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Chapter 6

Evaluation of Toxicity in Silver Catfish

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1. Introduction

The global population growth observed in the recent decades coupled with continual technological advancement and increase in the generation of new industrial products, including the manufacture of chemicals such as fertilizers and pesticides, has led to an expansion in the levels of xenobiotic compounds in aquatic ecosystems (Jesus; Carvalho, 2008).

The pollution of rivers and lakes with chemicals of anthropogenic origin may have adverse consequences: the waters become unsuitable for drinking and other household purposes, irrigation, and fish cultivation, and the animal communities living in them may suffer seriously. Massive fish kills are recorded rather frequently, and changes in the population of the fauna as a consequence of sublethal effects on ecologically important species have also been described (Koprocu; Aydin, 2004).

Insecticides are used extensively in agriculture and industry because it is easy to apply, cost effective, and in some situations, it is only a practical method of control. However, benefits of pesticides are not derived without consequences. They are one of the most potentially harmful chemicals and are released into the environment by direct applications, spraying, atmospheric deposition, and surface runoff. Given the fact that, insecticides are not selective and affect non target species as readily as target organisms, it isn't surprising that a chemical that acts on the insect's different systems will elicit similar effects in higher forms of life (Dogan; Can, 2011).

Levels of insecticides in superficial waters generally range far below lethal concentrations for aquatic organisms. However, sublethal adverse effects may result from expo-

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sure of aquatic organisms to insecticides at environmentally relevant concentrations (Das; Mukherjee, 2003).

Pesticides in the environment may be used as a model for the study of ecotoxicology, because they contaminate air, land and water, causing adverse effects that affect from bacteria to humans. It is well proven that these chemicals are toxic to aquatic arthropods, bees and fish (Santos et al., 2007). The effects of the use of pesticides are recognized worldwide and aggravated by misuse since part of this material is accumulated in plants and soil and much of it is transported to the rivers by rain (Tsuda et al., 1995; Wilson; Tisdell, 2001).

As a result of a great variety of human activities, the aquatic environment is becoming increasingly threatened by an alarming number of foreign chemicals or xenobiotics. Fish populations living in highly polluted areas often have high incidences of gross pathological lesions (Malins et al., 1988), associated with elevated levels of toxic contaminants in the sediments. However, pesticides applied to the land may be washed into surface waters and may kill or at least adversely influence the life of aquatic organisms.

Contamination of water with large amounts of pesticides leads to fish mortality or starvation by destruction of food organism, many toxicants have been shown to affect growth rate, reproduction and behavior, with evidence of tissue damage (Van Der Oost et al., 2003; Srivastav et al., 2002).

The poisoning of fish by pesticides can be acute or chronic and in general acute poisoning causes mass mortality. However, pollution is an often chronic process, apparently without any visible damage but sometimes producing several sublethal effects (Rodrigues, 2003).

The aim of this chapter was present a review based on some aspects of silver catfish's toxicology.

2. The fish

Silfvergrip (1996) conducted extensive taxonomic revision of the genus based on characters of internal morphology, and concluded that the genre *Rhamdia* consists of only 11 species among 100 previously described. According to the same author, quelen taxonomic division belongs to the following: Class: Osteichthues, Series: Teleostei, Order: Siluriformes, Family: Pimelodidae. Genre: Rhamdia, Species: quelen.

Rhamdia quelen, popularly known, as silver catfish is a species of fish found from southern Mexico to Argentina that displays the absence of teeth and scales with variable-length cylindrical wattles. This is an omnivorous species with an eating preference for fish, crustaceans, insects, plants and organic debris (Silfvergrip, 1996).

The silver catfish (*Rhamdia quelen,* Quoy & Gaimard) is an endemic South American fish species that with stands cold winters and presents fast growth rate in summer. These characteristics make catfish a suitable species for fish production in southern South America or any region with a temperate or subtropical climate. In aquaculture systems, at a density of two

to four fish/m^2 catfish reach a 600-800 g body weight in eight months. Our unpublished observations in experimental field trials and at fish farms have shown that this weight is easily reached, but high mortality rates (40-50%) might occur if small fish (1-3 g) are used to initiate the culture. However, when beginning with heavier juveniles (30-60 g), the final weight will still range from 600 to 800 g, but with mortality rates not exceeding 5-10%. Thus, the more reasonable order in silver catfish culture is: hatchery, larviculture (1-6 g), nursery (from 5-6 g to 30-60 g), and termination (from 30-60 g to 600-800 g) (Barcellos et al., 2001). This species can be considered eurytermal because the fry acclimated to 31°C withstand temperatures from 15 to 34°C (Barcellos et al., 2003).

3. Chemical contaminants in aquatic systems

Toxic compounds or natural anthropogenic are called xenobiotics. With the onset of the epidemiological investigations was to confirm the hypothesis of many xenobiotics to be dangerous to living things, as well as their respective offspring, exerting toxic effects in the short, medium or long term (Reys, 2001). These substances are persistent in the environment eventually absorbed and accumulated by living organisms, toxic effects on various organs and systems. Thus, it was noted that the use of xenobiotics without evaluation of risks to the ecosystem, constituted a potential threat to the health of people, animals and plants (Sanches, 2006).

