A review: Oxidative stress in fish induced by pesticides

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REVIEW

Abstract

The knowledge in oxidative stress in fish has a great importance for environmental and aquatic toxicology. Because oxidative stress is evoked by many chemicals including some pesticides, pro-oxidant factors' action in fish organism can be used to assess specific area pollution or world sea pollution.
Hepatotoxic effect of DDT may be related with lipid peroxidation. Releasing of reactive oxygen species (ROS) after HCB exposure can be realized via two ways: via the uncoupling of the electron transport chain from monooxygenase activity and via metabolism of HCB major metabolite pentachlorophenol. Chlorothalonil disrupts mitochondrial metabolism due to the impairment of NADPH oxidase function. Activation of spleen macrophages and a decrease of catalase (CAT) activity have been observed after endosulfan exposure. Excessive release of superoxide radicals after etoxazole exposure can cause a decrease of CAT activity and increase phagocytic activity of splenocytes. Anticholinergic activity of organophosphates leads to the accumulation of ROS and resulting lipid peroxidation. Carbaryl induces changes in the content of glutathione and antioxidant enzymes activities. The antioxidant enzymes changes have been observed after actuation of pesticides deltamethrin and cypermethrin. Bipyridyl herbicides are able to form redox cycles and thereby cause oxidative stress. Low concentrations of simazine do not cause oxidative stress in carps during sub-chronic tests while sublethal concentrations of atrazin can induce oxidative stress in bluegill sunfish. Butachlor causes increased activity of superoxide dismutase –catalase system in the kidney. Rotenon can inhibit the electron transport in mitochondria and thereby increase ROS production. Dichloroaniline, the metabolite of diuron, has oxidative effects. Oxidative damage from fenpyroximate actuation is related to the disruption of mitochondrial redox respiratory chain. Low concentration of glyphosate can cause mild oxidative stress.

Abbreviations:

AChE – acetylcholine esterase
ALA – aminolevulinic acid dehydratase
CAT – catalase
CYP1A – cytochrome P450, family 1, subfamily A
CYP450 – cytochrome P450
DCA – dichloroaniline
EROD – ethoxyresorufin-O-deethylase
$FADH_2$ – reduced flavin adenine dinucleotide
FG – gill cell line
$FMNH_2$ – reduced flavin mononucleotide
GPX – glutathione peroxidase
GR – glutathione reductase
GSH – glutathione
GSSG – glutathione disulfide
GST – glutathione S-transferase
G6PD – glucose 6-phosphate dehydrogenase
HAHs – halogenated aromatic hydrocarbons
HP – hydroperoxide
LOOH – fatty acid peroxides
LPO – lipid peroxidation
L-SH – low molecular mass thiol
MDA – malondialdehyde
MT – metallothioneins
NADH – reduced nicotinamide adenine dinucleotide
NADPH – reduced nicotinamide adenine dinucleotide phosphate $NADP^+$
NO· – nitric oxide
$ONOO^-$ – peroxynitrite
PAHs – polycyclic aromatic hydrocarbons
PMA – phorbol myristate acetate
POPs – persistent organic pollutants
PUFA – polyunsaturated fatty acids
RNS – reactive nitrogen species
RO· – alkoxyl radical
ROO· – peroxyl radical
ROS – reactive oxygen species
SOD – superoxide dismutase
XOD – xanthine oxidase
8-OHdG – 8-hydroxy-2′-deoxyguanosine
8-oxodG – 8- oxo-2′-deoxyguanosine

1. Oxidative stress

The oxidative stress is an inescapable component of aerobic life. In the healthy aerobic organism, a balance between the reactive oxygen species (ROS) production and the system to protect cells from ROS exists. Ascendancy of the ROS production results in defects that may cause cell or organism damages or death. This imbalance is referred to as oxidative stress (Davies, 1995).

The generation of ROS arises by many mechanisms in organism even under physiological conditions. There are so-called endogenous cellular sources of ROS. The most important of these sources are electron transport chains of mitochondria, endoplasmatic reticulum, the function of cytochrome P450 (Di Giulio & Meyer, 2008), the actuation of oxidative enzymes during catalysis (Fridovich, 1978; Halliwell & Gutteridge, 1999) and auto-oxidation of the key molecules of cellular function (Halliwell & Gutteridge, 1999) in animals, and chloroplasts as an alternative of mitochondria in plants (Di Giulio & Meyer, 2008).

ROS play also a positive role in the organism especially in the phagocytic activity of neutrophils and macrophages (Babior, 2000). Upon stimulation (e.g. from opsonized bacteria), these cells increase O_2 consumption up to 20 times resting levels and this is referred to as "the respiratory burst" (Di Giulio & Meyer, 2008). Processes which produce oxidative stress in aquatic organisms and mammals are similar. Many xenobiotics, such as pesticides, can induce the production of reactive oxygen species by several biochemical mechanisms such as the impairment of membrane-bound electron transport (e.g. mitochondrial, microsomal electron transport) and subsequent accumulation of reduced intermediates (Stolze & Nohl, 1994), redox cycling, photosensitization (Di Giulio & Meyer, 2008), facilitation of Fenton reaction, inactivation of antioxidant enzymes (Kono & Fridovich, 1983) and depletion of free radical scavengers (Winston & Di Giulio, 1991).

