Use of Chemicals and Other Biological Agents in the Management Practices Associated with Aquaculture

Introduction: Aquaculturists through out the world have used a wide array of chemicals and biological agents in an attempt to improve soil and water quality and thereby prevent or control different diseases. Some of these substances are thought to kill the disease causing agents or its potential carriers. Other substances are claimed to stimulate the immune system of culture organism and help them resist the disease. Substances that improve environmental conditions in ponds and thereby reduce stress also are thought to enhance the resistance of culture organism to disease. The agents used in this attempt may be applied to pond soil and water before culture organisms are stocked, applied to the water during the crop or added to the feed. The purpose of this review is to list the major chemicals and other substances used in aquaculture and comment on their food and environmental safety status and on the risks associated with handling them. The following groups of substances are be included: fertilizers, liming materials, oxidizing agents, antibiotics, plant extracts, coagulants, osmoregulators, algicides and herbicides, piscicides, water quality enhancers, probiotics and immunostimulants. The most common substances used in pond aquaculture are fertilizers and liming materials. Fertilizers are highly soluble and release nutrients that can cause eutrophication of pond waters. Some liming materials are caustic and can be hazardous to workers if proper precautions are not exercised. Liming materials do not cause environmental problems, and liming and inorganic fertilizer compounds do not present food safety concerns. These compounds or biological products quickly degrade or precipitate. They are not bioaccumulative and do not cause environmental perturbations in natural waters receiving aquaculture effluents. Most substances used in aquaculture to improve soil or water quality present little or no risk to food safety. The use of human wastes in aquaculture or the contamination of aquaculture systems with agricultural or industrial pollution could result in product contamination and food safety concerns.

Fertilizers: Fertilizers are applied to aquaculture ponds to increase plant nutrient concentrations, stimulate phytoplankton growth and ultimately enhance production of fish or crustaceans. The most common inorganic fertilizers are nitrogen and phosphorus compounds, but potassium, trace metals and silicate may be contained in some fertilizers. Fertilizers may be applied as individual compounds or they may be blended to provide a mixed fertilizer containing two or more compounds. Organic fertilizers or manures are animal wastes or agricultural by-products which, when applied to ponds, may serve as direct sources of food for invertebrate fish food organisms and fish or they may decompose slowly to release inorganic nutrients that stimulate phytoplankton growth. Primary chemical compounds used as fertilizers are highly water-soluble salts of nitrogen, phosphorus and potassium. They usually are applied in quantities necessary to increase concentrations of nitrate, ammonia, phosphate, potassium and silicate in pond waters. In addition to the primary nutrients, fertilizers usually contain a small percentage by weight of fillers and conditioners such as agricultural limestone, sand, rice hulls, granite dust or kaolin clay. Micronutrient elements are sometimes applied to ponds in much smaller quantities to supplement natural deficiencies. Because most micronutrients are not highly soluble, they are usually chelated. Fertilizer nutrients are absorbed by plants and enter the food web of aquaculture ponds. Seldom is more that 25% of the nutrients added to ponds in fertilizers recovered in aquatic animals at harvest. These nutrients occur as constituents of biomass such as protein, calcium, phosphorous in bones and minerals. None of the chemical fertilizers are known to be of any hazard for food safety. The fraction of the fertilizer nutrients not harvested in aquaculture products can be found in other pond organisms, dead organic mater and sediment or lost in outflowing water. Nitrate can be converted to nitrogen gas by denitrification and ammonia may be lost to the air by diffusion.

Because fertilizers increase nutrient concentrations in the water, they can cause nutrient enrichment when pond effluents are released into water bodies. If there are a few days between fertilizer application and water discharge to the surrounding environment, most fertilizer nutrients will have been absorbed by the pond organisms, absorbed by sediments or lost to the atmosphere through denitrification of ammonia volatilization. Ponds are extremely efficient in assimilating nutrients if the average hydraulic residence time is several weeks. Pond bottoms are not infinite sinks for nutrients, but by periodically draining and drying pond bottoms, their capacity to assimilate nutrients can be extended. Nitrogen fertilizers have the potential to increase ammonia concentrations in the water and excessive use can result in toxic ammonia concentrations within ponds. Ammonium fertilizers and urea are acidic in pond waters because nitrification releases hydrogen ions. Nitrate fertilizers are basic because of bicarbonate produced when denitrification occurs. Manures have much lower concentrations of nutrients than inorganic fertilizers. Because of their low nutrient content, manure application rates usually are much higher than those for chemical fertilizers. Decomposition of manures by bacteria requires oxygen and the amount of manure that can safely be added to a pond depends upon the biochemical oxygen demand of the manure. Adding manures to a pond increase the potential for low oxygen concentrations in pond water and pond effluents. Manures can sometimes be contaminated by heavy metals and present a low risk for food safety. In some parts of the world aquaculturists use human wastes as fertilizers, which increase the risk for contamination of aquatic animals with potential human pathogens. When possible, it is more desirable to use inorganic fertilizers than manures in aquaculture ponds. Manures can be more safely and efficiently disposed of as organic fertilizers and soil conditioners for terrestrial agriculture.

