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EFFECTS OF PESTICIDES AND ANTIBIOTICS ON PENAEID SHRIMP WITH SPECIAL EMPHASES ON BEHAVIORAL AND BIOMARKER RESPONSES

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Abstract—The purpose of the present study is to provide information on the current state of knowledge regarding the effects of pesticides and antibiotics used in aquaculture on penaeid shrimp, one of the most common aquatic products for human consumption, with a special emphasis on the use of behavioral, physiological, and biochemical response. These include behavior; feeding rate changes; respiration rate, oxygen consumption, and osmoregulation alterations; nucleic acids, protein, and glycogen synthesis; cholinesterase activity inhibition; ATPase activity; and oxidative stress responses. This paper also deals with residues of antibiotics and pesticides in penaeid shrimp. Antibiotics and pesticides used in aquaculture may have adverse effects on treated animals and human consumers health if they are not correctly used. As a complement to the measurement of antibiotic and pesticide residues in tissues, the use of behavioral and biomarker responses can provide more relevant biological information on the potential adverse effects of antibiotics and pesticides on penaeid shrimp health. Environ. Toxicol. Chem. 2010;29:929–938. © 2009 SETAC

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INTRODUCTION

Penaeid shrimp aquaculture is a commercially important industry worldwide. With the collection of wild seed in the 1970s and the supply of hatchery-produced postlarvae in the 1980s, production of farm-raised shrimp grew exponentially, and marine shrimp farming became one of the most outstanding success stories in the modern history of aquaculture. Shrimp culture has been adopted and consolidated as one of the largest profitable aquaculture activities all over the world. In shrimp farming systems, significant levels of drug and chemical products are used, including antibiotics and other therapeutic agents, pesticides, disinfectants, water and soil treatment, and feed additives. The use of drugs and chemicals is a major problem in shrimp aquaculture, with a significant potential to have a negative impact on the environment and human health. Contaminants resulting from the direct or indirect introduction of drugs and chemicals can cause changes at all levels of biological organization [1]. Research and practice in shrimp aquaculture are focusing on development and validation of technologies and methodologies allowing straightforward traceability of individual farm animals to ensure food safety for human consumers. In aquatic toxicology, the mechanistic evaluation of contaminants is a much younger area of investigation than in mammalian models, and only recently has it received significant attention. Traditionally, analyses of the contaminant concentrations in organisms have been carried out in order to monitor the presence of and exposure to contaminants. Today, biomarkers are recognized as useful tools for assessing the contaminant effects on aquatic organisms. Biomarkers are defined as quantitative measures of changes in the biological system that respond to exposure to or doses of substances that lead to biological effects and are potential tools for detecting exposure to or effects of contaminants and giving responses at different levels of biological organization: biochemical, physiological, organism, and population [2]. The aim of the present study is to summarize current knowledge on the effects of pesticides and antibiotics in penaeid shrimp and the potential use of biomarkers for assessing the contaminant exposure and effects on shrimp.

PESTICIDES AND ANTIBIOTICS USED IN PENAEID SHRIMP AQUACULTURE

Pesticides

The term ''pesticide'' applies to insecticides that target harmful or destructive insects, herbicides that target weeds, fungicides that target fungi, and various other substances such as rodenticides that target rodents and acaricides that target acarids. Pesticides are widely used to prevent and control the populations of organisms considered as pests in specific contexts. Some of them are also used in aquaculture, and it is likely that a large volume of pesticide residues accumulates in the environment. The safest pesticides should not affect nontarget species (usually in the soil zone) and do not persist in the environment [3,4]. In aquaculture, pesticides are used mainly to prevent and/or to control ectoparasites, endoparasites, and other organisms that may decrease production rates. This is particularly true in many Asian countries, which together produce over 90% of the nonfish aquaculture products that are distributed worldwide. Among pesticides, insecticides are of special concern, because their mechanisms of action are in general similar in pests and in species of economic interest. If not

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All Supplemental Data may be found in the online version of this article.

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correctly used, they can be toxic to shrimp at all their life stages. In addition, the presence of residues of the insecticide and/or of their metabolites in shrimp may have adverse effects on consumers' health. Biochemical responses in penaeids to contaminants were reviewed by Bainy [5] and Lignot et al. [6].

There are four major groups of insecticides in aquaculture: organochlorines (OCs), organophosphates (OPs), carbamates (CBs), and pyrethroids (PYR). Organochlorines can persist in the environment for several years and subsequently accumulate in aquatic organisms. They have been widely used for several decades, including in agriculture and in aquaculture, and, since the 1940s, they have been measured routinely in environmental monitoring programs [7]. Organochlorines act primarily by altering the movement of ions across the nerve cell membranes, thus changing the ability of the nerve to fire. At present, two other kinds of pesticides, OPs and CBs, have been largely used to replace OCs in several situations. These two classes of insecticides have the same mechanism of action: they inhibit the enzyme acetylcholinesterase, which breaks down the neurotransmitter acetylcholine [8]. The long-term ecological hazards associated with the use of OCs, OPs, and CBs propelled the introduction of a new generation of pesticides with a lesser degree of persistence. In this direction, synthetic pyrethroids have emerged as viable substitutes [9]. Pyrethroids alter normal neuronal function by inhibiting ion movements across the nerve cell membrane, by impairing intracellular calcium ion concentrations, and possibly by binding to γ-aminobutyric acid (GABA) receptors [10]. Some pesticides are very acutely toxic to penaeid shrimp, whereas others have low acute toxicity but may cause long-term health effects after chronic exposure.

