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Impact of Pesticide Toxicity on Selected Biomarkers in Fishes

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ABSTRACT

Modern agricultural practices result in indiscriminate use of various agrochemicals, which usually enter into the aquatic environment. The use of agrochemicals in the field has the potential to change the aquatic medium, affecting the tolerance limit of aquatic fauna and flora, as well as creating danger to the ecosystem. These agrochemicals adversely affect the non-target organisms, especially plankton and fish. The present study reports the acute and sublethal toxicity of pesticides on plasma protein, acetylcholinesterase, hormones, histopathology, changes in gill, ventillatory frequency and stress protein level of freshwater fishes. The alterations of the hormonal levels may be used as a potential biomarker and also can establish the ability of endocrine tissues to respond to their appropriate releasing factors. Heat Shock Proteins (HSPs) are detected in all cells, prokaryotic and eukaryotic. *In vivo* and *in vitro* studies have shown that various stressors transiently increase production of HSPs as protection against harmful insults. Increased levels of HSPs occur after environmental stresses, infection, normal physiological processes and gene transfer.

Key words: Pesticides, hormones, persistency, chlorpyrifos, xenobiotics, stress proteins, HSP70

INTRODUCTION

Pesticides have brought tremendous benefits to mankind by increasing food production and controlling the vectors of man and animal diseases. At the same time use of these pollutants has posed potential health hazards to the life of fishes. Pesticides are major cause of concern for aquatic environment because of their toxicity, persistency and tendency to accumulate in the organisms (Joseph and Raj, 2010). The impact of these pesticides on aquatic organisms is due to the movement of pesticides from various diffuse or point sources. These pesticides are posing a great threat to aquatic fauna especially to fishes, which constitute one of the major sources of protein rich food for mankind (Sharma and Singh, 2007).

BIOCHEMICAL CHANGES IN BLOOD

Blood is highly susceptible to internal and external environment fluctuations because it is the vehicle for the transport of such pollutants (Blaxhall, 1972). Metals are Transported in the blood stream by binding to specific plasma proteins (Joseph *et al*., 2010). The fish serves as bio-indicator of water quality and the impact of the pesticide can be well understood by analyzing either blood or serum of the fish, because blood is a pathophysiological reflecter of whole body (Sharma and Singh, 2004, 2006). The toxic effect of pesticides to the blood of fishes has been

studied by many researchers (Dawson, 1935). The toxicity of chloropyrifos has been observed on Erythrocyte Sedimentation Rate (ESR) (mm h^{-1}) in fish, *Channa punctatus* (Malla *et al.*, 2009). An increase in ESR (mm h^{-1}) has been reported in *Clarias batrachus* after exposure to savin (Kumar and Benerjee, 1990). Reduction in total serum protein content induces proteinaemia and may be correlated with reduced protein synthesis by liver (Sharma *et al.*, 2009). There was a significant decease in the total serum protein of cyprinus carpio at 0.1 and 0.001 mL curacron. A significant decrease was observed up to day 21 (Joseph and Raj, 2010). Spontaneous lesions of oocytes can also occur randomly under normal conditions, as reported in zebrafish studies and this is a phenomenon of fish pathology that has been broadly investigated (Rossteuscher *et al.*, 2008). The effects of assimilation of DDT from the water column by fish and this having a mild effect on the parental gonads and direct effects on the F1 generation survival and viability (Mlambo *et al.*, 2009).

ACETYLCHOLINESTERASE

AchE activity is more sensitive for OPs and carbamate pesticides than other contaminants, but the inhibition of this enzyme have been also used to indicate the exposure and effects of other contaminants in fishes. It has been shown that the addition of crude oil to brain homogenate in amounts equivalent to sediment concentration inhibited AChE activity in fishes (Rodriguez-Fuentes and Gold-Bouchot, 2000). Minier *et al.* (2000) reported that muscle AChE of flounder from polluted sites with high level of PAH was inhibited by 40%. Also, a reduction of 40% of brain AChE was observed in Mullus barbatus from three pollutes sites of Salento Apulia (Italy), related with presence of great variety of compounds (PAH, heavy metals and pesticides) present in the sediment (Lionetto *et al.*, 2003). The reduction in swimming performance in fish after exposure to Ops could be attributed to the inhibition of AChE (Rao, 2006; Rao *et al.*, 2005). The present results illustrate that after prolonged exposure to PF induced tissue-specific peroxidative damage in brain, gill, viscera and muscle tissues of O mossambicus and the most affected tissue was gill. Earlier studies show that LPO may be induced in various tissues by a variety of environmental pollutants (Ahmad *et al.*, 2000). Significant depression of cholinesterase activities in brain and liver tissues of O. niloticus following single and multiple exposure of chlorpyrifos (an organophosphate insecticide) and carbosulfan (a carbamate insecticide) in the laboratory was reported by Chandrasekara and Pathiratne (2005).