The introduction of toxic substances in the aquatic environment causes local and immediate effects, but can also lead to contamination of watersheds and commitment of underground reservoirs by infiltration through the soil. Numerous compounds have been detected in surface water, groundwater and water supply relating to agricultural activities and human cases of environmental contamination (Sanches, 2006).

3.1. Insecticides

The growing use of synthetic insecticides is intensifying global pollution risks. Insecticides are toxic and were designed to repel or kill unwanted organisms and when used for their different purposes they may be brought to water bodies killing or influencing the lives of aquatic organisms (El Sayed et al., 2007). The effects of the use of insecticides are recognized worldwide and compounded by their improper use (Tsuda et al., 1995; Wilson; Tisdell, 2001).

Organophosphates comprise a group of chemical compounds extensively used in farming as insecticides, which cause accidental poisoning in animals and men. The toxicity of these compounds is due especially to the respiratory and cardiac impairment in consequence of autonomic nervous system disorders.

The primary effect of organophosphates (Ops) on vertebrate and invertebrate organisms is the inhibition of the enzyme Acetylcholinesterase (AChE), which is responsible for terminating the transmission of the nerve impulse. OPs block the hydrolysis of the neurotransmitter

acetylcholine (ACh) at the central and peripheral neuronal synapses, leading to excessive accumulation of ACh and activation of ACh receptors (Peña-Llopis et al., 2003). The overstimulation of cholinergic neurones initiates a process of hyperexcitation and convulsive activity that progresses rapidly to *status epilecticus*, leading to profound structural brain damage, respiratory distress, coma, and ultimately the death of the organism if the muscarinic ACh receptor antagonist atropine is not rapidly administered (Shih; Mcdonough, 1997).

Melo (2004) showed that *Rhamdia quelen* (silver catfish) juveniles exposed for 96 hours to a sublethal dose (0.01 mL/L) of Folidol® 600, was target for the toxicant action and some alterations became evident after 4 hours of exposure. The alterations observed in the liver were reduction in the density of melanomacrophages, focuses of necrosis, enhancement in the density of hepatocytes, loss of the cellular contour of the hepatocytes, cytoplasmic granulation, reduction of the cytoplasmic vacuolization, mithocondrial disruption, disorganization of the rough endoplasmic reticulum, nuclear heterochromatization and decharacterization of the endothelium. These alterations could diminish the liver metabolism, and as a consequence, they could cause damages to the health of the *R. quelen* juveniles.

Deltamethrin (DM) and other pyrethroids have proven to be toxic to aquatic organisms, mainly to fish. Due to its lipophilic characteristics it can be highly absorbed by the fish gills, which partially explains the high sensitivity of these animals to DM exposure in concentrations up to a thousand times lower than in mammals (Rodrigues, 2003).

Galeb et al. (2010) and Montanha (2010) studied the behavior of silver catfish exposed to sublethal concentrations of DM and CM, respectively, and they have presented loss of balance, swimming alteration, dyspnea (they kept their mouths and opercula open). *Post-mortem* signs observed in the animals exposed to DM and CM were mainly darkening of the surface of the body, tail and wattles erosion and hemorrhagic spots on the body surface.

The main behavioral changes observed are represented by respiratory and neurological manifestations. Such results corroborate with Polat et al. (2002) and Ylmaz et al. (2004), who have tested Cypermethrin in guppies (*Poecilia reticulata*); Borges (2007), who have tested CM in silver catfish (*Rhamdia quelen*). These changes can be attributed to the neurotoxic effect of DM/CM by blocking sodium channels and inhibiting the GABA receptors in the nervous filaments which results in an excessive stimulation of the central nervous system that sometimes can lead to brain hypoxia (El- Sayed et al., 2007).

Galeb et al. (2010) studied the haematological response of silver catfish exposed by DM, it was a significant increase in the total leukocyte counts. Similar results were reported by El-Sayed et al. (2007) in Nile tilapia (*Oreochromis niloticus*) and Pimpão et al. (2007) in catfish (*Ancistrus multispinis*). Montanha (2010) showed similar results in silver catfish exposed to CM. The leukocytosis showed that these pesticides can generate inflammatory or stress responses.

It was observed decrease of serum levels of ALT (alanine aminotransferase), AST (aspartate aminotransferase) and FA (alkaline phosphatase) were observed in silver catfish exposed to DM (Galeb, 2010) and CM (Montanha, 2010).

Fipronil is a non-systemic, chiral phenylpyrazole insecticide registered for use to control ants, beetles, cockroaches, fleas, mole crickets, ticks, termites, thrips, and other insects in a variety of agricultural and residential uses. Its mechanism of action involves non-competitive binding to the GABA receptor, effectively blocking the chloride channel and resulting in paralysis (USEPA, 2011).

Their toxicity to fish varies with species and it is highly toxic to *Lepomis macrochirus* (LC_{50} = 85 g/L), the *Oncorhynchus mykiss* (LC_{50} = 248 g/L), the *O. niloticus* (LC_{50} = 42 mg/L), *Poecilia reticulata* (LC_{50} less than 100 g/L) and *Cyprinus carpio* (LC_{50} = 430 g/L). Low concentrations of fipronil are lethal to most species of fish that were tested, and is especially toxic to young fish. Studies indicate that the dose 15 mg/L reduced the growth of trout. In addition, this compound is also bioaccumulated fish.