The action of ROS results in lipid peroxidation, protein oxidations, modulation of gene expression, alterations of redox status as cellular effects, and certain diseases and premature aging as effects in stage of organism.

2. Antioxidant defences

These mechanisms of antioxidant defences include enzyme systems that act to remove ROS, low-molecular-weight compounds that directly scavenge ROS (in animals, some produced endogenously and others obtained from the diet), and proteins that act to sequester pro-oxidants, particularly iron and copper (Di Giulio & Meyer, 2008).

Antioxidant enzyme systems

The most important enzymes for the detoxification of reactive oxygen species in all organisms are superoxide dismutase (SOD), catalase, glutathione peroxidases (GPXs) and transferases (Di Giulio & Meyer, 2008), xanthine oxidase and glucose 6-phosphate dehydrogenase (G6PD).

Low-molecular-weight and other antioxidants

Glutathione (GSH) exists in two forms, as a reduced GSH and as an oxidized glutathione disulfide (GSSG). Normal healthy cells contain the reduced form, GSH, and exhibit GSH: GSSG ratios approaching or greater than 100:1. Decline in this ratio can serve as a marker of oxidative stress (Di Giulio & Meyer, 2008). Tissue glutathione levels are often depleted after short-term oxidant exposures but elevated after long-term exposures. Glutathione depletion sensitizes fish, as well as mammals, to the toxicity of pro-oxidant xenobiotics (Gallagher *et al.* 1992). Doyotte *et al.* (1997) and Zhang *et al.* (2004b) have reported that during a moderate oxidative stress, the GSH levels can increase as an adaptive mechanism by means of an increased synthesis.

Several other biomolecules with an antioxidant function are vitamins, including ascorbic acid (vitamin C), tocopherols (vitamin E components) and carotenoids (α-carotene, β-carotene, beta-cryptoxanthin) which are obtained through the diet in most of the animals, and the substances with primarily other than antioxidant function including coenzyme Q, estradiol, bilirubin,

lipoic acid, uric acid (Halliwell & Gutteridge, 1999), metallothioneins (MT) (Coyle *et al.* 2002) and melatonin (Reiter *et al.* 2008). The new findings refer to the antioxidant potential of glucomannan, a water-soluble polysaccharide isolated from yeasts. Bauerova *et al.* (2008) observed antioxidant effects of glucomannan isolated from *Candida utilis*.

3. Biomarkers of oxidative stress

As an introduction it must be said that no single biomarker sensitive and specific enough for oxidative stress has been identified (Di Giulio & Meyer, 2008). Generally, products of oxidative cell or tissue damages and an increased amount of antioxidant enzymes are observed. Many studies have cited GSH: GSSG ratios, levels of MT or lipid peroxidation, and activities of GR, GST and GPX as the most sensitive indicators but these markers have been completely unsuitable in other contexts (Di Giulio & Meyer, 2008). Field studies provide representative examples of biomarkers of oxidative stress associated with pollutants exposures. The important biomarkers of oxidative stress induced by pesticides from field studies were documented by e.g. Dorval *et al.* (2005), Eufemia *et al.* (1997) and Machala *et al.* (2001).

Commonly, biomarkers of oxidative stress can be divided into two groups: biomarkers for free radical damage in biological systems and factors of antioxidant defences.

Biomarkers for free radical damage in biological systems

The use of primary and secondary products of free radical damage as biomarkers is convenient for the monitoring of the oxidative pressure of the environment. The most widely used biomarkers are products of lipid peroxidation as aldehydes, especially malondialdehyde (MDA), ketones and the determination of diene conjugation from the polyunsaturated fatty acids (Valavanidis *et al.* 2006). Malondialdehyde is a main secondary lipid oxidation product of polyunsaturated fatty acids (PUFA).

The most important biomarkers of *in vivo* oxidative damage to DNA are products of the specific modifications and hydroxylations of purine and pyrimidine bases and products of damage to the deoxyribosephosphate backbone and protein-DNA cross-links (Valavanidis *et al.* 2006). Most of the studies have been focused on the detection of products of the hydroxylation of guanosine, namely of 8-hydroxy-2´-deoxyguanosine (8-OHdG) or 8- oxo-2´-deoxyguanosine (8-oxodG) and its free base 8-hydroxyguanine (Shigenaga & Ames, 1991). The amount of 8-OHdG in aquatic organisms is considered as a solid biomarker of oxidative stress in relation to environmental pollutants (Rodriguez-Ariza et al.1999; Steinert, 1999; De Almeida *et al.* 2003). By the hydroxylation of thymin two other markers arise - thymine glycol and thymidine glycol (Valavanidis *et al.* 2006).