HISTOPATHOLOGY

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory (Wester and Canton, 1991) and field studies (Hinton *et al.*, 1992; Schwaiger *et al.*, 1997; Teh *et al.*, 1997). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al.*, 2001). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta *et al.*, 2003) and serve as warning signs of damage to animal health (Hinton and Lauren, 1990).

GILL AS A BIOMARKER

The fish gill is a multifunctional organ involving gaseous exchange acid-base balance, ionic (Na^-, Cl^- and Ca_2^-) transport and nitrogenous waste excretion (Perry, 1997). The gills consist of

four branchial arches each bearing pairs of primary filaments in which rows of secondary lamellae are situated. The filaments and lamellae are covered by epithelial cells (i.e., pavement cell (PVC), Chloride Cell (CC) and mucous cell) supported by a complex system of blood vessels (Laurent, 1984). Hence, the gill epithelium provides an extensive surface of contact with the environment to facilitate ion transport and gaseous exchange. Gill surfaces are the first target of water-borne metals (Spicer and Weber, 1991). The micro-environment of the gill surface consists of an epithelial membrane which primarily contains phospholipids covered by a mucous layer (Bolis *et al*., 1984). The constituents of the gill epithelia will be fully ionised, resulting in negatively charged gill surfaces and potential gill-metal interaction sites (Reid and MacDonald, 1991; Joseph and Raj, 2010).

VENTILATORY FREQUENCY

Ventilatory Frequency (VF) represents a good alternative for alert or stressful conditions assessment in fish, because it has several advantages. Investigations have shown that VF is changed quickly in response to disturbances imposed, as demonstrated in fish subjected to social stress (Volpato *et al*., 1989; Alvarenga and Volpato, 1995), strobe light (Sager *et al*., 2000), predation risk (Barreto *et al*., 2003; Hawkins *et al*., 2004a, b; Queiroz and Magurran, 2005) and confinement (Barreto and Volpato, 2004; Brown *et al*., 2005). Moreover, VF is an inexpensive and easily measurable parameter, because it can be visually counted, so requiring no sophisticated equipment. It is also a non-invasive behavioural tool, avoiding painful or stressful technique to be used for stress assessment, such as blood sampling.

HORMONES

In fish, hormones are critical towards maintaining proper physiological function and amongst the many hormones found in fish the thyroid hormones (thyroxine (T4) and triiodothyronine (T3)) are known to play an important role in fish growth (Higgs *et al*., 1982; Miwa and Inui, 1985) and early development (Brown, 1997). When fish are exposed to stressors the levels of thyroid hormones have been demonstrated to be decreased (Pickering, 1993; Deane *et al*., 2001) and chemical pollutants have been reported to detrimentally affect thyroidal hormone status in a number of fish species (Xu *et al*., 2002; Brown *et al*., 2004; Scott and Sloman, 2004; Van der Ven *et al*., 2006).

STRESS PROTEINS

Exposure of living beings to sub lethal levels of environmental pollution has been shown to trigger several defense mechanisms at the cellular and molecular levels. Very important in this respect is the cellular accumulation of stress proteins which mainly act as molecular chaperones (Ellis and Vander Vies, 1991; Welch, 1993; Bauman *et al*., 1993; Feder and Hofmann, 1999). Among stress proteins, the HSP70 group (i.e., HSP72, HSP73) has been widely studied since it is regularly over expressed in response to a wide variety of natural, experimental or anthropogenic aggressors viz., heat shock, alcohols, oxidative stress, radiations, heavy metals, arsenic, pesticides and others (Sanders and Martin, 1993; Fulladosa *et al*., 2002; Gaubin *et al*., 2002; Delaney and Klesius, 2004). Besides, the stress-related induction of HSP is a general phenomenon observed in all animal species studied so far (Feder and Hofmann, 1999). Metallothioneins (MTs), ubiquitous low molecular weight cysteine-rich proteins, are also considered as stress proteins since they protect the cells against excessive metal uptake (Bauman *et al*., 1993) by virtue of their high proportion of -SH groups which sequester the metallic ions (Kagi and Schaver, 1988; Klaassen *et al*., 1999).