3.2. Drugs

The occurrence of pharmaceuticals for human and veterinary use has been detected in surface waters, sediments and drain in worldwide. Various substances such as anti-inflammatories, analgesics, antibiotics, hormones and antidepressants represent them. Although they have been subjected to pharmacokinetic studies, little information exists about the environmental fate and toxic effects in several organisms of aquatic fauna and flora, certainly affected (Stumpf et al., 1999; Fent et al. 2006).

Chemical toxicity distribution approaches have been employed for identifying Thresholds of Toxicological Concern for many industrial chemicals (Kroes et al., 2005), comparing the sensitivities of in vitro and fish models for estrogenicity (Dobbins et al., 2008), and predicting aquatic concentrations of ecotoxicological concern for chemical with common MOAs in plant models and invertebrates and fish (Dobbins et al., 2008).

4. Assessment of toxicity in fish by biomarkers

The biological response of an organism to xenobiotics following absorption and distribution starts with toxicant induced changes at the cellular and biochemical levels, leading to changes in the structure and function of the cells, tissues, physiology and behavior of the organism. These changes can perhaps ultimately affect the integrity of the population and ecosystem. For the biomonitoring and management of the aquatic ecosystems, these biological responses (biomarkers) have been proposed to complement and enhance the reliability of the chemical analysis data (Parvéz; Raisuddin, 2005).

There are very few pollutants that have been confirmed to cause adverse effects. In most cases, casual relationships have not been established to a large group of persistent pollutants, due the complex chemical contamination of environmental compartments, which difficulty to attribute harmful effects to any particular pollutant or category of pollutants.

Several definitions have been given for the term 'biomarker', which is generally used in a broad sense to include almost any measurement reflecting an interaction between a biologi-

cal system and a potential hazard, which may be chemical, physical or biological (WHO, 1993). A biomarker is defined as a change in a biological response (ranging from molecular through cellular and physiological responses to behavioral changes) that can be related to exposure to or toxic effects of environmental chemicals (Van Der Oost, R et al., 2003).

A bioindicator is defined as organism giving information on the environmental conditions of its habitat by its presence or absence or by its behavior, and an ecological indicator is an ecosystem parameter, describing the structure and functioning of ecosystems.

According to the WHO (1993), biomarkers can be subdivided into three classes:

- **Biomarkers of exposure:** covering the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism;
- **Biomarkers of effect:** including measurable biochemical, physiological or other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease;
- **Biomarkers of susceptibility:** indicating the inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance, including genetic factors and changes in receptors which alter the susceptibility of an organism to that exposure.

4.1. Histopathology

Histopathological characteristics of specific organs express condition and represent time-integrated endogenous and exogenous impacts on the organism stemming from alterations at lower levels of biological organization. Therefore, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters and, as an integrative parameter, provide a better evaluation of organism health than a single biochemical parameter. Histopathological biomarkers signal the effects of exposures both acute and chronic to toxic agents in high or low levels. The pathological changes may be adaptive or degenerative and will determine the survival or death of the organism. The organs most commonly damaged by toxic agents are gills, liver and kidneys, which may be also affected by bacteria, viruses and parasites. That is why a health assessment of the animal is important to differentiate damage induced by toxic agents and diseases. Pharmaceuticals of many categories may be detected on the environment contaminating water flows. In a study for the evaluation of the effect of the analgesic dipyron in surface waters, Pamplona (2009) observed important histological disorders in the kidneys of silver catfish. The fish were exposed to three concentrations of dipyrone (0.5, 5 and 50 μg/L) in the water for 15 days and then evaluated. According to the author, the histological parameters of the intoxicated animals showed important tissue damage in the silver catfish kidneys, such as necrosis, fibrous deposition, vacuolization and constriction of blood vessels of the renal parenchyma, hyperplasia, and even necrosis of great renal vessels and glomeruli in the higher concentration of dipyron. Such alterations were not observed in the control group.

Pesticides are the substances most extensively researched in the aquatic ecotoxicology due to the large amounts used in agriculture and livestock in the whole world. Melo (2004) exposed the silver catfish to the organophosphate methyl parathion at the sublethal concentration of 6 mg/L for 96 hours with the aim of histological and ultrastructural analysis of liver tissue. Melo (2004) suggested that the histological changes are related to the intoxication by methyl parathion that is likely to bring the individuals metabolic, cellular and subcellular problems. It was observed in *Rhamdia quelen* loss of the contour of the hepatocytes and endothelial cells, as well as changes in the nuclear chromatin. According to Melo (2004), changes in the organization of rough endoplasmic reticulum and mitochondrial disruption within four hours of intoxication and strongly eosin stained granulation in the cytosol within 24 hours of exposure could be noticed. Ultrastructurally, a high incidence of lipid droplets was observed in the cytosol of hepatocytes when compared with control within 48 hours of exposure to fipronil. Increased foci of necrosis in the liver of silver catfish after 48 and 72 hours of exposure in relation to the control group, which presents only occasional foci of necrosis, were noted, among other structural changes. After 96 hours of exposure, Melo (2004) describes cells indistinguishable contour, presence necrosis focus, in addition of damaged blood vessels, vacuolization of the cytosol and the presence of an unknown material strongly eosin stained in the cytoplasm of the hepatocytes. Blood leukocyte infiltration and congestion were observed in all animals analyzed. Melanomacrophage were found in various locations between the hepatocytes as well as pyknotic nuclei cells.