The protein oxidation is the last described damage of biological systems by free radicals. The measuring is focused mainly on carbonyl derivates of proteins, especially the oxidation products of tyrosine (i.e. dityrosine) (Huggins *et al.* 1993) and phenylalanine (Valavanidis *et al.* 2006). Detection of γ- glutamyl semialdehyde and 2-amino-adipic semialdehyde is another evidence of protein oxidative damage (Valavanidis *et al.* 2006).

Antioxidant defences as biomarkers of oxidative stress

Assaying antioxidant enzymes have been used as an indicator of the antioxidant status of the organism. The enzymes that were analyzed included superoxide dismutase, catalase, xanthine oxidase (XOD) and glutathione redox cycle enzymes, glutathione peroxidase, glutathione reductase (GR) and glucose 6-phosphate dehydrogenase.

In wild fish more specific markers of oxidative stress have not emerged because of the variety of factors playing role in this problem. They are sex and reproductive condition (Livingstone *et al.* 1995; McFarland *et al.* 1999; Meyer *et al.* 2003; Winzer *et al.* 2001, 2002a, 2002b), temperature (Heise *et al.* 2003; Olsen *et al.* 1999; Parihar & Dubey, 1995; Parihar *et al.* 1996), diet (George *et al.* 2000; Hidalgo *et al.* 2002; Mourente *et al.* 2000, 2002; Pascual *et al.* 2003), dissolved oxygen (Cooper *et al.* 2002; Hermes-Lima & Zenteno-Savin, 2002; Lushchak *et al.* 2001; Ritola *et al.* 2002b; Ross *et al.* 2001), salinity (Kolayli & Keha, 1999; Martinez-Alvarez *et al.* 2002), seasonal effects (Bacanskas *et al.* 2004; Ronisz *et al.* 1999) and physiological or genetic adaptation to pollution (Elskus *et al.* 1999; Hahn, 1998; Meyer *et al.* 2002; Roy *et al.* 2001). There are no studies that have reported large induction of antioxidant enzymes in fish after pro-oxidants exposure (Di Giulio & Meyer, 2008). In some cases, antioxidant enzymes have been depressed at the level of activity or expression after pro-oxidants exposure (Fujii & Taniguchi, 1999; Kim & Lee, 1997; Pedrajas *et al.* 1995; Radi & Matkovics, 1988; Stephensen *et al.* 2002; Zikic *et al.* 1997). The adaptation to oxidative stress in fish may also be a significant factor in some cases (Bacanskas *et al.* 2004; McFarland *et al.* 1999; Meyer *et al.* 2003). Hasspieler *et al.* (1994a, b) and Ploch *et al.* (1999) described the ability of resistance to contaminants in fish species due to different antioxidant defences.

The variability of antioxidant responses in wild fish may be associated with the fact that most fish populations are highly outbread with a high degree of genetic variability (Di Giulio & Meyer, 2008). Furthermore the various antioxidant isoenzymes, potentially good biomarkers, have been not detected due to the absence of specific substrates and antibodies (Di Giulio & Meyer, 2008).

An increase or inhibition of the antioxidant enzymes levels can depend on the intensity and the duration of the stressors actuation as well as the susceptibility of the exposed fish species (Oruc & Usta, 2007). In addition,

the fluctuation of the levels of antioxidant compounds in organ systems may be caused by a different concentration of xenobiotics in these systems due to the blood volume differences in the tissues of fish (Isik & Celik, 2008).

4. Pesticides evoking oxidative stress in fish

The pesticides that have been shown to cause oxidative stress include organochlorine pesticides, organofluorine pesticides, organophosphates, carbamates, pyrethroids, bipyridyl herbicides, triazine herbicides, chloroacetanilide herbicides and other pesticides.

The genesis of oxidative stress in fish organism is affected also by pesticides biodegradation products.

a) Organochlorine pesticides

These pesticides belong to persistent organic pollutants (POPs).They had been heavily used for agricultural purposes as insecticides and defoliants in 1960s, and they were banned in the 1970s in most of the developed countries. Their residues persist in the environment up to this day.

DDT is a hepatotoxic substance. Its hepatotoxicity is related to the accumulation of lipid peroxidation products or protein carbonyl derivates within hepatocytes (Grune, 2000; Dalle-Donne *et al.* 2003; Requena *et al.* 2003) and to the decrease of GSH concentration. The hepatocyte necrotic cell death has been observed after the administration of low concentrations of methylmercury or DDT in *Hoplias malabaricus* (traíra). The exposure to both chemicals at once produced greater effect than isolated exposure. This is the evidence for the protective effect of the increase of GST and G6PDH activities which thereby protected cells from lipid damage and cell death. Conversely, single DDT actuation caused the decrease of GST (Filipak Neto *et al.* 2008).