Liming materials: Liming materials are applied to pond waters and soils to neutralize acidity and increase total alkalinity. Increased alkalinity buffers water against drastic daily changes in pH common in eutrophic ponds. Increasing the pH of an acidic bottom sediment enhances the availability of phosphorus added in fertilizers. Some calcium and magnesium (Dolomite) from liming materials are absorbed by the pond biota to become normal constituents of plants and animals, adsorbed by the soil or dissolved in the water. The anionic component is either neutralized by hydrogen ions or it reacts with carbon dioxide to form bicarbonate that remains in the water to increase alkalinity. The pond sediments in coastal environments often are acidic and the liming of pond bottoms is done to neutralize acidity and stimulate microbial decomposition of the organic matter accumulated during the crop period. Burnt lime and hydrated lime are strong caustic materials and they should be handled cautiously. Contact with eyes can possible cause blinding and severe irritations can result from skin contact. If used excessively, these compounds increase water pH up to 10 or more and cause toxicity in aquatic plants and animals. The water pH will decrease to acceptable levels within a few days after applications of burnt or hydrated lime and ponds can be stocked. Agricultural limestone or dolomite is safer to use and considered to be the most effective liming material for ponds under normal circumstances. However, in ponds where severe disease problems were encountered in the previous crop, applications of burnt or hydrated lime to empty pond bottoms may be effective in destroying disease organisms in the soil before the next crop is stocked. None of the liming materials are known to be of any hazard for food safety.

Oxidizing agents : Oxidizing agents are used for controlling phytoplankton, killing disease organisms or oxidizing bottom soils. **Potassium permanganate** has been claimed to oxidize organic and inorganic substances and kill bacteria, thereby reducing the rate of oxygen consumption by chemical and biological processes. Some fish diseases are also treated by the application of potassium permanganate to fish in holding tanks or in ponds. In water, permanganate quickly oxidizes labile organic matter and other reduced substances and is transformed to relatively non-toxic manganese dioxide, which precipitates out. Potassium permanganate is toxic to phytoplankton and will reduce the production of dissolved oxygen by photosynthesis.

Peroxides and **chlorine** compounds are powerful oxidizing agents and they are strong irritants when highly concentrated. Calcium hypochlorite is sometimes applied to ponds to oxidize organic matter and reduce the biological oxygen demand. Treatments of pond water with large doses of chlorine may be an effective means of destroying disease organisms. Sodium, potassium and calcium nitrate are sometimes used as oxidizing agents. Nitrate compounds have a basic reaction with they are used by bacteria to support anaerobic decomposition (denitrification). Oxidants are transformed to inactive forms when they react with organic matter.

The most common chlorine compounds for disinfection are chlorine gas (Cl_2), calcium hypochlorite [$Ca(OCl)_2$] and sodium hypochlorite (NaOCl). When chlorine gas is added to water, it hydrolyzes to form hypochlorous acid (HOCl) and hydrochloric acid (HCl):

$$Cl_2 + H_2O = HOCl + H^+ + Cl^-$$

Hydrochloric acid is completely ionized and depending upon pH and temperature, hypochlorous acid ionizes to hydrogen ion (H^+) and hypochlorite ion (OCl^-):

$$HOCL = H^+ + OCl^-$$

Above pH 2, there will be essentially no Cl_2 in water. The ionization of HOCl reaches 50% at pH 7.4 and at higher pH values there will be more OCl^- than HOCl. When sodium or calcium hypochlorite is added to water they also form HOCl and OCl^- in relationship to pH and temperature. Hypochlorous acid and hypochlorite are called free chlorine residuals. The relative distribution of these two species is very important because the disinfecting efficiency of

HOCl is 40 to 80 times that of OCl^-. Chlorine readily oxidizes substances such as Fe^{2+}, Mn^{2+}, H_2S and organic matter and is reduced to nontoxic chloride ion. Once oxidation is complete, chlorine reacts with ammonia to form chloramines. After ammonia and other reduced substances have reacted with chlorine, continued addition of chlorine will result in a direct increase in free chlorine residuals (HOCl and OCl^-). The amount of chlorine that must be added to reach a desired level of residual is called the chlorine demand.