Ghisi (2010) studied the effects of the phenyl pyrazolefipronil in the gills of the silver catfish after 60 days of intoxication in the sublethal concentrations 0.05; 0.10 and 0.23 μg/L. The author reports hyperplasia, lamellar fusion and aneurysms in all groups treated, including the control group that can impair the gill function. However, Ghisi (2010) considers the injuries of low severity and possible regression if the source of stress is eliminated, since the concentration of fipronil used was very low.

Cattaneo (2009) studied the effects of 2,4 - dichlorophenoxiacetic acid (2,4-D) herbicide in *Rhamdia quelen* fingerlings in an acute toxicity assay. After acclimation, silver catfish were intoxicated with concentrations of 0, 400, 600 and 700 mg/mL of 2,4 – D for 96 hours. The author states that the histological analysis showed alterations in the liver of silver catfish after exposure to 2,4-D herbicide, such as abnormal arrangement of hepatocytes cords, cell membrane rupture and hepatocytes vacuolation.

The effects of the herbicide clomazone in teleost fish *Rhamdia quelen* were studied by Crestani et al. (2007). The silver catfish were exposed to the concentrations 0.5 and 1.0 mg/L of clomazone for 12, 24, 48, 96 and 192 h. Histological analysis showed vacuolation in the liver after herbicide exposure, some of that were completely restored after a recovery period. Ferreira (2010) studied the sublethal effects of glyphosate (1.21 mg/L), methyl parathion (0.8 mg/L) and tebuconazole (0.88 mg/L) herbicides to silver catfish. The fingerlings were intoxicated during 96 hours and then sampled after anesthesia. The mapathologicalcal changes in liver histology were: diffuse hepatocyte degeneration, bile stagnation, granules on the cytoplasm, hyperemia and vacuoles in the cytoplasm and nucleus of fingerlings exposed to

methyl parathion and tebuconazole. The liver of fish exposed to glyphosate did not show any visible histological changes.

4.2. Hematological and biochemical analyses

Hematological analysis in fish is not routinely used for fish diseases diagnosis. Hematology of fish lags behind that of other classes of vertebrates, but analysis of blood still can be informative about disease processes in teleosts and elasmobranchs (Clauss et al., 2008). The most important factor limiting accurate hematological analysis in different fish species is the variation in types, numbers, and appearance of leukocytes. In addition, the considerable variation in reported leukocyte values from healthy fish, even within a species, is also partly caused by differences in the methodology used as well as by subjective interpretation of cell types by the investigator. Nevertheless, some effort should be made to try to surpass these difficulties since these parameters can be a useful tool for evaluation of the effects of pesticides in the cellular components of blood and even in the immune system. Therefore, the analysis of hematological and biochemical parameters in fish can contribute to the assessment of the animal's health and also the habitat conditions (Pimpão et al., 2007).

Table 1 provides an overview of some hematological and blood chemistry results in catfish (*Rhamdia quelen*) exposed to toxic chemicals.

4.3. Enzymatic analyses

One of the more sensitive effect biomarkers is alteration in levels and activities of biotransformation enzymes. The activity of these enzymes may be induced or inhibited upon in fish exposure to xenobiotic. Enzyme induction is an increase in the amount or activity of these enzymes, or both and inhibition is the opposite of induction. In this case, enzymatic activity is blocked, possibly due to a strong binding or complex formation between the enzyme and the inhibitors.

The according by Van Der Oost, R et al. (2003) enzymatic analyzes to fish are used more:

- Phase I enzymes: Total cytochrome P450 (cyt P450); Cytochrome P450 1A ; Cytochrome b5 (cyt b5); Ethoxyresorufin O-deethylase (EROD) and arylhydrocarbon hydroxylase (AHH); NADPH cytochrome P450 reductase (P450 RED);
- Phase II enzymes and cofactors: Reduced and oxidized glutathione (GSH and GSSG); Glutathione S-transferases (GSTs); UDP-glucuronyltransferases (UDPGTs);
- Oxidative stress parameters: Superoxide dismutase (SOD); Catalase (CAT); Glutathione peroxidase (GPOX); Glutathione reductase (GRED); Non-enzymatic antioxidants
- Biochemical indices of oxidative damage: Lipid peroxidation; DNA oxidation;

Table 2 provides an overview of some enzymatic results in catfish exposed to toxic chemicals.

