagnostician to ensure accurate evaluation of the problem. It is important to note that if diagnosis is not accurate and "shotgun" treatments are performed, they usually are not effective and may actually do more harm than good.

Once an accurate diagnosis of the disease is obtained, the proper chemical for treatment needs to be selected. If the culturist submits specimens for diagnosis then usually a treatment of the disease is often suggested. If the culturist diagnoses the disease, a number of books are available on the subject of treatment of fish diseases or they can consult an extension agent for a recommended treatment. Presently there are only five registered drugs and seven low priority drugs from

which to choose.

Is the treatment economically feasible?

It is easy to see that diseases cause economic losses due to mortality, but diseases also cause losses due to treatment expense, reduced growth rates during and after disease outbreaks, and the postponement or loss of opportunity to sell the fish. Once the aquaculturist sees a problem, accurately diagnoses it, and selects a treatment, he must assess the value of the diseased fish. If the value of the treatment expense is greater than that of the crop of fish, the fish farmer should not treat and should accept his losses. Conversely, if the value of the treatment expense is minimal in comparison to the value of the crop of fish then a treatment is warranted.

What are the chances that the treatment will be successful?

As discussed previously, the earlier the detection of a disease or parasite problem, the more likely of a successful treatment. Also, if time allows, take a subsample of the diseased fish. Initially, treat this sample of fish to determine the effectiveness of the drug before exposing the whole crop to the therapeutant (Stickney 1979). This could save the farmer time and money if the treatment is ineffective.

What application alternatives are available?

There are several different ways to treat using chemicals. Dip treatments consist of placing small numbers of fish in a strong solution of a chemical for a short period of time. This method of treatment is somewhat dangerous because there is only a small difference between a dose which is effective and a dose which kills fish. In a bath treatment, the inflowing water is turned off and the correct amount of chemical is added directly to the holding unit. After a specified time, the inflowing water is turned on and the treated water is flushed out. In this method oxygen concentrations must by monitored because inflowing water is cut off. Also, for this type of treatment to be effective the drug must be uniformly distributed throughout the tank.

A variation of the bath treatment, the indefinite bath, is generally used in ponds. In this method, a low concentration of the chemical is added to the pond and is left to naturally dissipate. One disadvantage to this method is that if the pond to be treated is large then the amount of chemical needed to treat the pond would be expensive.

A flush treatment consists of adding a treatment solution at the upper end of a holding tank and allowing the chemical to flush through. This method has been widely used in the culture of salmonids and is only applicable in raceways, tanks, or troughs.

For certain diseases, such as systemic bacterial infections and certain internal parasite infestations, treatment requires that the drug be introduced into the fish's body. This can be accomplished two ways: medicated feed or injections. For certain diseases, the medication must be introduced into the

fishes stomach. One way to do this is to feed medicated feed, which can be commercially purchased. Another way to introduce medication to the stomach is to weigh out the correct amount of drug, put it in a gelatin capsule and then insert the capsule into the fishes stomach with a balling gun (Piper et al. 1983).

For small numbers of fish which are very valuable, such as broodstock, perhaps the best way to treat is by injection. Injections of medication can be inserted into the body cavity or directly into the muscle. Although injections work rapidly, a major disadvantage of this method is the chance of damaging internal organs.

Are there complicating factors?

Before treating a body of water with a chemical, the aquaculturist must consider how environmental factors such as temperature, pH, turbidity, alkalinity and rain can influence the effectiveness of a treatment. Also, the aquaculturist must consider what effects the treatment may have on phytoplankton, zooplankton, and bacteria. There are three fishery chemicals which interact heavily with water quality variables: formalin, potassium permanganate, and copper sulfate.

Formalin, which is registered by the FDA, is a chemical widely used for the control of fungi on fish eggs and external parasites (Boyd 1990). Water temperature is known to have a effect on the toxicity of formalin on fish. As a general rule, for warmwater fish at temperatures above 70°F, formalin becomes very toxic, and

for coldwater species, fish become very sensitive to formalin at temperatures above 50°F (Piper et al. 1983). So, if is necessary to treat with formalin above these temperatures, it would be prudent to use low concentrations and to repeat the treatment on successive days (Piper et al. 1983). Also, formalin is highly toxic to phytoplankton and extreme caution is warranted when treating ponds with moderate to heavy phytoplankton blooms. If a pond with a heavy bloom is treated, then a dieoff of algae will occur and a subsequent oxygen depletion will present a new problem to the culturist.