Antibiotics

The term ''antibiotic'' today covers a wide spectrum of substances, including synthetic compounds (e.g., sulphonamides and quinolones) and natural compounds (e.g., penicillins, tetracyclines, and macrolides), which have the capacity to kill or to inhibit the growth of micro-organisms. In penaeid shrimp aquaculture, antibiotics are used during both production and processing, mainly to prevent (prophylactic use) and treat (therapeutic use) bacterial diseases.

Aquatic animals are given antibiotics as a component of their food and occasionally in baths and through injection. Antibiotics can leach from the nonconsumed food and diffuse into the sediment, or they can be washed by currents to distant sites [11]. Another problem created by the excessive use of antibiotics in aquaculture is the presence of residual antibiotics in commercialized aquatic products, which may cause direct human health concerns [12,13]. In addition, the unintentional consumption of antibiotics is leading to the development of antibiotic resistance, which is considered to be one of the most serious risks to human health. Recognition of the risks associated with the direct and indirect effects on human health of both active and passive consumption of antibiotics has led to bans on the use of certain antibiotics in animal food production. The European Union (EU) has produced legislation establishing safe maximum residue limits (MRL) for residues of antibiotics in animal tissues [14]. The monitoring is necessary to ensure that antibiotics are not present at levels that may pose health risks to the public [15].

EFFECTS OF PESTICIDES AND ANTIBIOTICS IN PENAEID SHRIMP

Effects of pesticides on behavior and feeding rate

A variety of animal behaviors can be observed during pollutant exposure, including avoidance of the pollution gradient and changes in feeding and reproduction activity and predator avoidance [16]. Behavioral assays provide biologically relevant endpoints to evaluate sublethal exposure effects and may compliment traditional toxicity testing. Today, the relationship between many exposure-related behavioral alterations observed in the laboratory and their relevance to ecological effects observed in the field remains poorly understood. Several studies in penaeid shrimp have indicated that penaeid shrimp exposed to sublethal concentrations of pesticides show several behavioral alterations, such as restlessness and hyperexcitability, tremor in the appendages, uncoordinated swimming movements, spasms, and violent action of chelate legs [17–20]. These changes, in general, were more apparent with increasing concentrations of pesticides and were assumed to be a direct consequence of the action of the pesticides on the nervous system.

Feeding depression has been found as a sensitive endpoint for measuring the response of the organism to chemical exposure [21]. Food is the basic requirement for growth and other physiological functions of the body [22]. Feeding rate has been proposed in several studies as a sensitive indicator of toxic stress in both freshwater and marine species [23–25]. García de la Parra et al. [20] observed that, in whiteleg shrimp (*Litopenaeus vannamei*) exposed to sublethal concentrations of 0.66, 0.83, 1.01, 1.18, and 1.35 mg/L methamidophos for 24 h under the experimental conditions of 25°C, 32 ppt, 12:12 h light:dark photoperiod, the feeding rate did not show significant differences among treatments. However, Comoglio et al. [26] observed that oral exposure to methyl parathion 0.62 and 1.31 μg/g of food for 7 d under the experimental conditions of 24°C, 34 ppt, 12:12 h light:dark photoperiod increased the feeding rate in the same species because of the need for increased energy to meet the demand for higher maintenance costs. Thus, effects of pesticides on shrimp feeding rate may depend on the species, the pesticide, and the assay conditions, including temperature, exposure route, and exposure time, among other factors.

Effects of pesticides on oxygen consumption and osmoregulation

It has been demonstrated that osmoregulation capacity (OCap) decreased in kuruma prawns *(Penaeus japonicus)* exposed to 0.50, 0.75, and 1.00 μg/L of fenitrothion for 96 h and in blue shrimp (*Litopenaeus stylirostris*) exposed to 6, 8, 10, and 12 μg/L fenitrothion for 24 h [27,28]. In addition, Galindo-Reyes et al. [29] worked with the OCap of whiteleg shrimp (*Litopenaeus vannamei*) exposed to different OPs and OCs at different salinities. Results indicated that, at the highest salinity (50 ppt), DDT and lindane as well as chlorpyrifos reduced the OCap of the whiteleg shrimp, whereas diazinon, methyl parathion, and azinphosmethyl increased OCap compared with controls. At the lowest salinity (10 ppt), all the assayed pesticides, except for DDT, reduced the OCap of the shrimp, whereas, at 30 ppt, there was no clear difference between the effects produced by OCs versus OPs.

Respiration rate decreased in whiteleg shrimp exposed to 12 mg/L diazinon, 7 mg/L methyl parathion, and 10 mg/L azinphosmethyl [30]. Moreover, decrease in oxygen consumption and increase in respiratory rate were found in whiteleg shrimp exposed to 0.0006 mg/L lindane, 0.0105 mg/L chlordane, and 0.0025 mg/L DDT for 48 h or exposed to 0.19 μg/L lindane, 0.27 μg/L chlordane, and 0.13 μg/L DDT for 48 h [31,32]. However, the authors also indicated an increased respiration rate in shrimp treated with chlorpyrifos at a concentration of 0.0044 mg/L, whereas it decreased at a concentration of 0.12 μg/L. Changes in oxygen consumption and respiratory rate caused by the pesticides indicate alterations that may affect animal growth.