Chlorination is a commonly used method for destruction of pathogenic and other harmful organisms in drinking water. In disinfection, enough chlorine is added to meet the chlorine demand and provide free chlorine residuals. Some organic constituents of water react with chlorine residuals to form toxic compounds such as trihalomethanes, dioxins and polychlorinated biphenyls (PCBs). These compounds are suspected carcinogen and they may contaminate food. Dioxins and PCBs are thermally stable molecules that resist oxidation and hydrolysis and persist in the environment. EPA mentions that both dioxin and PCB molecules rarely cause acute toxicity in humans, but they have marked effects after repetitive or chronic exposures. The major threat associated with their presence in shrimp is the increasing concerns in international markets over contaminated food and KloSant is the better alternative to bleaching powder. The implementation of more stringent regulations for food importation will ultimately lead to the rejection of shrimp containing dioxins or PCBs. Of course, the main source of contamination for dioxins and PCBs in cultured shrimp is not thought to result from the use of chlorine in hatcheries or production ponds, but from the use of fish oil in the preparation of feed.

Chlorine dioxide –ClO_2 (KloSant) have advantages over chlorine gas and calcium hypochlorite for use in water containing ammonia and organic matter in appreciable concentrations. Chlorine dioxide is greater than chlorine in disinfecting power. It has an extremely high oxidation potential, which accounts for its potent germicidal powers. Chlorine dioxide residuals and end products are believed to degrade quicker than chlorine residuals. Formation of halogenated organic compounds has not been observed with the use of chlorine dioxide, as has been the case with chlorine and hypochlorites. There is no evidence that any of these compounds other than chlorine compounds leave harmful residues in the water or accumulate in the tissues of aquatic organisms, so no food hazard is associated with them. Although chlorine compounds have been reported to form residual compounds that are suspected carcinogens, they have a long history of safe and beneficial use in the disinfection of drinking water. Therefore, the use of chlorine compounds in aquaculture does not pose a significant food safety risk, but there is some possibility of environmental contamination by reaction products of chlorine in effluents.

Formaldehyde solution (or formalin)-Microlin is a general disinfectant used as a germicide, fungicide or preservative in various industries. Its main mode of action is to form covalent cross-links with functional groups on proteins. Formalin will cause irritation of the respiratory system and skin reactions in humans. It is suspected to be a carcinogen. In the context of aquaculture, the chemical may be applied to the entire pond volume, but more commonly, treatment is limited to puddles of water in the bottom of ponds after harvest. It is also used as a disinfectant in hatcheries. Formaldehyde is thought to be degraded by natural processes before shrimp are stocked for the next crop. No food safety hazard is thought to be associated with the use of formaldehyde solution.

The germicidal effect of **iodine** (SparkDin)is based on its concentration. It is commonly used to disinfect nauplei and larvae in hatcheries and farms. Iodine is thought to be degraded by natural processes in water and should not pose a threat to the environment.

Quaternary ammonium compounds (BKC) (BioNex) are used in hatcheries and farms to disinfect larvae, tanks and other equipment. These compounds are sometimes added to ponds at 400g/ha in attempts to kill bacteria. Quaternary ammonium compounds disrupt cell membranes and are most active against gram-negative bacteria. There is no evidence on the possibility that these compounds, their reaction products or their degradation products are bioaccmulative or pose any threat to the environment.

Antibiotics : **Furazolidone** is a common antibacterial used by humans as a prophylactic treatment, in aquaculture, it is used to disinfect larvae and as a prophylactic agent during transport. Other antibiotics are also used for the treatment of fish and shrimp diseases in hatcheries and grow-out ponds or applied prophylactically to prevent outbreaks of disease. The use of antibiotics in shrimp culture raises several issues of concern to human health, product quality and the environment. Several studies on salmon farms have shown that antibiotic residues can be extremely persistent in marine sediments and may lead to the development of bacterial antibiotic resistance. Use of chloramphenical has caused increased bacterial resistance in shrimp hatcheries. The ecological implications of such antibiotic misuse are not known, but at the very least there is a direct threat to shrimp farming created by the emergence of antibiotic resistant pathogens. There is also concern that transfer of such resistance to human pathogens could have serious human health implications. Antibiotics can leave residues in shrimp flesh that may lead to rejection of products in export markets. Residues of the antibiotic oxytetracycline were detected in farm-reared shrimp from Thailand, India and caused rejection of shipment to Japan.