HCB. There are two alternatives of the HCB-induced ROS production. The first source of ROS is the uncoupling of the electron transport chain from a monooxygenase activity (Ferioli *et al.* 1984). The second source of ROS is the metabolism of pentachlorophenol, one of the HCB major metabolites (Wang *et al.* 2001). The concentration range of 2-200 μg L^{-1} caused the increase in lipid peroxidation and the content of reactive oxygen species in common carp (*Cyprinus carpio*) (Song *et al.* 2006). Exposure to 2 μg L^{-1} of HCB evoked slight induction of hepatic SOD activity and GSH content in common carp after 10 day exposure with subsequent inhibition after 20 days of the exposure (Song *et al.* 2006). The results demonstrate the adaptation of the liver to slight oxidative damage during the shorter exposure period (i.e. 10 days) and HCB-induced oxidative stress in the organ during the longer exposure period (i.e. 20 days). A different situation was observed in the brain. SOD activity and GSH content depressed after 5, 10 or 20 days of the treatment, and the brain tissue suffered from a severe oxidative stress – even after as short as 5 days of the exposure (Song *et al.* 2006). The brain was more sensitive than the liver towards the oxidative effect of this pesticide. It is possible that the brain is the most important target organ of HCB action in fish (Song *et al.* 2006). The possible explanation lies in a high mitochondria oxidative metabolism of the brain (Soengas & Aldegunde, 2002), in large amount of easily oxidized polyunsaturated fatty acids, and in the relatively low antioxidant potential of this organ (Mates, 2000).

Chlorothalonil (TCIN). According to the study by Baier-Anderson & Anderson (2000), TCIN is able to suppress H_2O_2/hypochlorous acid production and interferes with the NADPH oxidase function. The dose-dependent suppression of zymosan- or PMA (phorbol myristate acetate)-stimulated $\cdot O_2^-$ production was observed after *in vitro* exposure of macrophages from striped bass (*Morone saxatilus*) to TCIN. Both stimulants of $\cdot O_2^-$ production serve as activators of NADPH oxidase. TCIN probably interferes with the NADPH oxidase function in the activation of protein kinase C which is a mutual point of both activation pathways of NADPH oxidase (through zymosan and PMA) (Baier-Anderson & Anderson, 2000). Multiple thiol groups of NADPH oxidase enzyme complex may be attacked by TCIN, and this leads to the loss of the NADPH oxidase function and thereby TCIN disrupts mitochondrial metabolism (Baier-Anderson & Anderson, 2000). Furthermore TCIN was able to suppress macrophage function in striped bass and GSH has a great importance in the detoxification of TCIN in fish liver (Baier-Anderson & Anderson, 2000).

Endosulfan elevated levels of GPX, GST, GSH and LPO in the liver, gill and kidney of the green snakehead (*Channas punctatus*). On the other hand CAT decreased in all these organs (Pandey *et al.* 2001). This decrease in CAT activity could be due to the excess of superoxide radicals (Kono & Fridovich, 1982).

In the plasma of Nile tilapia (*Oreochromis niloticus*) exposed to 7 ppb of this insecticide for 96 h the treatment lead to elevated LPO (Tellez-Banuelos *et al.* 2009). In this experiment, the acute exposure to the sublethal concentration of endosulfan provoked an unspecific activation of spleen macrophages in *O. niloticus* with the sequential increase in the phagocytic activity of splenocytes, production of oxygen reactive species and LPO (Tellez-Banuelos *et al.* 2009).

b) Organofluorine pesticides

Etoxazole belongs to a new generation of acaricides. Acetylcholine esterase (AChE) inhibition was correlated with etoxazole concentrations and the significant statistical increase of MDA level was found in the liver of *Oreochromis niloticus* at the concentration 0.81 ppm after 7 days of the exposure (Sevgiler *et al.* 2004). According to this author, there may be a significant correlation between the increase in lipid peroxidation and

the inhibition of AChE activity in the liver of *O. niloticus*. Thus, it may be considered that etoxazole mediated lipid peroxidation relates to its anticholinergic activity (Sevgiler *et al.* 2004).

c) Organophosphates

Organophosphates might induce oxidative stress but the information on such ability is still incomplete (Oruc & Usta, 2007). Toxicity of organophosphates is caused mainly due to the inhibition of acetylcholine esterase. Cholinergic hyperactivity after the AChE inhibition initiates the accumulation of free radicals leading to lipid peroxidation, which may be the initiator of cell injury (Yang *et al.* 1996). Although the main mode of action of organophosphates is the AChE inhibition, the increase in lipid peroxidation and changes in ATPase activity were observed in context to the toxicity of these pesticides (Hazarika & Sarkar, 2001). The long-time administration of organophosphates caused a gradual exhaustion of SOD, GR, GPX and GST or the increase of antioxidative defence systems (Gupta *et al.* 1992; Begchi *et al.* 1995; Gultekin *et al.* 2000).