Effects of pesticides on nucleic acids and glycogen synthesis

The use of nucleic acids (RNA:DNA) has been suggested to estimate instantaneous growth and assess the nutritional status of aquatic organisms. This relationship is based on the assumption that RNA content per cell varies with growth because RNA serves as both a template and an organizer for protein synthesis. Furthermore, DNA content per cell is assumed to be constant in normal somatic cells within a given species, and this amount is not altered by starvation or other stresses. A decrease in cellular RNA results in a decrease in the potential maximum rate of protein synthesis and may consequently limit growth [33]. Galindo-Reyes et al. [31] indicated that nucleic acid content decreased in the whiteleg shrimp (*Litopenaeus vannamei*) exposed to 0.19 μg/L lindane, 0.12 μg/L chlorpyrifos, 0.27 μg/L chlordane, and 0.13 μg/L DDT for 7 d. Although RNA analysis is useful for the estimation of growth rate in a wide variety of organisms, one limitation of its use for estimation of penaeid shrimp growth is that penaeid may undergo several molts [34]. Thus, more studies are needed to determine the correlation between changes in RNA concentration and changes in growth in penaeid shrimp and other invertebrates exposed to pesticides.

Generally, in invertebrates, depletion of glycogen reserves has been attributed to increasing energy demand associated with toxic stress caused by pollutants [35]. Reddy and Rao [17,18] reported that the hepatic glycogen content decreased when penaeid prawn (*Metapenaeus monoceros*) were exposed to 10, 20, 30, and 40 μg/L methyl parathion for 5 d, and hepatic glycogen was more rapidly utilized in shrimp exposed to sublethal concentrations of methyl parathion than that in the muscle. Later, Galindo-Reyes et al. [31] reported that whiteleg shrimp exposed to 0.19 μg/L lindane, 0.12 μg/L chlorpyrifos, 0.27 μg/L chlordane, and 0.13 μg/L DDT had decreased glycogen synthesis. These authors explained that the decrease in the glycogen content might be due to decreased glycogen synthetase activity and increased glycogen utilization.

Galindo-Reyes et al. [32] indicated that, in whiteleg shrimp, the protein content decreased to 29, 37, 38, and 42% with exposure to 10.5 μg/L chlordane, 4.4 μg/L chlorpyrifos, 0.6 μg/L lindane, and 2.5 μg/L DDT for 48 h. Later, Galindo-Reyes et al. [36] showed that protein content decreased in blue shrimp exposed to DDT, azinphosmethyl, permethrine, methyl parathion, chlorpyrifos, malathion, endosulfan, and carbaryl. The weakest protein reduction was found in shrimp exposed to carbaryl, whereas permethrin resulted in the highest protein reduction. The decrease in percentage of protein in muscle of up to 42% in shrimp exposed to DDT signifies a marked reduction in nutritive and commercial quality of product [32].

Effects of pesticides on acetylcholinesterase activity

The inhibition of acetylcholinesterase (AChE) activity has been used as a biomarker to detect OP and CB exposure, because the toxicity of these pesticides is due to the inhibition of AChE [8,37]. The activity of AChE and other cholinesterases (ChEs) in tissues may be inhibited for days to weeks [38,39], even if the animals no longer contain detectable traces of pesticides.

The inhibition of AChE caused by pesticides has been studied in the ventral nerve cord in pink shrimp (*Penaeus duorarum*) exposed to 1,000 ppb of malathion for 48 h [40], in the nervous tissue of penaeid prawn (*Metapenaeus monoceros*) exposed to 0.4, 1.2 ppm of phosphamidon for 48 h, to 0.04, 0.12 ppm of methyl parathion for 48 h [41], or to 40 μg/L of methyl parathion for 5 d [17], in whiteleg shrimp (*Litopenaeus vannamei*) exposed to fenitrothion, azinphosmethyl, methyl parathion, chlorpyrifos, diazinon, and methamidophos [19,20,26,28]. As mentioned above, the mechanism of the toxic action of OPs and CBs is based on the inhibition of the enzyme AChE. However, Galindo Reyes et al. [29] indicated that AChE in penaeid shrimp is also sensitive to OCs.

Lignot et al. [28] observed no significant changes in the AChE activity in whiteleg shrimp treated with sublethal concentrations of fenitrothion. On the other hand, AChE activity was decreased in blue shrimp (*Litopenaeus stylirostris*) treated with this insecticide.

In most experimental aquatic toxicology studies, water is the main exposure route. However, it is important to take into account that many organisms are benthic and take food directly from the sediments. The toxicity of compounds being ingested may be dominated by oral exposure. Comoglio et al. [26] conducted an experiment to determine the sublethal effects of methyl parathion via food (0.62 and 1.31 μg/g food for 7 d) on AChE in whiteleg shrimp. Results showed that the AChE activity was significantly lowered after the pesticide treatment. Roque et al. [19] also showed that the AChE activity in this species decreased in relation to the concentration of methyl parathion in food. Thus, results from these authors showed that food-borne exposure to pesticides could be used to measure its toxic effects in penaeid shrimp.

García de la Parra et al. [20] found that AChE inhibition in whiteleg shrimp did not completely depend on the concentration of methamidophos tested, insofar as a slight increase in activity was observed at the highest tested concentration. The maximum level of inhibition was 48.7% in the group exposed to 1.17 mg/L methamidophos, whereas a slight increase in activity was observed at the highest tested concentration (1.35 mg/L). These authors also indicated that inhibition levels equal to or greater than 50% are generally considered to induce irreversible effects, whereas inhibitions below this value are considered reversible, assuming that organisms are able to recover their normal functions after the toxic insult. However, this approach, although practical, has some obvious limitations, in that it does not consider the nature of the chemical, the sensitivity of the species, or the linkages with other vital functions of the organisms. Behavioral, biochemical/molecular, and physiological alterations represent responses at different levels of organization, some of which are determinants of the survival of the organism and of population fitness. Thus, AChE should be used in association with other biochemical, physiological, and molecular responses to provide valuable supplementary information (Supplemental Data, Tables S1, S2, S3).