Plant extracts: The plant extracts like KILOL, garlic, extract of passion fruit and neem are used in aquaculture as disinfectants. KILOL is made from extracts of grapefruit seed and contains a mixture of ascorbic acid and large amounts of amino trace elements. It is applied to aquaculture ponds, either directly or mixed with lime and is claimed to be a general water quality enhancer and bactericide. It is also added sometimes to fish or shrimp feed. Both major compounds of KILOL are considered generally safe if added to human food. Garlic and extract of passion fruits are also applied to ponds as potential bactericides. These compounds should not cause environmental harm or pose worker safety or food contamination concerns. Natural insecticides derived from products of the neem tree (*Azadirachta indica*) are also used occasionally. They are active against nematodes, fungi and ostracods.

Coagulants : Coagulants are applied to pond waters to flocculate suspended clay particles and cause them to precipitate in order to clear the water of turbidity. Calcium sulfate (gypsum) dissolves in water to increase calcium and sulfate concentrations. Calcium and sulfate may be absorbed in small amount by plants and animals to become normal biological constituents. Aluminum and ferric iron ions from aluminum sulfate and ferric chloride applications quickly precipitate as aluminum and iron oxides. Never-the-less, these two compounds have a strongly acidic reaction in water because of the hydrolysis of iron and aluminum. Because of their high potential to create acidity, they should be stored indoors and risks of spills minimized. Aluminum sulfate and ferric chloride both should be handled with care because skin irritation can result from contact. None of these coagulants are bioaccumulative and should not be of any hazard to food safety.

Osmoregulators : These substances are applied to water to increase the salinity or the calcium concentration and improve conditions for osmoregulation by certain culture species. They are simple salts that dissolve in the water and have little influence on the composition of aquatic animal products. The two most common osmoregulators are sodium chloride and calcium sulfate.

Algicides and herbicides : Algicides and herbicides are applied to ponds to reduce the abundance of nuisance aquatic plants. Excessive phytoplankton may result in chronically low dissolved oxygen concentrations during the night and blue-green algae are responsible for off-flavor in fish and crustaceans. Larger aquatic plants create dense communities that interfere with feeding and harvest. A number of herbicides are used to control weeds in aquatic ecosystems, but only a few of them have been cleared for use in aquaculture ponds. Because of their expense and lack of effectiveness relative to mechanical and biological control techniques, their use is seldom justified in aquaculture. A variety of algicides has been used in ponds, but four, copper sulfate, chelated copper compounds, simazine and potassium ricinoleate, have been employed most extensively. Copper inhibits both respiration and photosynthesis in algae. High doses of copper sulfate may be acutely toxic to fish but copper compounds quickly precipitate from water as copper oxide and toxicity can be avoided if the dose does not exceed one hundredth of the total alkalinity concentration of the water to be treated. Organisms may absorb some copper, but concentrations in tissues are no greater than those normally found in native plants and fish. Decapod crustaceans also have the ability to regulate essential trace elements, such as copper and zinc, at least to some degree. No food hazard is associated with copper sulfate use. Copper is often chelated with citric acid or triethanol amine to increase its solubility and residual time in water. These organic compounds are degraded fairly by bacteria in ponds and they do not accumulate in plants and animals.

Simazine (2-chloro 4,6 bis ethylamino-S-triazine) is a powerful photosynthesis inhibitor and is extremely toxic to phytoplankton but non-toxic to fish at the treatment rates used for algal control. Bacteria degrade Simazine, its half-life is about 2 weeks and it is not known to be bioaccumulative. Potassium ricinoleate is a saponified fatty acid. It is degraded by bacteria and is not bioaccumulative. Some times dyes are used in aquaculture to shade the pond bottom and limit light for underwater weeds. These are food dyes that have a half-life of a month or more. They are not bioaccumulative or directly toxic to plants and animals. In summary, copper sulfate, chelated copper compounds and simazine are very effective algicides. They may cause problems in ponds with low dissolved oxygen concentrations immediately after treatment, but they have not been reported to cause ecological problems in receiving waters. None of these algicides are known to be bioaccumulative and should not be of any hazard to food safety.