Dichlorvos. MDA content increased dose dependently following dichlorvos exposure in stinging catfish (*Heteropneustes fossilis*) (Vadhva & Hasan, 1986). The concentration 1–5 mg L^{-1} of dichlorvos increased dose-dependently CAT activity in the liver, GSH level in the brain, muscle and heart, SOD in every organ esp. liver, and lipid peroxidaton in the brain in brown bullhead (*Ictalurus nebulosus*) after 24 hours of the exposure (Hai *et al.* 1997). In contrast, the same concentrations for the same treatment period caused GSH decrease in the liver and muscle, LPO, GSH, GPX, CAT increase in the brain, SOD and CAT increase in the liver and CAT increase in the kidney of common carp (*Cyprinus carpio*) (Hai *et al.* 1997). It is evident that the oxygen radical effect and the subsequent tissue oxidative damage upon dichlorvos treatment are not as intensive in common carp tissues as in catfish. It may by due to the different life styles of those two species (Hai *et al.* 1997). Oxidative stress in *Anguilla anguilla* was observed after the exposure to this pesticide (Pena-Llopis *et al.* 2003). A sublethal concentration of dichlorvos (0.17 mg L^{-1}, 20 % of the 96h LC_{50}) declined and oxidised muscular GSH, declined the GSH: GSSG ratio, which is a proof of the process of oxidative stress. AChE and GR activities were inhibited in the brain.

Trichlorfon (TRC). Thomaz *et al.* (2009) described glutathione S-transferase (GST) depletion and hydroperoxide (HP) accumulation after 96 h TRC exposure (0.5 mg L^{-1}) in the heart of Nile tilapia (*Oreochromis niloticus*). The heart is the most sensitive organ when compared to the liver and gills. The antioxidant mechanisms in the gills and liver were able to remove ROS from TRC actuation, preventing the increase of LPO. This might indicate a tissue-specific adaptation to the oxidative conditions after 96 h of TRC exposure. The ROS production and LPO might play a role in TRC-induced cytotoxicity. The enzymatic responses shown in Nile tilapia after TRC exposure can be related to the innate antioxidant potential and defensive system presented by different tissues as it is in the case of paper mill effluent exposure (Ahmad *et al.* 2000).

Diazinon. Oruc & Usta (2007) described the increase of SOD and GPX activity, as well as MDA content, in the gill and muscle of *Cyprinus carpio* after diazinon exposure when compared to the control fish. The slight decrease of GPX was shown in the kidney after diazinon exposure. Low activities of GPX in the kidney of diazinon-exposed fish demonstrate the inefficiency of these organs in neutralizing the impact of peroxides (Ahmad *et al.* 2000). Furthermore, diazinon exposure significantly increases lipid peroxidation in the muscle of *C. carpio*. The diazinon-mediated lipid peroxidation is coupled with the induced anticholinergic activity of this pesticide. The toxicity of diazinon mediates free radical induced oxidative cell injury that tends to elevate lipid peroxidation in the gill and muscle tissues of *C. carpio*. Additionally SOD activity increased in the tissues of *C. carpio* after diazinon exposure, and the increase was more distinct in the gill (Oruc & Usta, 2007). This increase in the SOD activity indicates the increase in $\cdot O_2^-$ production (Zhang *et al.* 2004a). Oruc & Usta (2007) attributed the dose-dependent decrease of CAT activity in the muscle of *C. carpio* to the recorded high SOD activity due to the diazinon-induced $\cdot O_2^-$ production.

Diazinon exposure decreases Na^+/K^+ -ATPase activity in the muscle and kidney of *C. carpio*. Two different mechanisms can lead to Na^+/K^+ -ATPase inhibition. The first is a direct effect of ROS and MDA level, and the second an indirect effect of changes in membrane fluidity (Oruc & Usta, 2007).

Methylparathion (MP).The experiment described by Isik & Celik (2008) in rainbow trout with the administration of MP and diazinon has shown the significant increase of MDA content in the liver and muscle tissues after 24h and 48h periods, GSH depletion in the liver and gills after the same periods, and GSH increase in the muscle after 48 h exposure to both pesticides and after 72 h period of diazinon exposure. The cellular oxidative stress due to MP can cause the fluctuation of SOD activity. The decrease in GR activity in the tissue is caused partly by the variation in glutathione concentration in MP and diazinon treated cells and partly by the slow GSH regeneration. The decline in GSH content in the tissue can be due to its utilization to tackle the prevailing oxidative stress under the influence of ROS generated from MP and diazinon oxidative actuation. The decrease in the intracellular level of GSH and GR activity caused by MP and diazinon leads to the oxidative imbalance and induces oxidative damage of cells.

The experiment in *Brycon cephalus* exposed to the sublethal concentration (2 mg L^{-1}) of methylparathion for 96 hours was performed by Monteiro *et al.* (2006). There was observed the significant induction of super-

oxide dismutase, catalase and glutathione S-transferase activities in the gills, liver and white muscles while the decrease in glutathione peroxidase activity and increase in LPO values were restricted to the gills and white muscle. The increase of SOD and CAT activities probably responded to the increased superoxide anions ($\cdot O_2^-$) and H_2O_2 levels (John *et al.* 2001). The observed decrease of GPX in the gills and white muscle could be related to the $\cdot O_2^-$ production (Bagnasco *et al.* 1991) or to the direct action of pesticides on the enzyme synthesis (Bainy *et al.* 1993).