As a consequence, MTs should be logically responsive to metal poisoning. Studies carried out in low vertebrates are already numerous and expression of stress proteins in different fish species in response to various stressors has been investigated by many authors (Segner, 1998; Iwama *et al*., 1998, 1999). For instance, several heat shock proteins have been detected after exposure of various kinds of fish cells to heat shock, arsenate and several metal ions (Kothary and Candido, 1982; Heikkila *et al*., 1982; Misra *et al*., 1989; Currie *et al*., 1999, 2000). Overexpression of MTs has also been studied in different fish species (Carbonell *et al*., 1998; Dethloff *et al*., 1999) and its use as a biomarker for monitoring metal pollution in the environment was proposed (Hamilton and Mehrle, 1986). Stress protein mRNA were recently shown to be actively produced in red blood cells of the brook trout submitted to a heat shock (Lund *et al*., 2003). Besides, fish erythrocytes display high levels of aerobic metabolism that supports many cellular processes, including an active protein synthesis (Currie and Tufts, 1997). Heat shock proteins (hsps), in particular hsp 60 and hsp 70, have been suggested as suitable biomarkers of the exposure to and effects of environmental contaminants (Sanders, 1990). The heat shock proteins are families of proteins which are classified by their molecular weight. They are also known as molecular chaperones (Ellis,1987) for their constitutive roles in protein synthesis.

HEAT SHOCK PROTEINS

Organisms respond to proteo toxicity with the expression of stress proteins which are able to repair partly denatured proteins. Proteins belonging to the Heat Shock Protein (HSP) families have been demonstrated to play a critical role in the stress response of fish and it has been extensively reported that expression profiles of HSP families can be modulated by abiotic (Iwama *et al*., 1998; Basu *et al*., 2002), biotic, heavy metal (Iwama *et al*., 1998) and organic pollutants. The role of HSP during stress is related to a cytoprotective function as these proteins can act to prevent and repair protein damage (Ananthan *et al*., 1986). Heat shock protein levels have been shown to be modulated in fish cells and tissues upon exposure to a vast array of stressors (Iwama *et al*., 1998). Of all the heat shock protein families, HSP70 has been the most comprehensively studied, due to its major importance in cytoprotection (Ryan and Hightower, 1994) and recently it was shown that elevated HSP70 is critical in protection of sea brim cells against chemical induced apoptosis (Deane *et al*., 2001). The accumulation of these heat shock proteins has been linked to the intensity of stress, these proteins have been regarded as a suitable biomarker in assessing reactions of biota to environmental and physiological stressors (Hightower, 1991; Sanders, 1993).

DISCOVERY OF HSPS

The first report on HSPs appeared in 1962 after Drosophila salivary gland cells were exposed to 37°C for 30 min and then returned to their normal temperature of 25°C for recovery, a puffing of genes was found. All HSP-70s bind ATP (Chappell *et al*., 1987; Milarski and Morimoto, 1989) have occurred in the chromosome in the recovering cells (Ritossa, 1962) accompanied by an increase in the expression of proteins with molecular masses of 70 and 26 kDa (Tissieres *et al*., 1974).

CLASSIFICATION OF HSPS

The best understood HSPs are those with molecular masses of 60, 70, 90 and 110 kDa. These major HSPs are expressed at 378°C in the absence of heat shock. HSP-70 and -90 are observed in all organisms, whereas HSP-110 is present mainly in mammalian cells. A second group of HSPs (sometime referred as minor HSPs) are induced under conditions of glucose deprivation and include

glucose-regulated proteins (GRP) 34, 47, 56, 75, 78, 94 and 174 kDa (Sciandra and Subjeck, 1983). A third group of HSPs is the low-molecular-mass HSPs; these have molecular masses of about 20 kDa; these are found at elevated levels in heated *Drosophila* cells (Tissieres *et al*., 1974) and ischemic cardiomyocytes (Mestril *et al*., 1994). A small portion of their amino acid sequence is similar to mammalian a-Crystalline (Ingolia and Craig, 1982). HSP-70s are highly conserved and demonstrate a 60-78% base identity among eukaryotic cells and a 40-60% identity between eukaryotic HSP-70 and *Escherichia coli* DnaK, similar to the HSP-70 (Bardwell and Craig, 1984; Craig, 1985; Lindquist, 1986; Caplan *et al*., 1993).