	Diets containing aflatoxins *				Treatment with diflubenzuron in 24-hour immersion baths **				***
Chemical substance	**Control**	**41ppb/ aflatoxin/ kg**	**90ppb/ aflatoxin/ kg**	**204ppb/ aflatoxin/ kg**	**Control**	**0,01 mg/ l^{-1}diflubenzuron**	**0,1 mg/ l^{-1}diflubenzuron**	**1,0 mg/ l^{-1}diflubenzuron**	**None**
Erythrocytes (x 10^6/µL)					2,71 ± 0,3	2,67 ± 0,4	2,56 ± 0,3	2,6 ± 0,4	1,95 ± 0,40
Hemoglobin (g/dL)	8,2 ± 0,5	5,5 ± 0,3	4,0 ± 0,3	3,5 ± 0,2					6,73 ± 1,15
Hematocrit (%)	24,51 ± 1,51	13,16 ± 1,47	11,75 ± 0,41	11,16 ± 0,88	32,7 ± 3,62	32,9 ± 2,61	31,1 ± 2,8	30,1 ± 1,7	26,5 ± 5,3
Leucocytes (x 10^3/µL)					13,4 ± 2,7	13,2 ± 2,9	12,6 ± 2,1	12,2 ± 2,4	
Lymphocytes (%)					62,1 ± 5,7	61,9 ± 6,8	62,4 ± 6,3	62,6 ± 6,4	11,61 ± 6,57
Neutrophils (%)					25,3 ± 5,5	26,9 ± 5,8	25,9 ± 6,7	25,9 ± 6,7	6,02 ± 3,33
Granulocytic especial cell (%)					2,7 ± 1,2	2,0 ± 0	2,2 ± 0	4,0 ± 2,0	1,24 ± 1,96
Monocytes (%)					11,6 ± 2,9	10,9 ± 4,5	11,1 ± 3,5	10,6 ± 4,3	1,13 ± 1,09
Eosinophils (%)					2,0 ± 0	2,0 ± 0	2,0 ± 0	2,0 ± 0	0,02 ± 0,14
Glucose (mg/dL)	45,00 ± 6,28	36,40 ± 2,42	24,40 ± 6,69	23,20 ± 5,93					

* Vieira, et al. (2006).

**Mabilia; Souza (2006)

***Tavares-Dias et al. (2002)

Table 1. Some hematological and blood chemistry results in catfish (*Rhamdia quelen*) exposed to toxic chemicals.

Species	Pollutants	cyt P450	CYP1A	AHH	EROD	cyt b5	P450 RED	References
Channel catfish	PCB Aroclor1254	=		++	++	=	=	Ankley et al., 1986
Ictaluruspunctatus	BKME	=			++			Mather-Mihaich;DiGiulio, 1991
	PAH (BNF)				++			Hasspieler et al., 1994
	2,4D+ Picloram							Gallagher and Di Giulio, 1991
	2 Aminocanthracene (AA)				-			Watson et al.,1995
	PAH (BaP)		+	+				Ploch et al., 1998
	PCB126		++		++			Rice and Roszell, 1998
Species	**Pollutants**	**SOD**	**GPOX**	**GSSG**	**CAT**	**LPOX**	**GSH**	**References**
Catfish*Ictalurusnebulosus*	OPP (dichlorvos)	=	=		=	+	=	Hai et al., 1995
Channel catfish *Ictaluruspunctatus*	BKME	=	=		+	=	-	Mather-Mihaich and Di Giulio, 1991
	PAH			+	+		+	Di Giulio et al., 1989

Van Der Oost, R et al. (2003)

Symbols: --, strong inhibition (<20% of control); -, inhibition; =, no (significant) response; +, induction; ++, strong induction (>500% of control);

Table 2. Laboratory studies on responses of organic trace pollutants on fish enzymes

4.4. Genetic analyses

Genotoxic substances produce chemical or physical changes inDNA, commonly measured as breaks or DNA adducts, respectively (Nacci et al., 1996). The exposure of an organism to genotoxic substances can induce a cascade of events such as changes in the structure of DNA and the expression of damaged DNA using modified products. Thus, biological consequences may be triggered in cells, organs, body and finally in community and population (Lee; Steinert, 2003; Van Der Oost et al., 2003).

Many biomarkers have been used as tools for the detection of exposure and to evaluate the genotoxic effects of pollution. Among the major genetic biomarkers, we can mention the evaluation of the frequency of chromosomal aberrations, analysis of the frequency of sister chromatid exchanges, formation of DNA adducts, DNA breakage assessed by measuring the comet assay and micronucleus frequency and nuclear morphological abnormalities (Bombail et al., 2001).

According to the studies of Pamplona (2009) by the Comet Assay, widely used to measure DNA damage in aquatic organisms, after the intoxication of silver catfish by dipyrone, the

genetic damages occurred in the lowest concentration of dipyron (0.5 μg/L). DNA fragmentation may be triggered by the release of free radicals in response to a stressor such as a xenobionte that may damage the nucleus chromatin, or by lipoperoxidation that causes the loss of cellular membrane integrity, subjecting the attack of the nucleic acid by oxygen radicals.

In a study of genotoxicity by copper sulphate in four different doses (5, 30, 50 and 500 mg/Kg) using the catfish, Costa (2011) induced DNA breakage triggered by comet assay. The fish were intoxicated by trophic copper sulphate during 60 days and suffered genetic damage in brain tissue, kidney, liver and blood tissues. Blood was less sensitive tissue to levels of copper used in this study. The piscine micronucleus test developed by Costa (2011), however, showed no significant changes in relation to the negative control, as well as the polychromatic erythrocytes frequency in relation to the total number of erythrocytes. There was an increase in the frequency of viable cells in blood tissue, according to the author.

Piancini (2011) performed the piscine micronucleus test and the comet assay in erythrocytes and gill cells of the silver catfish to evaluate the genotoxic effects of atrazine in concentrations 2, 10 and 100 μg/L for 96 hours. The author observed an increased frequency of nuclear morphological changes at all concentrations, a dose-dependent effect of atrazine on DNA trough the comet assay in erythrocytes: damage was significantly higher on 10 and 100 μg/L concentrations compared to control. In gills, there was no difference between treatments. Piancini (2011) observed a dose-dependent effect of atrazine in relation to genotoxicity and its capacity of damaging the DNA of the silver catfish in concentration considered safe by the Brazilian legislation. The same author evaluated the genotoxic effects of copper on *Rhamdia quelen* through the piscine micronucleus test and the comet assay in erythrocytes and gills. The silver catfish were exposed to concentrations of 2.0; 9.0 and 18 μg/L of cuprous chloride for 96 hours. Dose-dependent breaks to the DNA were detected by the comet assays in all treatments, as well as in gills.