The considerable decline in the reduced GSH level may be related to its increased utilization and its conversion into oxidized glutathione and an inefficient GSH regeneration (Monteiro, 2006). Low hepatic LPO content suggests that this organ is probably more resistant to the oxidative stress due to the large spectrum of antioxidant mechanisms and high vitamin E levels found in the liver of *Brycon cephalus* (Wilhelm-Filho and Marcon, 1996). The results indicate that the gills and white muscle are more sensitive organs to oxidative stress in comparison to the liver. The main reason for these differences could be the different rate of free radical generation and different antioxidant potentials in the tissues. The antioxidant system of these tissues is not as efficient as that of the liver.

Fenthion. The sublethal concentration (0.45 mg L^{-1}) of fenthion in the brain of *Oreochromis niloticus* caused the elevation in GSSG content after 96 h and GPX activity increase after 24 h (Piner *et al.* 2007). The induction of GPX activity could be related to the scavenge of H_2O_2 and lipid peroxides by utilizing GSH (Piner *et al.* 2007).

Azinphosmethyl. Administration of this pesticide together with 2, 4-dichlorophenoxyacetic acid resulted in the significant elevations of MDA level in the kidney, brain and gill tissue of Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*). In the gill, brain and kidney, CAT activity was higher in *O. niloticus* than in *C. carpio*. In the brain and kidney, SOD activity was higher in *O. niloticus* than in *C. carpio*. In the gill tissues, activity of SOD and GST did not show any significant difference between species. Increased GST activity in the kidney was similar both in *O. niloticus* and *C. carpio*. GPX was increased more in the gill of *C. carpio* than that in *O. niloticus.*

The results suggest that the most affected tissues were the kidney and gill. Elevated GST activity may relate to the better protection against pesticide toxicity. GPX activity may be increased due to the increased production of H_2O_2 derived from $\cdot O_2^-$ (Oruc *et al.* 2004). CAT together with the high SOD activity provided an abundant protective effect (Oruc *et al.* 2004).

The higher CAT activity could reflect the high H_2O_2 production from the increased oxygen consumption (Ritola *et al.* 2002a). The gill of *O. niloticus* showed the highest changes in SOD activity among the tissues (Oruc *et al.* 2004).

The increase in SOD activity could reflect the increased generation of ROS. In this study, 2,4-D azinphosmethyl and their combined treatment were unable to stimulate the lipid peroxidation process in the exposed fish. Low levels or absence of LPO in the tissues is a marker of the protective effects of antioxidative enzymes (Oruc *et al.* 2004).

Azinphosmethyl in the sublethal concentrations (2.5 and 5 μg L^{-1}) caused GSH content decrease in the liver and kidney of juvenile rainbow trout after 24 h and 48 h of the exposure and renal and hepatic CAT activity decrease after 48 h and 96 h of the exposure (Ferrari *et al.* 2007). The inhibition of renal and hepatic CAT is related to the GSH decrease in these organs (Ferrari *et al.* 2007).

Chlorpyriphos (CPF). Mosquito fish (*Gambusia affinis*) exhibits the inhibition in activities of CAT, SOD and GR and the increased LPO and MDA level after the exposure to 297 μ L^{-1} (LC_{50}) for 96 h (Kavitha & Venkateswara, 2008). These facts suggest that the ROS-induced damage plays main role in the toxic effect of CPF. The consistent decrease in the antioxidant enzymes was due to the excessive generation of free radicals generated by CPF. The normal levels of antioxidants could not quench the excess of free radicals and thereby increased lipid peroxidation.

Monocrotophos treatment resulted in the decrease of CAT activity in the liver of asian stinging catfish (*Heteropneustes fossilis*) (Thomas & Murthy, 1976). Kavitha & Venkateswara (2007) described the induction in activities of CAT, SOD, GR and the increase of MDA concentration in viscera of mosquito fish (*Gambusia affinis*) after the exposure to 20.49 mg L^{-1} (LC_{50}) for 96 h. It is possible that the ROS-induced damage may be one of the main toxic effects of monocrotophos.

d) Carbamates

Carbaryl is an insecticide which causes GSH content decrease in the liver and kidney of juvenile rainbow trout (*Oncorhynchus mykiss*) after 24, 48 and 96 h of the exposure to 3 mg L^{-1}. The same concentration caused the increase of hepatic and renal GST after 24 h but the decrease after 96 h, the increase in hepatic CAT activity after 24 h of the exposure. Carbaryl is able to induce hepatic cytochrome P450 (CYP1A) and thereby increase GST level (Ferrari *et al.* 2007).