Effects of pesticides on the activity of ATPases

Sodium-potassium adenosine triphosphatase (Na^+,K^+-ATPase) is a membrane-bound enzyme found in animal cells. Its most important feature is the coupling of the free energy stored within the adenosine triphosphate (ATP) molecule to the translocation of Na^+ ions [42]. Satyavathi and Rao [43] have reported four different ATPase activities in the plasma membrane and mitochondrial fractions of Indian prawn (*Penaeus indicus*), Mg^{2+}-dependent ATPase, Na^+,K^+-stimulated ATPase, Na^+-stimulated ATPase, and K^+-stimulated ATPase, although the stimulation caused by Na^+ or K^+ could be due to the effect of each cation acting on a single ATPase. Often, ATPase activity is used as a sensitive indicator of heavy metal toxicity, although there is evidence that organic pollutants can inhibit ATPase activity in concentration-based experiments [42]. However, Na^+,K^+-ATPase activity increased in animals exposed to various concentrations of DDT [44]. Results from Comoglio et al. [26] for whiteleg shrimp (*Litopenaeus vannamei*) indicated that Na^+,K^+-ATPase activity in control and in 1.31 μg/g methyl parathion were significantly higher than in 0.62 μg/g methyl parathion. However, the total ATPase activity and the Mg^{2+}-ATPase activity were significantly higher in pesticide treatments. Giesy et al. [45] proposed a nonspecific multitest assay to study toxic stress produced by pollutants. These procedures could include ATPase activity modification as a complement of other biochemical, histological, and metabolical tests.

Antibiotic residues in penaeid shrimp

A number of reports, press releases, and ongoing investigations have raised questions about the safety of antibiotic usage in aquaculture [46–51]. Establishing the exact level of antibiotics use and potential dangers is difficult because of lack of data.

Chloramphenicol (CAP), a broad-spectrum antibiotic, is a very effective veterinary drug and is used to treat animal pathogens that have become resistant to other commonly used antibiotics. Chloramphenicol can cause potentially fatal aplastic anemia and leukemia. Governments around the world have established zero-tolerance policies, which means that no residues in food, food-producing animals, or feed products are permissible. However, traces of CAP have been detected in shrimp and other aquatic products. Wang et al. [47] published one of the few studies on the residue elimination of CAP in Chinese shrimp (*Penaeus chinensis*) fed medicated feed containing 2 g CAP/kg for 3 d. Results showed that the elimination half-life of CAP was 10.04 h. The peak concentrations of CAP arrived at 1.5 h posttreatment. These authors also indicated that the predicted withdrawal periods for CAP in Chinese shrimp was after 139.7 h.

Nitrofurans are synthetic broad-spectrum antibiotics that are frequently used in animal production for their excellent antibacterial properties. In long-term studies with experimental animals, nitrofurans and their metabolites showed carcinogenic and mutagenic characteristics. The nitrofuran drugs, furaltadone, nitrofurantoin, and nitrofurazone, were banned from use in food animal production in the EU in 1993, and the use of furazolidone was prohibited in 1995. The furazolidone metabolite 3-amino-2-oxazolidinone (AOZ) may persist for considerable periods in the tissue of animals after treatment [48]. Results from Tu et al. [49] showed that the maximum concentrations of AOZ were 874 μg/kg in black tiger shrimp (*Penaeus monodon)* treated with 4 g furazolidone/kg food for 7 d. After 28 d posttreatment, the concentration of AOZ dropped to 115 μg/kg, which showed that AOZ was quite difficult to eliminate from the shrimp muscle. Thus, very long withdrawal periods are required to deplete the AOZ residue levels to zero. Therefore, it is very important to use AOZ as a target to analyze for identifying the use of furazolidone in penaeid shrimp in order to reduce the risks of adverse effects on human consumers.

The quinolones are a group of synthetic antibiotics used in the treatment of various bacterial infections, particularly in aquaculture. Enrofloxacin and ciprofloxacin (quinolones) have been used extensively to treat bacterial infections in fish [50]. However, both enrofloxacin and ciprofloxacin residues may be able to be transferred through the food chain to humans. Because the excess amount can harm people, the EU prescribed a maximum residue limits (MRL) of 100 μg/kg for both enrofloxacin and ciprofloxacin in the edible tissues of aquatic animals. The results from Xu et al. [51] showed that, when Chinese shrimp (*Penaeus chinensis*) were treated with 50 mg enrofloxacin/kg food for 7 d, the maximal concentrations of enrofloxacin and ciprofloxacin in muscle were 1.68 μg/g and 0.07 μg/g, respectively. The predicted withdrawal time for Chinese shrimp was 12 d. In black tiger shrimp (*Penaeus monodon*) treated with 4 g enrofloxacin/kg food for 7 d under laboratory experiment, the maximum concentration of enrofloxacin was 441 μg/kg. This level decreased rapidly to 17 μg/kg at 7 d postmedication. Ciprofloxacin was not detected in any samples at any sampling time [49]. In contrast to the laboratory experiment, Tu et al. [49] conducted an experiment in black tiger shrimp at the same enrofloxacin dosing regime in a field study. Results showed that the residues of enrofloxacin in shrimp muscle could be detected only in two (75 and 131 μg/kg) of three samples from intensive-use ponds at 7 d medication. Residues could not be detected at harvest. Tu et al. [49] also showed that enrofloxacin residues were not detected in any samples from improved extensive ponds. In the present study, ciprofloxacin was not detected in any samples at any sampling time. This result indicates that the use of such a single dose of enrofloxacin in shrimp farms stopped one month before harvest ensures that the European Union MRL of 100 μg/kg is far from being exceeded. Even if enrofloxacin can be rapidly eliminated from shrimp muscle, farmers should be made aware of the issues surrounding the use of antibiotics such as disease resistance of shrimp pathogens and possibly of humans as well as an important impact on the environment in regions where shrimp farms are located.