Ghisi (2010) did not observe micronuclei in the catfish cells, but did confirm nuclear morphologic changes, after 60 days of intoxication in the sublethal concentrations 0.05; 0.10 and 0.23 μg/L of fipronil. The control group was not significantly different from the group intoxicated by the lower concentration of the insecticide. The higher concentrations were showed significantly higher among of micronuclei compared to the control group, not intoxicated by fipronil, suggesting that the pesticide in higher concentrations (0.10 e 0.23 μg/L) may induce damages to the DNA of *Rhamdia quelen*. The comet assay, also performed by Ghisi (2010) in the same study, showed no significant difference between the intoxicated groups and the control, corroborating the results of histopathological analysis.

The aim of the study performed by Salvagni et al. (2011) was to determine, by micronucleus testing on silver catfish, the risk of genotoxic impact by the use of pesticides such as glyphosate, cyhalothrin, atrazine, simazine and azoxystrobinon in farms located in the Lambedor River watershed in Guatambu, State of Santa Catarina. Blood samples were collected from several species of fish, including *Rhamdia quelen*, captured in 10 different dams existing in agricultural rural properties. Micronucleate erythrocytes were found in blood samples of silver catfish close to the overall average of the other species. Salvagniet at. (2011) believe through *in vivo* piscine micronucleus testing, that water from the Lambedor watershed can

be considered genotoxic, with emphasis on the degree of genotoxicity from pollution in the area, since spontaneous formation of micronuclei in fish is normally very rare. Ferraro (2009) also exposed the silver catfish to different molecules of pesticides and the combinations of them in the aim to determine the bioindicator potential of this teleost species. After the exposure of the fish to the pesticides glyphosate (1.58 e 3.16 mg/L), tebuconazole (0.4 e 0.8 mg/L) and a mixture of them (3.16 e 0.8 mg/L) for 5, 10 and 15 days, were proceeded the micronucleus test and the comet assay in blood samples collected from the fish. DNA damage was confirmed by both techniques in all treatments, pointing the suitability of the species for biomonioring. In a similar way, Ramsdorf (2011) studied the genotoxic effects of fipronil (0.05; 0.10 and 0.23 μg/L), lead nitrate (0.01; 0.03 and 0.10 mg/L) and naphthalene (0.005; 0.06 and 3 mg/L) in the water for *Rhamdia quelen* during 60, 30 and 28 days, respectively. In silver catfish the concentrations 0.10 and 0.23 μg/L of fipronil increased the frequency of micronucleus, nuclear morphological changes, and damages to DNA observed after comet assay. After the intoxication by lead nitrate, it was observed that the concentrations 0.03 e 0.1 mg/L increased the levels of DNA breaks, as well as in all concentration of naphthalene tested for the species.

5. Impact of toxic agents in the reproductive system

The use of fish embryos is gaining popularity for research in the area of toxicology and teratology. Particularly embryos of the zebrafish, catfish and rainbow trout offer an array of different applications ranging from regulatory testing to mechanistic research.

Recently, much attention has been given to possible adverse effects resulting from exposure of aquatic animals to chemicals during the prenatal and perinatal (Tramujas et al., 2006; Montanha; Pimpão, 2012).

The impairment of reproductive function in humans and animal species has been of particular concern in recent years. Many factors can interact with the components and the reproductive function and cause infertility and other functional and structural changes. Illness, stress, hormonal changes and exposure to chemicals, are some factors that contribute to the emergence of reproductive system disorders (Sanchez, 2006).

Fish are one of the most thoroughly studied organisms in terms of effect of substance with estrogenic activity in the development of abnormalities in the reproductive system. According to Sumpter (1998), research on how estrogenic substances affect the sexual system of fish began in the 1980s (Bila; Dezotti, 2003).

The fecundity, the spawning period and type-specific characteristics are essential for the maintenance of any species of fish (Gomiero et al., 2007; Montanha; Pimpão, 2012). Reproduction in fish, as in other vertebrates, is affected by environmental factors, social and nutritional (Parra et al., 2008; Montanha, 2010). Reproductive parameters are more complex indicators of exposure and accumulation of chemicals, hampered for several reasons, the two main effects of pollutants on reproduction caused directly and indirectly and physiological process (Bernardi et al., 2008).

Few data exist regarding the potential sublethal effects of pesticides on reproduction and long-term viability of fish populations (Moore; Waring, 2001).

In 60 years, Rachel Carson published her famous book considered a landmark in the history of environmental pollution, Silent Spring, calling attention to the reproductive failure in birds and fish caused by bioaccumulation of persistent organochlorinepesticides such as diclorofodifeniltricloroetano (DDT). This work brought out the fears of modern society in relation to the introduction of synthetic substances in the environment and renewed public interest in science and government toxicology (SANCHEZ, 2006).