e) Pyrethroids

Deltamethrin belongs to synthetic pyrethroids that are the most popular and widely used insecticides all over the world. Deltamethrin exposure (0.75 μg L^{-1} for 48 h) caused the significant increase in LPO levels in all tissues of green snakehead (*Channa punctatus*), namely the liver, kidney, and gill and the increase in the activities of GST and GPX in the liver and kidney, while the significant decrease was observed in the activities of GST and GPX in the gills. The increase in GST activity was concomitant with the increase of glutathione

content in the liver and kidney. CAT activities were depressed in all organs of *Ch. punctatus* (Sayeed *et al.* 2003). CAT inhibition may be induced by superoxide radicals (Kono & Fridovich, 1982). Furthermore, Sayeed *et al.* (2003) described higher levels of GSH in the liver, kidney and gills, the significant increase of total thiol, non-protein thiol, and protein thiol in the liver, while the significant decrease of these parameters was observed in the gill. Gills are the primary site for the absorption of deltamethrin. It is not surprising that we noted the high level of LPO coupled with the depletion of antioxidant enzymes in this organ.

Cypermethrin. In *Oreochromis niloticus* and *Cyprinus carpio* exposed to the concentration of cypermethrin 3 μg L^{-1} during 10 days, SOD and CAT activities and MDA levels in the liver were increased. GPX activity increased in the liver of *O. niloticus* while it decreased in *C. carpio*. The changes of GPX activity may be related to the $\cdot O_2^-$ excess resulting in the SOD activity increase in exposed fish (Uner *et al.* 2001). However, in the kidney of both fish species SOD, GPX, CAT activities and MDA levels are increased, except CAT activity in *O. niloticus* (Uner *et al.* 2001). In view of the fact that the antioxidant enzyme activities in the liver of control group were higher than in the kidney while after the exposure they were higher in the kidney it is hypothesized that the kidney participates in the detoxification of cypermethrin or its metabolite (Uner *et al.* 2001).

f) Bipyridyl herbicides

Bipyridyl herbicides are known for their potential to make redox cycles and cause oxidative stress (Kappus & Sies, 1981).

Paraquat is a redox active chemical (Di Giulio & Meyer, 2008). Figueiredo-Fernandes *et al.* (2006) described effects of gender and temperature on the oxidative parameters in the liver of Nile tilapia exposed to paraquat. In their study males showed higher SOD values than females at both temperatures (17 °C, 27 °C). SOD activity in both genders was increased compared to the control group. On the other hand GR activity was highest in females at both temperatures, while no significant differences were found between males. GR is able to reduce paraquat to its cation radical (Richmond & Halliwell, 1982) which causes the oxidation of GSH leading to an increased GR activity (Stephensen *et al.* 2000). GST activity in males showed higher values than in females at both temperatures (Figueiredo-Fernandes *et al.* 2006). It is evident that gender had an effect on SOD and GST activities while no significant differences were observed in enzymatic activities after the exposure to different temperatures. However, slightly higher enzyme activities have been detected in both genders at 17 °C (Figueiredo-Fernandes *et al.* 2006). The higher temperature could block enzymes activities.

g) Triazine herbicides

Simazine is a selective chloro-S-triazine herbicide that was registered as an algicide for the use in aquariums, swimming pools, and ponds.

The effect of simazine exposure to common carp was described by Oropesa *et al.* (2009). In their field study there were observed increased GSH levels in the hepatopancreas and increased tissue MDA levels in carps from polluted reservoirs. In their laboratory toxicity test the highest GSH levels were also found in the hepatopancreas. MDA content remained in the range of levels in the control carps. However, the lipid peroxidation phenomenon was not observed in carps exposed to low concentrations (up to 45 μg L^{-1}) of simazine under subchronic conditions. Thereby it is evident that the presence of simazine in natural waters at the concentrations usually reported in the literature or expected after a correct application in agriculture does not cause oxidative stress in carps (Oropesa *et al.* 2009).

Atrazine. The exposure to atrazine leads to the stress oxidative phenomenon due to the ROS generation in bluegill sunfish (Elia *et al.* 2002). In addition, it has been described that this herbicide might cause the damage to the cell membranes since an increase of haemoglobin concentration in the plasma after the subacute exposure to this triazine was observed in mozambique tilapia (*Tilapia mossambica*) (Prasad *et al.* 1991). The measurement of tissue GSH and MDA levels has been suggested as a tool for the assessment of the exposure and cellular toxicity of atrazin in fish since sublethal concentrations of this herbicide induced oxidative stress in bluegill sunfish (*Lepomis macrochirus*) after the acute exposure (Elia *et al.* 2002).

