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Responses of Biochemical Markers in the Fish *Prochilodus lineatus* Exposed to a Commercial Formulation of Endosulfan

Carla Bacchetta · Jimena Cazenave · María Julieta Parma

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Abstract Among the most extensively used compounds for the pest control in Argentinean crops is the organochlorine endosulfan. The sublethal effects of the commercial endosulfan formulation on hematology and lipid peroxidation (LPO) of the neotropical fish *Prochilodus lineatus* were investigated. Firstly, we calculated acute toxicity (LC_{50}) in order to define sublethal concentrations (0, 1.2, and 2.4 μg L^{-1}). Hematological and oxidative stress responses were assessed at 24, 48, and 96 h. Endosulfan exposure significantly diminished the hemoglobin concentration, mean cell hemoglobin, and total plasma protein and increased white blood cells count and plasma glucose after 96 h. Exposed fish showed an alteration of the differential leukocytes count, evidenced by more thrombocytes and monocytes and less lymphocytes and neutrophils. Endosulfan increased LPO levels in intestine, liver, and brain in both sublethal concentrations. The present results suggest that endosulfan produces biochemical and physiological alterations, including immunological disorders, and it is a good inductor of oxidative stress in *P. lineatus*.

Keywords Fish · Biomarkers · Hematology · Oxidative stress · Pesticide

C. Bacchetta · J. Cazenave · M. J. Parma (✉)
Laboratorio de Ictiología, Instituto Nacional de Limnología (INALI-CONICET-UNL),
Paraje El Pozo, Ciudad Universitaria UNL,
3000 Santa Fe, Argentina
e-mail: mjparma@inali.unl.edu.ar

J. Cazenave · M. J. Parma
Departamento de Ciencias Naturales,
Facultad de Humanidades y Ciencias,
Universidad Nacional del Litoral (FHUC-UNL),
Paraje El Pozo, Ciudad Universitaria UNL,
3000 Santa Fe, Argentina

1 Introduction

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-ethano-2,4,3-benzodioxathiepin-3-oxide) is a broad spectrum organochlorine insecticide which controls insects by contact action and by ingestion of treated plant material, on field cereal crops, oilseeds, coffee, vegetables, and fruit crops. Endosulfan reaching the water column will have a propensity to be absorbed into benthic sediment, which may eventually become a source of endosulfan redistribution into the overlying waters. Due to its semivolatility, endosulfan is present in the global background atmosphere and is capable of undergoing long-range transport to remote environments, including the Arctic (Weber et al. 2009). Due to its toxic effects, the US EPA (2002) has classified endosulfan as highly toxic to both freshwater and marine fish and defined the level for protection of freshwater aquatic life as 0.22 μg L^{-1}. However, endosulfan concentrations from 0.2 to 13.5 μg L^{-1} have been found in

water bodies near rice fields in neotropical wetlands of Argentina (Silva et al. 2005), exceeding the maximum permitted concentration.

Studies carried out on the toxicity of endosulfan have shown that 96 h medium lethal concentration (96-h LC_{50}) values vary by several orders of magnitude among fishes, ranging between 0.4 and 12.8 μg L^{-1} (Vittozzi and De Angelis 1991; Jonsson and Toledo 1993; Capkin et al. 2006; Magesh and Kumaraguru 2006; Pandey et al. 2006; Ballesteros et al. 2007; Hii et al. 2007; Tellez-Bañuelos et al. 2009). Differences in sensitivity to endosulfan can be observed between different fish taxa. The families Cichlidae (*Oreochromis niloticus*, Nile tilapia) and Channidae (*Channa punctatus*, spotted murrel), both belonging to order Perciformes, seem to be predominantly tolerant, with the highest 96-h LC_{50} values (12.8 and 7.7 μg L^{-1}). On the contrary, families Symbranchidae, Chanidae, Salmonidae, and Anablepidae have higher sensitivity to endosulfan, with 96-h LC_{50} values between 0.4 and 1.7 μg L^{-1}.

Current water quality criteria for priority pollutants were mainly derived from laboratory toxicity tests using standard test species. However, it is known that some pollutants may be more or less toxic to native fish species compared to the standard ones (e.g., *Oncorhynchus mykiss*, *Pimephales promelas*, *Lepomis macrochirus*, *Danio rerio*, *Oryzias latipes*) because of interspecific differences in sensitivity (Buhl 1997). Thus, the setting of realistic water quality criteria requires information on the response of native endangered species to pollutants. Despite the fact that endosulfan is widely used in neotropical regions, little is known about its toxicity on neotropical fish species. However, some information is available about its effects on fish from other regions. Exposure to sublethal concentrations of waterborne and dietary endosulfan has induced a wide diversity of effects in fish, including neurotoxicity and genotoxicity (Dutta and Arends 2003; Ballesteros et al. 2009a), liver toxicity (Mishra and Schukla 1994; Glover et al. 2007; Ballesteros et al. 2009b), hematological and histopathological changes (Nowak 1996), and alterations in the immune and endocrine systems (Bisson and Hontela 2002; Coimbra et al. 2005; Harford et al. 2005).