In the mid-70s, researchers found that other chemicals, such as the Kepone and PCBs (polychlorinated biphenyls), also had hormonal effects. Thereafter the toxicological effects of the mixture of individual congeners have been studied mainly in fish, mammalian cells and even humans (Sanchez, 2006).

The structural integrity of the gonads can be altered by xenobiotics (Mayon et al., 2006). Chemical agents that can affect the endocrine system are called endocrine-active chemicals (ECAs), expression adapted to Portuguese as "endocrine disruptors" (Sanchez, 2006).

The Environmental Protection Agency of the United States (USEPA) alternatively defines endocrine disrupters as chemicals that lead to toxic results as various types of cancer and a wide range of adverse effects on the reproductive system (Sanchez, 2006).

The pollution in the aquatic environment can affect the reproductive potential, thus reducing the spread of fish species. This can occur by the possible interaction between the gametes and water pollutants, such as the blockage of the micropyle, which prevents the entry of sperm in the fertilization process (Hilbig et al., 2008). In addition, solutions containing certain levels of pollutants can directly interfere with sperm motility and morphology, and subsequently at fertilization (Hilbig et al., 2008; Witeck et al., 2011).

In teleost fish, such as quelen (catfish), with external fertilization, which occurs when spawning, the gametes are released into the environment for fertilization to occur. At that moment, the gametes are exposed to various contaminants in the water, including heavy metals mercury, zinc, lead, copper and cadmus. These trace elements, at certain levels, affect the motility of spermatozoa and fertilization of oocytes (Witeck et al., 2011).

The gonadotropins stimulate gonadal maturation and release of steroid hormones from the gonads. The steroid hormones and the pituitary determine the development of sexual characteristics and various influencing courtship and parental care (SANCHEZ, 2006).

In teleosts, there are two gonadotropin: R gonadotropin (GTH I) and II gonadotropin (GtH II). In females, the GTH I stimulates gonadal growth, gametogenesis and the entry of vitellogenin in the oocyte. GTH II is important for the final maturation of oocytes and spawning. In males, GTH II acts in the testis, acting on testosterone production (Sanchez, 2006).

Endocrine disrupters are chemicals, natural or synthetic compounds also contained in fungicides, pesticides and insecticides, which have estrogenic and antiandrogenic action. Some of these compounds can maintain their chemical nature for many years contaminating the wa-

ters, being accumulated in fish. Many are potentially harmful to health since they are difficult excretion, may accumulate in the body and cause changes in all systems, especially in the endocrine system (Lara et al., 2011).

Recently, the monitoring of drug residues in the environment has been gaining great interest due to the fact that many of these substances are frequently found in effluents from sewage treatment plants (STPs) and natural waters. Some groups of residual drugs deserve special attention, among them are estrogen, because of its potential to adversely affect the reproductive system of aquatic organisms such as the feminization of male fish found in rivers contaminated with effluent disposal TEE. This was observed in fish species such as *Cyprinus carpio* and *Rutilus rutilus*. Similar effects (induction of hermaphroditism or complete feminization) were also observed when fish species *Oryzias latipes* were exposed to estrogen 17 β-estradiol (Bila; Dezotti, 2003).

Currently, there is growing concern about the presence of pharmaceuticals in aquatic environments and their possible environmental impacts. Ternes et al. (1999) identified the presence of various estrogens in sewage effluent and wastwater treatment plant (WWTP) in Germany, Brazil and Canada (Bila; Dezotti, 2003).

These compounds are characterized by mimicking the structure and function of natural sex steroids leading to endocrine disorders, which can result in abnormal sexual and reproductive function in animals and humans (LARA et al., 2011).

Deltamethrin, a pyrethroid pesticide widely used, is listed by the USEPA as endocrine disruptor, interfering with the reproductive system (Tramujas et al., 2006). Concentrations of 0.01 and 0.1 mg/L of deltamethrin presented toxic to fertilization of eggs of silver catfish (*Rhamdia quelen*). Since the concentrations 0.1, 0.5 and 1.0 mg/L proved to be toxic in the hatching and survival rates of 12 and 24 hours for eggs and larvae of silver catfish (Montanha, 2010). Tramujas et al. (2006) reported histological changes in levels in the gonads of aquatic organisms subjected to different concentrations of deltamethrin.

In recent years, monitoring the impacts of pesticides and other pollutants on sperm production of various kinds of research has been aimed (Mabilia et al., 2008). According to Moore and Waring (2001), even at low levels, cypermethrin (synthetic pyrethroid) in water caused a negative effect on reproductive functions in Atlantic salmon (*Salmo salar*) (Begum, 2005; Mabilia et al., 2008; Montanha, 2010; Montanha; Pimpão, 2012), causing a reduction in fertilization rate (Moore; Waring, 2001). Tramujas et al. (2006) reported a reduction in egg hatching rate of zebrafish (*Danio rerio*) exposed to deltamethrin. Rodrigues (2007) reported hatching in early zebrafish (*Danio rerio*) exposed to dichlorodiphenyltrichloroethane (DDT), with consequent reduction in survival rate. Aydin et al. (2005) and Rodrigues (2007) reported mortality of larvae of carp (*Cyprinus carpio*) exposed to cypermethrin and zebrafish (*Danio rerio*) exposed to DDT, respectively after 12 hours of exposure.