Potassium permanganate and copper sulfate are not currently registered by the FDA; however, discussion is worthy because very little data is needed for approval and these two chemicals are on the highest priority list for registration (Stefan 1992). Potassium permanganate has been used widely in the past to treat external protozoa parasites, monogenetic trematodes and external fungal and bacterial infections (Piper et al. 1983). It has been found that the toxicity of potassium permanganate ($KMnO_4$) is lessened in water with plankton blooms (Boyd 1990). Thus, it is necessary to treat with enough $KMnO_4$ to satisfy the $KMnO_4$ demand of the water and to provide a residual amount which will be toxic to disease organisms (Boyd 1990). $KMnO_4$ gives the water a pink color and upon breaking down the color changes to brown (Boyd 1990). It is recommended that if the color change takes place before twelve hours after the $KMnO_4$ has been applied it will be necessary to repeat the treatment (Piper et al. 1983). To resolve this problem, a method devised by Boyd (1990) is very useful. Boyd suggested treating water samples of one liter with

0,1,2,3,4,5,6,8, and 12 ppm of $KMnO_4$. The lowest concentration which still has a pink color after fifteen minutes is determined to be the $KMnO_4$ demand of the pond. Later, Tucker revealed that the result of this test multiplied by 2.5 gave a reliable treatment rate for bacterial diseases in fish (Boyd 1990).

Copper sulfate has been used in the past as an effective algicide. As mentioned with other compounds, the effectiveness of copper sulfate is dependent upon water quality variables. The most important factors regulating toxicity is the alkalinity and pH of the water. The toxicity of copper sulfate to fish decreases with increasing pH. Also, copper sulfate is more toxic in water of low alkalinity rather than water of high alkalinity (Boyd 1990). It should be noted that because of the high correlation between hardness and alkalinity, some biologists have mistakenly thought that the toxicity of copper sulfate interacted with hardness, not alkalinity (Boyd 1990). The decomposition of algae following the application of copper sulfate may cause oxygen depletions later, thus oxygen concentrations must be monitored after treatment. In addition, if heavy rains occur after a treatment, safe levels of treatment may become lethal because of a lowered total alkalinity (Boyd 1990).

CONCLUSION

Due to the potential of pathogens to devastate fish populations, there is a need for the aquaculturist to have registered therapeutants available. The limited number of

currently approved therapeutants is a result of the lengthy and costly NADA process which has deterred chemical manufacturers from pursuing sponsorship of new animal drugs. Producers can become a part of the solution to this problem by utilizing the compassionate INAD policy. The data generated by producers encourages further research on drugs of highest priority. This reduces the expense and amount of time required that sponsors must invest in registration efforts. The end result is a greater number of approved therapeutants available to the aquaculturists.

Table 1 : Therapeutic Drugs Approved for Use in Aquaculture of Food Fish

Drug	Species	Indication	Dosage regimen	Limitations/Comments
Oxytetracycline monoalkyl trimethyl ammonium (Terramycin by Pfizer, Inc.)	Pacific salmon	Mark skeletal tissue	250 mg/kg/day for 4 days	–Salmon < 30 g –In fedd as sole ration –7 day withdrawal time –Also hydrochloride form
	Salmonids	Control ulcer disease, furunculosis, bacterial hemorrhagic septicemia, and pseudomonas disease (*Hemophilus piscium, Aeromonas salmonicida, A. liquefaciens, Pseudomonas*)	2.5 to 3.75 g/100 lb/day for 10 days	–In mixed ration –Water temperature not below 48.2°F –21 day with drawal time
	Catfish	Control bacterial hemorrhagic septicemia and pseudomonas disease (*A. liquefaciens, Pseudomonas*)	2.5 to 3.75 g/100 lb/day for 10 days	–In mixed ration –Water temperature not below 62° F –21 day withdrawal time
	Lobster	Control gaffkemia (*Aerococcus viridans*)	1 g/lb medicated feed for 5 days	–In feed as sole ration –30 day withdrawal time
Sulfadimetthoxine + ormetopriim (Romet-30 by Hoffman-La Roche, Inc.)	Salmonids	Control furunculosis (*Aeromonas salmonicida*)	50 mg/kg/days for 5 days	–In feed –42 day withdrawal time
	Catfish	Control enteric septicemia (*Edwardsiella ictaluri*)	50 mg/kg/days for 5 days	–In feed –3 day withdrawal time
Tricaine methanesulfonate (MS-222, Finquel by ARgent Chemical Labs)	Fish (Iclauridae, Salmonidae, Esocidae, Percidae)	Sedation/anesthesia	15 to 330 mg/L (fish)	–Powder is added to water –Concentration depends upon desired degree of anesthesia, species, size, water temperature and softness, stage of development; preliminary tests of solution should be made with a few fish –21 day withdrawal time (fish); inboratory or hatchery use only in other poikilotherms –Water temperature over 50° F
	Other aquatic pikilotherms		1:1000 to 1:20000 (other poikilotherms)	