The decrease of GSH levels was observed in rainbow trout (*Oncorhynchus mykiss*) and sand trout (*Pseudaphritis urvillii*) exposed to the sublethal concentrations of atrazine and cyanazine (Davies *et al.* 1994).

h) Chloroacetanilide herbicides

Butachlor. The concentrations 1, 2 and 2.5 ppm induced the significant increase in LPO and MDA level in the liver, kidney, gills and heart of the African catfish (*Clarias gariepinus*) after 24 h exposure (Farombi *et al.* 2008). It is hypothesized that ROS may be coupled with the metabolism of butachlor leading to lipid peroxidation (Farombi *et al.* 2008). The concentration-dependent increase of SOD and CAT activities was observed in the liver and kidney of *C. gariepinus*, whereas concentration-dependent decrease of these enzymes was detected in the gills and heart. The increase of SOD in the liver and kidney may be a response to the production of superoxide anions. SOD converts the superoxide radical to H_2O_2. The increase of CAT activity in the same organs may be due to the SOD-stimulated H_2O_2 production since CAT is responsible for the detoxification of hydrogen peroxide to water (Farombi *et al.* 2008). System SOD-CAT is considered to be the first line of the defence against oxidative stress (Pandey *et al.*

2003) and the increase of these antioxidant enzymes in the kidney indicate an important role of kidney in the butachlor detoxification (Farombi *et al.* 2008).

In the same study the GST activity and GSH level were increased in the liver whereas in the kidney, gill and heart they were decreased. The decrease in GSH level and GST activity was caused by the ROS overproduction which depleted GSH and inactivated GST (Farombi *et al.* 2008).

i) Other pesticides

Rotenone is another insecticide that produces ROS by the inhibition of electron transport in mitochondria (Li *et al.* 2003a).

Diuron is a biologically active and persistent pollutant found in the soil, water, and sediments, presenting slight toxicity to mammals and birds as well as moderate toxicity to aquatic invertebrates (Giacomazzi & Cochet, 2004). However, the principal product of its biodegradation, DCA (3,4-dichloroaniline) shows higher toxicity and is also persistent in the soil, water, and groundwater (Giacomazzi & Cochet, 2004). Previous studies suggested that DCA-induced free-radical generation and antioxidant depletion might cause oxidative stress and lipid peroxidation in the liver of crucian carp (Li *et al.* 2003b) which can be illustrated by the high incidence of necrosis and other degenerative lesions found in the liver.

Fenpyroximate is used as an acaricide. The fenpyroximate toxicity lies in its effects on the mitochondrial redox respiratory chain (Barrientos & Moraes, 1999; Motoba *et al.* 1992). Sublethal concentration of this pesticide exhibited the significant increase of SOD, CAT and GPX activities in the gill cell line (FG) cells of marine flounder (*Paralichthys olivaceus*) during the 48 h exposure period. The concentration 300 nM in the FG cells increased SOD, CAT, GPX activity within the first 12 h of the exposure, then those activities decreased. The concentration 12 nM in the gill during 6 h raised the level of all three enzymes, in the liver it raised GPX level after 12 h, SOD and CAT after 24 h. Results showed that the background activities of the above three antioxidant enzymes tested in the liver tissue were about 3–6 times higher than those in the gill tissue of flounder (Na *et al.* 2009). This fact suggests higher sensitivity of the gill tissue to fenpyroximate compared to the liver tissue. Additionally, fenpyroximate can cause the significant increase of the activities of the antioxidant enzymes SOD, CAT and GPX both *in vivo* and in exposed FG cells (Na *et al.* 2009).

Glyphosate is an active ingredient of the preparation Roundup which has herbicide effect. Lushchak *et al.* (2009) recorded following changes in goldfish (*Carassius auratus*) after Roundup exposure (2.5–20 mg L^{-1} for 96 h): the increase of LOOH concentration in the kidney (at 10 mg L^{-1}), the decrease of low molecular mass thiol (L-SH) levels in the brain (at 2.5 mg L^{-1}) and in the liver (at 20 mg L^{-1}), the reduction of SOD, GR and G6PDH activities in the brain, liver and kidney, the increase of CAT activity in the liver and kidney and the decrease of GST activity in the liver. The decrease of SOD, GR, GST, G6PDH and the small decrease in the L-SH levels may indicate that in goldfish tissues Roundup induces mild oxidative stress (Lushchak *et al.* 2009). The small increase of hepatic catalase activity was described in *Prochilodus lineatus* exposed to 10 mg L^{-1} of Roundup for 24 h, which could be due to the Roundup-mediated oxyradical production (Langiano & Martinez, 2008).

5. Conclusion

Apparently a manifestation of oxidative stress varies in terms of fish species and organs. Factors explaining these variations were presented above. In the future the study of oxidative stress should be focused on the searching for more convenient biomarkers of this process. It is necessary to identify antioxidant genes present in fish, their regulation and their biochemical statistics. Results from the studies in mammals are a great source of information for fish ecotoxicology. It is necessary to develop and apply new methods of detection because traditional methods frequently provide non-objective results, because they often assess acute toxicity observable at high concentrations that are not normally encountered in the environment.

The changes in concentration or levels of biomarkers of oxidative stress in fish may be helpful in assessing the risk of environmental contaminants and the safety of fish meat for human nutrition.

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