DDT at concentrations of 50 μg/L, and cause premature death hatching of larvae of *Danio rerio*, as well as parathion, from which 20 μg/L cause a decrease in the growth rates of fish of the same species (Rodrigues, 2007).

Cypermethrin proved toxic to the catfish during embryonic development in concentrations of 0.001, 0.01 and 0.1 mg/L relative to the fertilization rate and hatching. As regards the survival rate at 12 hours of exposure to cypermethrin after hatching, the concentration 10 mg/L performed toxic to larvae of catfish and 24 hours, concentrations of 0.001 to 10 mg/L (Montanha, 2010).

Some studies have reported the influence of heavy metals in fish breeding, referring to the influences of contaminants on the quality of the gametes on the fertilization of the oocytes and the quality of the larvae (Hilbig et al., 2008).

The water contamination by lead, the moment of fertilization to the first 8 hours of incubation eggs artificially catfish gray (quelen) at a concentration greater than 0.25 mg/L cause a deleterious effect on the percentage of fertilized eggs (Hilbig et al., 2008).

Water contamination fertilization with cadmus at levels up to 28.65 mg/L does not reduce rates of fertilization and hatching. However, concentrations above 20 mg/L cause reduction in sperm motility catfish (quelen) (Witeck et al., 2011).

In the study by Routledge et al. (1998), two fish species, *Oncorhynchus mykiss* and *Rutilus rutilus*, were exposed for 21 days at concentrations of 17 β-estradiol and estrone environmentally relevant. According to these researchers, the results confirmed that estrogens identified in effluents are present in sufficient amounts to cause the synthesis of vitellogenin, a protein that plays an important role in the reproductive system of female oviparous vertebrates, in fish species. Kang et al. (2002) clearly show that exposure to estrogen concentration of 17 β-estradiol environmentally relevant (in the range of 30-500 ng/L), for three weeks, induces high levels of vitellogenin hermaphrodite and the incidence of male fish species *Oryzias latipes* (Bila; Dezotti, 2003).

According to studies by Panter et al. (1998), low concentrations of 17 β-estradiol and estrone caused effects in male fish species *Pimephales promelas*, as vitellogenin synthesis and testicular inhibition (Bila; Dezotti, 2003).

Exposure to pesticides and other toxic substances during the prenatal and perinatal, can alter the reproductive system of fish without compromising growth and viability of offspring, but cause functional changes that become apparent later in adulthood (Tramujas et al., 2006; Montanha; Pimpão2012). Many xenobiotics are known to affect reproduction in many organisms (Mayonet al., 2006; Montanha, 2010).

6. The fish and the fish embryo toxicity test as an animal alternative method in hazard and risk assessment and scientific research

Biological and ecological responses to contaminant stressors may range from changes at the molecular level, where genetic integrity and subcellular processes are evaluated, to population and community levels where dynamics and structure of entire foodwebs can be affect-

ed. Indicators of stress at several levels of biological organization have been used to evaluate effects of contaminants at the organism level.

A variety of *in vitro* and *in vivo* assays with fish are being used as model systems for toxicological, biochemical and developmental studies (Powers, 1989). Fish species such as rainbow trout *(Oncorhynchus mykiss),* carpas, catfish and Japanese medaka *(Oryzias latipes)* have been used extensively as test organisms for studies of carcinogenesis. A variety of teleost species have been used to study the development of early life stages of fish and the teratogenic effects of environmental contaminants (Wisk; Cooper, 1990). Fish respond in a manner similar to mammalian test species to chemicals that induce peroxisome proliferation in hepatocytes (Yang et al., 1990), and oxidative damage in hepatocytes (Washburn; Di Giulio, 1989). Hepatic cytochrome P-450 monooxygenases in fish also metabolize many carcinogens in a manner analogous to mammalian organisms (Stegeman; Lech, 1991). The advantages of using fish as model organisms include the ease with which teleosts, especially small aquarium species, can be held in the laboratory and exposed to toxic chemicals. Since fish often respond to toxicants in a manner similar to higher vertebrates, they can be used to screen for chemicals that have the potential to cause teratogenic and carcinogenic effects in humans. However, the main application for model systems with fish is to determine the distribution and toxic effects of chemical contaminants in the aquatic environment. Industrial and urban discharges are responsible for high concentrations of toxic substances in the aquatic environment, although in many countries, regulatory activity is beginning to limit point-source discharges of toxic chemicals to our water resources.

Animal alternatives research has historically focused on human safety assessments and has only recently been extended to environmental testing. This is particularly for those assays that involve the use of fish. A number of alternatives are being pursued by the scientific community including the fish embryo toxicity (FET) test, a proposed replacement alternative to the acute fish test. Discussion of the FET methodology and its application in environmental assessments on a global level was needed. With this emerging issue in mind, the ILSI Health and Environmental Sciences Institute (HESI) and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) held an International Workshop on the Application of the Fish Embryo Test as an Animal Alternative Method in Hazard and Risk Assessment and Scientific Research in March, 2008.

7. Conclusion

Environmental pollution is of current environmental concern. It is therefore necessary to find solutions to determine environmental contamination, because the organisms are continuously exposed to a variety of anthropogenic toxicants daily released to the environmental.

The silver catfish have been used for bioindicator of environmental contamination for many researches and can be used to aquatic biological systems. Moreover, a considerable expansion of knowledge in using various model species which are easily cultured and have lesion induction times of relatively short duration, such silver catfish.

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