INDUCTION AND FUNCTIONING OF HEAT SHOCK PROTEINS

Heat shock protein expression also be induced by the presence of denatured proteins (Edington *et al*., 1989). Increased expression of heat shock proteins has also been called a stress response, because hsps can be increased or induced after exposure to some environmentally-relevant stressors, including contaminants such as heavy metals (Cd, Cu, Cr, Hg, Ni, Pb and Zn), tributyltin,organophosphate and organochlorine pesticides and other organic contaminants including benzene, 1-chloro-2,4-dinitrobenzene, 2,4-dichloroaniline, 2,4-dinitrobenzene, hexachlorobenzene, pentachlorophenol and trichloroethylene (Sanders, 1993). The common mode of action of these diverse stressors seems to be that they are proteotoxic (Hightower, 1991), resulting in damage to proteins. Basal expression of hsps has shown to be essential in the early embryonic development and in resting cells. However, over expression of hsp70 in normal drosophila cells slowed down the growth rate, suggesting that over expression of Hsps in various cells under normal physiological conditions may be of no significance. Molecular chaperones orchestrate when and where proteins fold and unfold in the cell andif there is serious misfolding and aggregation caused by environmental stress or pathology, they can act as sensors to direct these cells to apoptosis. In maintaining the protein homeostasis, Hsps serve at various levels, such as: (1) protect other proteins against aggregation; (2) prevent protein aggregation; (3) assist the folding of nascent proteins or refolding of damaged proteins; (4) target severely damaged proteins to degradation and (5) in case of excessive damage, sequester damaged proteins to larger aggregates (Soti *et al*., 2005). The importance of chaperones in promoting and maintaining the native confirmation of cellular proteins is of utmost importance due to the toxic consequences of protein misfolding and aggregation. Cellular accumulation of stress proteins which mainly act as molecular chaperones (Ellis and Vander Vies, 1991; Welch, 1993; Bauman *et al*., 1993; Feder and Hofmann, 1999). Among stress proteins, the HSP70 group (i.e., HSP72, HSP73) has been widely studied since it is regularly over expressed in response to a wide variety of natural, experimental or anthropogenic aggressors (heat shock, alcohols, oxidative stress, radiations, heavy metals, arsenic, pesticides and others) (Welch, 1993; Sanders and Martin, 1993; Fulladosa *et al*., 2002; Gaubin *et al*., 2002; Delaney and Klesius, 2004). Studies carried out in lower vertebrates are already numerous and expression of stress proteins in different fish species in response to various stressors has been investigated by many authors (Segner, 1998; Iwama *et al*., 1998, 1999). For instance, several heat shock proteins have been detected after exposure of various kinds of fish cells to heat shock, arsenate and several metal ions (Kothary and Candido, 1982; Heikkila *et al*., 1982; Misra *et al*., 1989). Protein aggregation associates a number of acute and chronic neurodegenerative conditions. Hsps reduce the risk of formation of toxic oligomeric assemblies of the respective disease proteins such as tau and amyloid-b in Alzheimers disease, a-synuclein in parkinsons disease huntingtion in Huntingtons disease (Wyttenbach and Arrigo, 2006). Prions are

nothing but infectious particles of protein, which cannot be inactivated by measures that modify nucleic acids. Hsp family members, especially Hsp 140, were shown to inhibit prion protein aggregation independent of ATP. Overexpression of MTs has also been studied in different fish species (Carbonell *et al.*, 1998; Dethloff *et al.*, 1999) and its use as a biomarker for monitoring metal pollution in the environment was proposed (Hamilton and Mehrle, 1986). Stress proteins mRNA were recently shown to be actively produced in red blood cells of the brook trout submitted to a heat shock. Besides, fish erythrocytes display high levels of aerobic metabolism that supports many cellular processes, including an active synthesis of protein. A correlation between levels of heavy metals and metallothioneins in human blood (Szitanyi *et al.*, 1996). The possibility of using MTs gene activation in Peripheral Blood Lymphocytes (PBLs) in humans as a biomarker of susceptibility to cadmium exposure (Lu *et al.*, 2001). Studies carried out on mammalian PBLs of grey seal demonstrated that MTs levels can be induced by Zn (Pillet *et al.*, 2002). However, when using fish as a biological target, only effect of copper in blood and biochemical parameters were analyzed (Dethloff *et al.*, 1999).

STRESS RESPONSE BY HSPS

All HSP-70s bind ATP (Chappell *et al.*, 1987; Milarski and Morimoto, 1989). HSPs are bound to HSFs that reside in the cytosol of mammalian cells under unstressed conditions (Schlesinger, 1990). Under stress conditions such as heat shock and ischemia, HSFs are separated from the HSPs. When HSFs are then phosphorylated by PKC or other serine/threonine kinases, they form a homotrimeric structure (Kroeger *et al.*, 1993). The trimers enter the nucleus, bind to HSEs located on the promoter region of HSP genes and become further phosphorylated by HSF kinases (Price and Calderwood, 1991). Transcription is then initiated,followed by translation. The newly synthesized HSPs bind to HSFs to prevent further synthesis of HSPs (Li *et al.*,1995).

CONCLUSION

Long term exposure of organisms to pesticides means a continuous health hazard for the population. So, human population is at high risk by consuming these toxicated fishes. This implies that one should take the necessary precaution in the application of pesticides to protect the life of fish and other aquatic fauna. It is known that both environmental and pathological stresses cause an increase in HSPs of host cells. It is believed that levels of HSP-70s might be used as a measure of stress. These organisms can be used to detect the environmental stressors. It is likely that approaches using molecular biology techniques will revolutionize toxocological applications that are cheaper and do not require the use of animals to detect environmental stressors.

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