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Formalin (Paracide-F by Argent Chemical Labs and Formalin-F by Natchez Animal Supply)	Salmonids, catfish, largemouth bass, bluegill	Control protozoa and monogenetic trematodes (*Ichyopthirius, Chilodonella, Costia, Scyphidia, epistylis, Trichodina* spp. and *Cleidodiscus; Gyrodactylus Dactylogyrus* spp.)	Tanks and raceways Above 50° F: Uc to 170 ul/L, up to 1hr. Below 50° F: Up to 250 ul/L, up to 1 hr. Earthen ponds: 15 to 25 ul/L indefinitely	--Aqueous solution is added to water --Drug must not be subjected to temperature below 40° F --Do not apply to ponds when water is warmer than 80° F, there is a heavy phytoplankton bloom, or dissolved oxygen is less than 5 mg/L --Ponds may be retreated in 5 to 10 days if needed --Do not treat ponds containiing striped bass --Drug should be applied to eggs in constat flow water supply of incubating facilities; treatmen; may be repeated as often as needed to prevent fungal growth
	Salmonid and esocid eggs	Control fungii of the family Saprolegniaceae	1000 to 2000 ul/L fo 15 minutes	
Sulfamerazine (by American Cyanamid Co.)	Rainbow, brook, and brown trout	Control furunculosis	-10 g/100 lb/day for up to 14 days	--In feed --21 day witthdrawal time --Not currently available

Approval applies only to the specific drug which is the subjeect of a new animal drug application (NADA); active ingredients from other souces (e.g. bulk drug from a chemical company or similar compounds made by companies other than those speccified in the NADA) are not approved new animals drugs.

Approval applies only to use of the drug for the indications and manner specifiied on the label.

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Table 2: Low Regulatory Priority Aquaculture Drugs

DRUG	APPLICATION
Carbon Dioxide	Gas for anesthetic purpose in cold, cool, and warmwater fish.
Sodium Bicarbonate	142 to 642 ppm dip for 5 minutes as a means of introducing carbon dioxide into the water to anesthetize fish.
Acetic Acid	1,000 to 2,000 ppm dip for 1 to 10 minutes as a parasiticide for fish.
Sodium Sulfite	15% solution for 5 to 8 minutes on fish eggs to improve their hatchability.
Sodium Chloride	0.5% to 1% solution for an indefinite period as an osmoregulatory aid for the relief of stress and prevention of shock in fish; 3% dip for 10 to 30 minutes as a parasiticide.
Calcium Chloride	Used to increase water hardness to 150 ppm calcium carbonate equivalents for an indefinite period as an osmoregulatory aid during the holding and transportation of fish.
Providone Iodine	100 ppm dip for 10 minutes as an egg surface disinfectant after water hardening.

Adapted from Beaulieu, 1992; Center for Veterinary Medicine, U.S. Food and Drug Administration, 1992; Office of Surveillance and Compliance, Center for Veterinary Medicine, 1992; Stefan, 1992.

Table 3: Physical and Chemical Characteristics Data Requirements

- Color
- Physical state
- Odor
- Melting point (CR)
- Boiling point (CR)
- Density, bulk density, or specific gravity
- Solubility
- Vapor pressure
- Dissociation constant
- Octanol/water partition coefficient (CR)
- PH (CR)
- Stability
- Oxidizing or reducing action (CR)
- Flammability (CR)
- Explodability (CR)
- Storage stability
- Viscosity (CR)
- Miscibility (CR)
- Corrosion characteristics
- Dielectric breakdown voltage (CR)

Conditionally required studies are noted with (CR).