Piscicides : The most common piscicides are teaseed cake, saponin (SapoNex-Aqua), derris root powder (rotenone), mahua oilcake, lime, potassium permanganate and ammonium fertilizer. The teaseed cake contains 5.2–7.2% saponin, a glucoside that causes hemolysis in organisms. The higher sensitivity of finfish than crustaceans to the glucoside has made it an effective piscicide in shrimp ponds. The concentrations of these compounds used for eradicating fish vary widely. The entire pond volume is sometimes treated, but usually treatment is limited to puddles of water that remain in the bottom of ponds after harvest. These compounds are degraded through natural processes before the fish and shrimp are stocked for the next crop. No food safety hazards are thought to be associated with piscicides.

Water quality enhancers : The group of chemicals called water quality enhancers comprises products used in attempts remove ammonia and improve water quality in ponds. The most commonly used are zeolite, KILOL and probiotics. Zeolite is an aluminosilicate clay of high cation exchange capacity and is applied to aquaculture ponds. Farmers apply zeolite with the aim to reduce ammonia concentration through ion exchange, providing physical cover over sediments to prevent leaching of metabolites into the water column, removing suspended solids and improving water colour and algal blooms. These functions are believed to be achieved by either flocculation of suspended solids, ionic exchange and absorption of ammonium ions by zeolite or the prevention of toxic metabolites leaching from pond sediments by covering the sediments with a layer of zeolite. Zeolite will settle to the pond bottom and it does not cause food safety problems or environmental threats.

Probiotics : The common probiotics used in pond management are live bacterial inocula (non-pathogenic organisms) and fermentation products rich in extracellular enzymes. Claims about the potential benefits of probiotics in aquaculture ponds include: enhanced decomposition of organic matter; reduction in nitrogen and phosphorus concentrations; better algal growth; greater availability of dissolved oxygen; reduction in blue-green algae; control of ammonia, nitrite and hydrogen sulfide; lower incidence of disease and greater survival and better fish and shrimp production (BioRemid, TerraGard, Spark-PS – Neospark). The addition of probiotics to aquaculture ponds should not result in any damage to the fish and shrimp crop or to the environment. No food safety hazards are thought to result from probiotics.

Immunostimulants: The immuno-stimulants are used to boost the fish or crustacean immune system. It includes extracts of Chanca piedra (*Phyllantus niruri*), *Yucca schidigera* (Sarsaponin), vitamin mixes, products containing glucan, probiotics, extracts of other natural products and homeopathic products and these products are mixed with the feed. In general, immunostimulants should not cause any hazard to the environment or food safety problems.

Summary: Most chemicals used in aquaculture management pose little or no food safety risk. However, some farmers my apply insecticides and antibiotics to ponds and these substances can be bioaccumulative and present a food safety hazard. Accidental contamination of aquaculture products by pesticides and heavy metals does not appear to be a major risk. Although aquaculture chemicals are generally not a food hazard, some of the compounds pose risks to workers who apply them to ponds. Enrichment of water in aquaculture ponds is desirable, but the direct discharge of such waters may not always be environmentally sound. Management practices that will reduce potential problems from aquaculture effluents should be promoted and implemented on a larger scale. The adoption of best management practices by aquaculturists is a practical way to approach environmental management of aquaculture. Most substances used to improve water quality or to stimulate the immune system of fish or crustacean present little or no risk to the environment or food safety. Aquaculture farmers who use these substances should follow product labels regarding dosage, withdrawal period, proper use, storage, disposal and other constraints including environmental and human safety precautions. Also, careful records should be maintained regarding use of chemicals in ponds, as suggested by the Hazard Analysis and Critical Control Point (HACCP) method. A greater effort must be made to prepare lists of acceptable chemicals and recommendations for the use of these chemicals. Some chemicals are necessary in aquaculture and a system for using them in a safe and publicly acceptable manner must be implemented by the aquaculture industry worldwide.

Conclusion : In future, as natural fish stocks are getting depleted, it is likely that we will have to rely increasingly on aquaculture for the production of fish and crustaceans for human consumption. Therefore, it is important that the sustainability of this industry is maintained and it is essential that this be done with the minimum of impact on the aquatic environment. Improved aquaculture practices coupled with the more effective assessment and need-based use of scientifically approved chemotherapeutants and other biological agents should at least go part way in meeting these objectives.