Oxolinic acid (OA) is another quinolone antibiotic commonly used in aquaculture prophylactically or as a disinfectant to prevent diseases. The pharmacokinetic profile of OA has previously been extensively studied in finfish species, but there are few studies on the pharmacokinetics and residues of OA in penaeid shrimp [52,53]. Results from these studies indicated that the distribution half-life of OA in black tiger shrimp (*Penaeus monodon*) was 0.84 h, whereas it was 0.59 h in kuruma shrimp (*Penaeus japonicus*). Elimination half-life is an important parameter used to characterize drug disposition. The elimination half-life of OA from black tiger shrimp hemolymph was 17.7 h, which was almost half that found for kuruma shrimp (33.2 h). Uno et al. [53] showed that a maximal hemolymph concentration of OA of 4.20 μg/ml was observed at 4 h after oral administration of 50 mg/kg body weight in back tiger shrimp, whereas it was 17.8 μg/ml at 7 h at the same dosing regime in kuruma shrimp [52]. These data indicate that the absorption rate in black tiger shrimp is more rapid than that in kuruma shrimp, whereas the extent of absorption in the former is lower than that in the latter. Results from Samuelsen [54] estimated that more than half of the OA applied may be left free in the surrounding environment. The bioavailability of OA was reported to be only 32.9% in kuruma shrimp [52] and as low as 7.9% in black tiger shrimp [53].

Oxytetracycline (OTC) is a commonly used antibiotic to fight bacterial infections in fish farming, and it has the potential to be used in farm-raised shrimp for the treatment of vibriosis and necrotizing hepatopancreatitis infections. Oxytetracycline is widely used because of its low toxicity and broad-spectrum antibiotic activity against a wide range of gram-positive and gram-negative bacteria [55]. The majority of bacteria that affect shrimp include numerous species of *Vibrio* such as *Vibrio harveyi*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio anguillarum*, and *Vibrio alginolyticus*. Oxytetracycline has commonly been used in shrimp aquaculture to prevent and treat those bacterial diseases [47]. Legislation in some countries prescribed an MRL for OTC used in aquatic animals. For instance, the MRL for OTC is 0.1 μg/g in the EU, 0.2 μg/g in the United States, and 0.2 μg/g in Japan in edible tissues [53,56]. There have been a number of studies of OTC pharmacokinetics in penaeid shrimp [47,53,56–61]. The concentration of OTC in blue shrimp (*Litopenaeus stylirostris)* treated with 1.5 g OTC/kg food for 14 d remained between 3.3 and 5.2 μg/g of shrimp tail muscle from day 4 through day 14 of the feeding period. This level decreased to 0.4 μg/g at 5 d after treatment end [57]. Nogueira-Lima et al. [56] showed that the maximal tissue residue level in whiteleg shrimp (*Litopenaeus vannamei)* treated with 4 g OTC/kg for 14 d was 17.21 μg/g after 7 d of medicated feed (in the laboratory) and 4.38 μg/g after 12 d of medicated feed (in the pond). Reed et al. [60] injected 11.1 μg OTC/g of tissue intravascularly in white shrimp (*Litopenaeus setiferus)* and found that OTC levels in the hemolymph had immediately risen to 15 μg/ml, falling to 1 μg/ml 40 h later. Recently, in whiteleg shrimp (*Litopenaeus vannamei)* fed with medicated feed of 5 g OTC/kg for 14 d followed by nonmedicated feed for another 14 d, the maximal OTC concentrations were 33.54, 194.37, and 18.79 μg/g for muscle, hepatopancreas, and hemolymph, respectively. Ten days after treatment, the drug content in the shrimp tail muscle was below the detection limit for the method (0.01 μg/g of OTC) [61]. This is in agreement with the findings of Mohney et al. [57]. Unlike vertebrates, for which metabolism and excretion of drugs take place in separate organs such as the liver and kidney, shrimp possess only a hepatopancreas, which plays several roles, such as collection, metabolism, and excretion of antibiotics [62]. Chiayvareesajja et al. [63] proposed the hepatopancreas to be the major route for OTC elimination (60%) in whiteleg shrimp. There is little information on OTC content in the hepatopancreas possibly because of the complexity of the tissue, as stated by Reed et al. [60].