Table 4: Residue Chemistry Data Requirements

- Nature of the Residue
  - Plants (CR)
  - Livestock (CR)
- Residue analytical method
- Magnitude of the residue
  - Crop field trials
  - Processed food/feed (CR)
  - Meat/milk/poultry/eggs (CR)
  - Potable water
  - Fish
  - Irrigated crops (CR)
  - Food handling (CR)
- Reduction of residue
- Proposed tolerance

Conditionally required studies are noted with (CR).

Table 5: Environmental Fate Data Requirements

Degradation studies-lab

- Hydrolysis
- Photodegradation - in water, on soil, in air (CR)

Metabolism studies-lab

- Aerobic soil (CR)
- Anaerobic soil (CR)
- Anaerobic aquatic
- Aerobic aquatic

Mobility studies

- Leaching and adsorption/desorption
- Volatility - lab and field trials (CR)

Dissipation studies-field

- Soil (CR)
- Aquatic (sediment)
- Forestry (CR)
- Combination and tank mixes (CR)
- Soil, long-term (CR)

Accumulation studies

- Rotational crops - confined and field (CR)
- Irrigated crops (CR)
- In fish
- In aquatic non-target organisms

Conditionally required studies are noted with (CR).

Table 6: Toxicology Data Requirements

Acute testing

- Acute oral toxicity, rat
- Acute dermal toxicity
- Acute inhalation toxicity, rat
- Primary eye irritation, rabbit
- Primary dermal irritation
- Dermal sensitization
- Acute delayed neurotoxicity, hen

Subchronic testing

- 90-day feeding studies, rodent and nonrodent
- 21-day dermal (CR)
- 90-day dermal (CR)
- 90-day inhalation, rat (CR)
- 90-day neurotoxicity:
  - Hen (CR)
  - Mammal (CR)

Chronic testing

- Chronic feeding, rodent and nonrodent
- Oncogenicity study, rat and mouse preferred
- Teratogenicity, 2 species
- Reproduction, 2-generation

Mutagenicity testing

- Gene mutation
- Structural chromosomal aberration
- Other genotoxic effects (CR)

Special testing

- General metabolism
- Dermal penetration (CR)
- Domestic animal safety (CR)

Conditionally required studies are noted with (CR).

Table 7: Reentry Protection and Spray Drift Data Requirements

Reentry protection studies

Foliar dissipation (CR)
Soil dissipation (CR)
Dermal exposure (CR)
Inhalation exposure (CR)

Spray drift studies

Droplet size spectrum (CR)
Drift field evaluation (CR)

Conditionally required studies are noted with (CR).

Table 8: Wildlife and Aquatic Organisms Data Requirements

Avian and mammalian testing

Avian oral LD_{50}-preferably mallard or bobwhite
Avian dietary LC_{50}-preferably mallard and bobwhite
Wild mammal toxicity (CR)
Avian reproduction-preferably mallard and bobwhite (CR)
Simulated and actual field testing-mammals and birds (CR)

Aquatic organism testing

Freshwater fish LC_{50}-preferably rainbow and bluegill
Acute LC_{50} freshwater invertebrates-preferably Daphnia
Acute LC_{50} estuarine and marine organisms (CR)
Fish early life stage and aquatic invertebrate lifecycle (CR)
Fish life cycle (CR)
Aquatic organism accumulation (CR)
Simulated or actual field testing-aquatic organisms (CR)

Conditionally required studies are noted with (CR).

LD_{50} means the lethal dose of the test article that produces 50% survival of the test animals.

LC_{50} means the lethal concentration of the test article that produces 50% survival of the test animals.

Table 9: Nontarget Insect Data Requirements

Nontarget insect testing-pollinators

Honey bee acute contact LD_{50} (CR)
Honey bee-toxicity of residues on foliage (CR)
Honey bee subacute feeding study (CR)
Field testing for pollinators (CR)

Nontarget insect testing-aquatic insects

Acute toxicity to aquatic insects (CR)
Aquatic insect life cycle study (CR)
Simulated or actual field testing for aquatic insects (CR)
Nontarget insect testing-predators and parasites (CR)

Conditionally required studies are noted with (CR).

LD_{50} means the lethal dose of the test article that produces 50% survival of the test animals.

Table 10: Efficacy Data Requirements

In Vitro — demonstration of the effective concentration of NAD to inhibit or kill pathogen, including:
- -time relationships
- -dose relationships

In Vivo — demonstration of the effective concentration (or effective dose) for control of the pathogen in laboratory infected fish, including:
- -time relationships
- -dose relationships

Production conditions: demonstrate efficacy of NAD in at least three epizootics

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