The sulfonamides are antimicrobial agents commonly used in infection therapy in aquaculture. The presence of sulfonamide residues in food of animals or in the environment constitutes a potential hazard for human because of the increasing incidence of microbial resistance and the risk of allergic reaction [64]. Maximal residue limits for sulfanamides in tissues have been established in many countries. In the EU, the United States, and Canada, an MRL of 100 mg/kg was set. In Japan, a zero residual level is applied. Therefore, sensitive methods for monitoring these residues are required to ensure that the animal tissues are safe for human consumption. However, there are few studies on the residues of this group in penaeid shrimp. A study on the pharmacokinetics of sulphamethoxazole (SMZ), belonging to the sulfonamide group, has been reported for Chinese shrimp (*Penaeus chinensis)* by Wang et al. [47]. The data showed that SMZ was distributed quickly to the shrimp muscle. The peak concentration of SMZ in the muscle of Chinese shrimp (3.44 μg/g) was detected at 2 h after the last oral administration. Sulphadimethoxine (SDM) reached the peak concentration of 10.0 μg/g 4 h after a single oral administration in the muscle of whiteleg shrimp (*Litopenaeus vannamei)* [65]. The depletion half-life of SMZ in Chinese shrimp was 5.68 h, whereas this value for SDM in whiteleg shrimp muscle was 5.3 h. Thus, the absorption of SMZ in Chinese shrimp was more rapid than that of SDM in whiteleg shrimp, and the depletion of SMZ was a little bit slower than that of SDM. These authors explained that the main possible factor influencing the elimination rate of drugs was water temperature. In the Chinese shrimp experiment, water temperature remained at 22 to 23°C, while it was 26 to 27°C in the whiteleg shrimp experiment. The SDM concentration in feed for whiteleg shrimp was different from the SMZ concentration in feed for Chinese shrimp.

The pharmacokinetics and bioavailability of ormetoprim (OMP) in combination with SDM were examined in whiteleg shrimp (*Litopenaeus vannamei)* by Park et al. [65]. Results showed that disposition half-life of OMP was 7.8 h after intrasinus injection. The bioavailability of OMP after oral administration was 32%. Hemolymph and muscle tissue concentrations of OMP were below detection limits after 24 h. Thus, the ideal aquaculture antibacterial would be rapidly and completely absorbed. It would be distributed to the desired tissues and have a relatively short half-life [66]. Based on these criteria, OMP in combination with SDM has good potential as a shrimp antibacterial because of rapid absorption, moderate bioavailability, and rapid and extensive tissue distribution.

Florfenicol (FF) is a broad-spectrum, primarily bacteriostatic antibacterial with a range of activity similar to that of CAP. In aquaculture, FF demonstrated potent activity against a wide range of fish pathogens in vitro and in vivo through oral administration or intramuscular injection [67]. The drug has been authorized in many countries for use in aquaculture, including Canada, Japan, Norway, and the United Kingdom. In the first report on the use of FF in black tiger shrimp (*Penaeus monodon*), Tipmongkolsilp et al. [68] showed that the maximal concentration of florfenicol-amine (FFA), the marker residue of

FF, detected in the hepatopancreas was 0.7 μg/g tissue 1 h after oral medication and was 0.05 μg/g tissue in the muscle 4 h after oral medication (0.8 g/kg feed for 5 d). Seven days after treatment termination, the drug residue in the shrimp hepatopancreas and muscle was lower than the detection limits for the methods used (0.01 μg/g). Shojaee-AliAbadi and Lees [69] suggested that the optimal dosage for a bacteriostatic drug should maintain concentrations at the site of infection in excess of MIC90 (minimum inhibitory concentration required to inhibit the growth of 90% of organisms) for the entire medication period.

The pharmacokinetics of antibiotics may be affected by parameters such as shrimp species, water temperature and salinity, route of administration, and other experimental conditions. For this reason, the extrapolation of pharmacokinetic data obtained in one species to another species, living under different conditions, should be performed with caution [70,71].

Some researchers have suggested that differences in certain pharmacokinetic parameters between crustacean and finfish may be due to different anatomical volumes, plasma protein, and tissue binding of a drug [72]. First, the shell is a structure that is not present in finfish and has been demonstrated to be a site of a drug deposition in crustaceans [65]. Second, the volume of hemolymph in crustaceans compared with other animals is also different. In crustaceans, hemolymph volumes are approximately 22% of the total body weight [72] compared with finfish species, in which blood volume comprises approximately 5% [73,74]. Third, the degree of plasma protein and tissue binding varies greatly depending on aquatic species [60,75] and drug used [73].

PESTICIDE RESIDUES IN PENAEID SHRIMP

The increasing use of pesticides has also led to widespread public concern because pesticide residues could eventually end up in aquaculture products. Organochorines continue to be the group of chemicals used in control of agricultural pests and vectors of diseases such as malaria, even though many new broad-spectrum pesticides have been developed in recent years. Moreover, the lipophilic nature and low chemical and biological degradation rates have led to their accumulation in biological tissues [76]. The Cl atoms in the organochorines make these compounds very stable in the environment.

In the first report on accumulation of DDT in pink shrimp (*Penaeus duorarum*) and white shrimp (*Litopenaeus setiferus*), Nimmo et al. [77] showed that in pink shrimp of 3.6 to 3.8 g, total body residues was 0.15 ppm after 22 d of exposure to 0.14 ppb. Residues of DDT in the hepatopancreas (0.13 ppm) were greater than those in ventral nerve (0.06 ppm), heart (0.03 ppm), digestive tract (0.04 ppm), gills (0.02 ppm), exoskeleton (<0.01 ppm), muscle (<0.01 ppm), and total body (<0.01 ppm) after 56 d exposure to 0.05 ppb. However, in pink shrimp of 21.1 g exposed to 0.12 ppb for 28 d, residues of DDT in the hepatopancreas (40.4 ppm) were the highest, whereas in gills, digestive tract, ventral nerve, heart, exoskeleton, and muscle they were 2.2 ppm, 1.97 ppm, 1.86 ppm, 1.69 ppm, 0.66 ppm, and 0.19 ppm, respectively. In contrast to pink shrimp, blue shrimp of 28 g had residues of DDT in the hepatopancreas (11 ppm) greater than those in gills (0.36 ppm), digestive tract (0.04 ppm), ventral nerve (0.55 ppm), heart (0.46 ppm), exoskeleton (0.86 ppm), and muscle (0.02 ppm) after 18 d exposure to 0.2 ppb. The authors also determined the loss of DDT from hepatopancreas and other organs in pink shrimp. Shrimp were exposed to 0.17 ppb DDT for 5 d and subsequently held in pesticide-free water. Results indicated that a loss of DDT (98% from hepatopancreas and 100% from other organs) occurred after the shrimp had been placed in clean water for six weeks. These authors also showed that the principal functions of the hepatopancreas are nutrient absorption, digestion, and accumulation of waste products. Thus, the hepatopancreas plays an important role in body metabolism. Nimmo et al. [77] also stated that hepatopancreas plays a major role in accumulating, neutralizing, and eliminating harmful chemicals under both normal and pathological conditions. It receives blood draining the gastrointestinal tract; consequently, it is exposed to toxins absorbed from gut. This capacity for absorption of the hepatopancreas might explain the ability of shrimp to accumulate many times more DDT than was found in the water. The amount in the hepatopancreas is a better estimate of residual DDT in shrimp than total-body residue, because the concentration per unit weight is always higher.

POTENTIAL USE OF BIOMARKERS FOR ASSESSING THE EFFECT OF CONTAMINATION IN PENAEID SHRIMP

Biomarkers are useful as early warning tools for potentially adverse effects. Biomarker responses may provide a temporary and spatially integrated measure of bioavailable contamination [78]. Biomarkers can provide information on the effect of exposure to mixtures of chemicals with which toxicological interactions can occur. Despite a wealth of information on physiological and biochemical biomarkers through which environmental stressors produce adverse effects in penaeid shrimp, they have not been examined to the same extent as in fish. Therefore, comprehension of the mechanisms related to the sublethal effects caused by different chemicals on shrimp metabolism would help us in developing sensitive and precise diagnostic tools with which to assess the toxic effects, thus contributing to better pond management and sustainable aquaculture.

Some biomarkers are not sensitive enough to detect contaminant exposure or effects at environmentally realistic concentrations, and some biomarkers require more technical expertise than others. It is also well understood that no single biomarker has emerged as a widely used biomarker for contamination without some limitation, so a set of biomarkers is essential [79]. Measurements at biochemical or physiological levels detect more quickly and specifically the presence of several toxic compounds, allowing their utilization in a prospective way, until that deleterious effect reaches higher organization levels. To assess exposure to or effects of contamination, the following set of biomarkers may be examined in penaeid shrimp.

Behavioral toxicology is a useful biomarker of sublethal contamination [80,81]. Behavioral endpoints that integrate endogenous and exogenous factors can link biochemical and physiological processes, thus providing insights into individual- and community-level effects of environmental contamination.

The nutritional alterations produced by stress caused by contamination can have critical consequences on most vital processes. There is evidence that variations in the levels of digestive enzymes could be related to developmental cycles and external factors [82,83]. Therefore, the significant alterations in feeding rate resulting from exposure to contamination could partially be explained by a possible alteration in the concentrations of digestive enzymes or changes in the rate of utilization of reserves. Some other studies suggest that exposure to a contamination can increase the feeding rate, but this response may be due not to a stimulatory effect but rather to the result of the need for increased energy to cope with metabolic damage [84]. Some authors have indicated that feeding rate is a sensitive endpoint that allows rapid assessments and is physiologically and ecologically relevant [85,86].

Hemolymph osmolality can be considered as a possible biomarker for penaeid shrimp exposed to pesticides [29]. Exposure to contaminants usually results in a decrease of the OCap. Osmoregulatory capacity is the difference between the osmotic pressures of the hemolymph and of the external medium, at a given salinity. Osmoregulatory capacity can be proposed as a reliable biomarker for monitoring the effect of pesticides in penaeid shrimp, but it must be aided by coupling these changes with other physiological changes.

The change in oxygen consumption and respiration rate caused by pesticides are metabolic alterations. Because metabolism is also influenced by several endogenous and environmental factors [87], it may be difficult to relate changes in oxygen consumption and respiration rate to pesticide intoxication.

The use of nucleic acid has been proposed as a biomarker for the assessment of potential genotoxic effects [88]. However, the techniques considered are expensive and time consuming. Moreover, the use of nucleic acid to estimate toxic effect of xenobiotics in crustaceans has had limited success, because crustaceans change physiologically throughout the molt cycle and exhibit pronounced biochemical changes associated with specific molt stages. Total protein content has also been used as biomarker because the metabolism is directly affected by many xenobiotics. However, it has also been indicated that a reduction of protein concentration in shrimp exposed to pesticides may be due to a normal toxic stress, which produces an increase in energy metabolism. In addition, decreases in the rate of glycogen synthesis in penaeid shrimp exposed to pesticides indicated an inhibitory effect on biochemical processes related to carbohydrate synthesis or a higher consumption of energy from carbohydrate in order to withstand the stress caused by pesticides. Thus, additional research has to be performed to define clear relationships between these biomarker responses and the health of shrimp.

With respect to neural functions, one of the enzymes of interest is AChE [89]. Many insecticides are toxic because they inhibit the animal nervous system enzyme AChE. A major concern is that multiple forms of AChE might obscure the correlation between symptoms and inhibition of AChE activity [90]. Thus, to be used as a biomarker, candidate species should be screened carefully for the anatomical location of multiple forms. In addition to AChE activity varying in its form and location, the amount of AChE activity in a given tissue can also vary in response to characteristics of the organism such as the developmental stage and age. In addition, it is unknown whether inhibition observed in field studies was due to pesticides, other factors, or a combination of both.

Adenosine triphosphatases play important roles in intracellular functions and in all types of physiological activities. However, ATPase activity does not seem to be a valuable parameter in the determination of physiological damage at sublethal concentrations, mainly when this enzymatic activity is the only indicator used. In this sense, it is suggested that ATPases can be used as biomarkers in penaeid shrimp in combination with other physiological and biochemical biomarkers (Supplemental Data, Tables S1, S2, S3).

FURTHER DEVELOPMENTS

Insofar as many environmental contaminants exert toxic effects related to oxidative stress, this phenomenon is another important feature for biomarker development. The study of oxidative stress broadly includes biological phenomena associated with the generation of reactive oxygen species (ROS) and molecular systems designed to protect cells from ROS, referred as ''antioxidant defense systems.'' These ROS, which are continually generated as byproducts of normal aerobic metabolism, can also be produced to a greater extent under stress and pathological conditions as well as being taken up from the external environment. Thus, oxidative stress is an imbalance between the production of ROS and the ability of cells to reduce their levels. The main reason to study oxidative stress in aquatic organisms is to understand not only whether animals are detrimentally affected by exposure but also the mode of action of toxicants. Oxidative stress has been studied in a vast number of situations and organisms in aquatic environments [91,92]. However, studies have focused principally on fish species and on marine bivalves exposed to OPs, CBs, and pyrethroid.

Systems toxicology has been defined as the application of new ''-omics'' approaches to traditional toxicological studies [93]. It refers to the interaction between genes and environmental stressors and combines the studies of transcriptomics, proteomics, and metabolomics. Molecular biomarkers that will be developed using this approach have the potential of providing early detection of environmental stress, inferred mechanism of action, and relatively efficient monitoring of the environment [94]. Transcriptomics is based on the application of DNA microarrays that allow the expression of hundreds to many thousands of genes to be monitored simultaneously, providing a broad and integrated picture of the way in which an organism responds to a changing environment. Proteomics research has provided certain protein expression signatures, which are specific sets of proteins, differentially expressed, potentially indicating specific toxicity profiles. It has undergone tremendous advances over the past few years, and technologies have noticeably matured. However, the use of proteomics in environmental toxicology is still in its infancy as a result of a number of drawbacks such as the limited number of organisms fully covered in sequence databases. Biomarker discovery remains a challenging task because of the complexity of the samples (e.g., serum, other bodily fluids, or tissues) and the wide dynamic range of protein concentrations [95]. Some authors have reported that environmental stress such as variation in salinity and temperature [96] or exposure to environmental contaminants such as heavy metals, xenoestrogen, and chlorinated compounds [94,97,98] has an impact on protein expression in different tissues of relevant aquatic organisms. Although gel-free methods using surface-enhanced laser desorption/ionization (SELDI) mass spectrometry have been investigated [99–101], environmental biomarker discovery still relies mainly on 2D gel electrophoresis and, to our knowledge, is lacking for aquaculture production [102–104]. However, there is no doubt that using a proteomic approach in combination with other biomarkers at physiological, behavioral, and biochemical levels will give a more precise set of biomarkers for assessing the exposure of penaeid shrimp to drugs and chemicals.

CONCLUSIONS

Recent research has provided evidence that current aquacultural production practices could lead to exposures to various pesticides and antibiotics. However, such chemicals can also impair shrimp health and can accumulate inside muscle and, therefore, should be forbidden for sale. Normally, to assess exposure to pesticides and antibiotics, their presence in penaeid shrimp is determined. This strategy can sometimes lead to conflicting results. For example, it is possible that there are elevated residues but no physiological or biological effects because of low bioavailability. Furthermore, these measurements give information only about those antibiotics and pesticides that are included in the analysis and can vary as a result of many factors that are not linked to antibiotic and pesticide contamination. Undoubtedly, a set of biomarkers, using proteomics and more classical biochemical and physiological approaches, should be developed as an early warning signal to prevent excess use of chemicals in shrimp farming. Thus, to achieve sustainability, the aquaculture sector should require an impact assessment tool as an integral component of a sound management plan.

We suggest that the problem of the use of antibiotics and pesticides should be dealt through unified local and global preventive approaches. Heavy use of antibiotics and pesticides must be reduced drastically and replaced with improved culture techniques. Future research should focus on the development of new biomarkers, with which information from several techniques and several levels of biological organization are used to provide a better assessment of contamination in the environment.

SUPPLEMENTAL DATA

Table S1. Effects of different pesticides on physiological and biochemical responses in penaeid shrimp.

Table S2. Pharmacokinetics/residues observed in penaeid shrimp exposed to different antibiotics.

Table S3. Residues observed in penaeid shrimp exposed to different pesticides. (1,165 KB DOC)

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