Blood offers an important profile to study the toxicological impact on animal tissues. To measure the response to environmental stressors, some changes in blood composition (hematocrit, hemoglobin content, red and white cell count, plasma biochemistry) are used widely to assess the overall condition of fish (Niimi 1990; Leatherland et al. 1998; Handy and Depledge 1999; van der Oost et al. 2003).

A fundamental aspect of aerobic life is the potentially dangerous sequels that might be engendered when antioxidant defenses are overcome by prooxidant forces (Winston and Di Giulio 1991). Changing the balance toward an increase in the prooxidant over the capacity of the antioxidant is defined as oxidative stress. This situation can make an organism suffer oxidative damage in terms of lipids, proteins, or DNA (Monserrat et al. 2007). Being lipids the key component in the cell membrane makes it necessary to understand the mechanisms and consequences of lipid peroxidation (LPO) in biological systems (Kelly et al. 1998). Malondialdehyde, a breakdown product from lipid peroxidation, indicates lipids oxidative damage from a variety of toxicants (Kohen and Nyska 2002) and can be used as a suitable biomarker in fish (Oakes et al. 2004; Crestani et al. 2007; Zhang et al. 2008).

Prochilodus lineatus (Pisces, Prochilodontidae) is the most abundant fish species and the main resource in commercial fisheries in the Middle Paraná River (Rossi et al. 2007). Besides, this fish represents an appropriate species for environmental monitoring, as it is a bottom-feeder animal which is in contact with xenobiotics in water and sediment (Camargo and Martinez 2006; Vanzella et al. 2007; Carvalho and Fernandes 2008; Cavalcante et al. 2008; Cazenave et al. 2009).

The present study was designed to evaluate sublethal changes in hematological and oxidative stress markers on different tissues of *P. lineatus* exposed to waterborne endosulfan. Moreover, this work is aimed at enlarging the endosulfan toxicity database of aquatic vertebrates.

2 Materials and Methods

2.1 Experimental Design

Juvenile *P. lineatus* (n=132) were collected from an unpolluted area of the Paraná River (31°42′ S, 60°45′ W, Argentina). The average weight and standard

length of fish were 59.9±2.8 g and 13.5±0.2 cm, respectively. For acclimation purpose, fish were held in 150-L tanks in well-aerated dechlorinated water for 2 weeks and were fed once daily, with dry commercial pellets.

Tests were conducted in 25-L glass aquaria, under static conditions following the recommendations of the OECD Guidelines for Testing of Chemicals (OECD 1992). The experiments were carried out in 12:12-h light–dark cycles, and the test water characteristics were pH 6.7±0.1, total hardness 315± 7.1 ppm CO_3Ca, and temperature 24±1°C. Fish feeding was suspended 24 h before the beginning of tests. Endosulfan test solutions were prepared from a commercial formulation containing 35% active ingredient (Zebra Ciagro®, Ciagro S.A. Argentina) since endosulfan in such grade is frequently employed in field practices. Endosulfan concentrations in water was quantify at the beginning of each experiment by GC-ECD, according to US EPA (1989), showing recoveries >95% of the nominal value. For lethal 96-h toxicity test, preliminary range-finding tests were performed to establish a mortality range and to define test concentrations.

The lethal 96-h toxicity test consisted of exposing fish to five concentrations of endosulfan (2.2, 2.8, 3.5, 4.4, and 5.5 μg L^{-1}). One additional group served as a control and was kept in tap water. Each concentration and the control group consisted of five individuals and were tested by duplicate. Mortality of test organisms was recorded when opercular movements stopped, and dead individuals were removed instantly. Accurate records of mortality counts were maintained at a regular interval of 12 till 96 h. As an estimate of relative lethal toxicity, LC_{50} values and their corresponding 95% confidence limits were calculated for 24, 48, 72, and 96 h time points using US EPA probit software 1.5 free version (US EPA 1992).

For the sublethal toxicity test, fish were exposed to 0 (control), 1.2 (50% 96-h LC_1), and 2.4 μg L^{-1} (96-h LC_1) endosulfan. Test and control groups were tested by duplicate. At 24, 48, and 96 h, eight control and exposed fish of each concentration were removed from test aquaria and anesthetized with benzocaine according to Parma de Croux (1990). After blood sampling, fish were sacrificed and dissected. Gills, liver, intestine, and brain were immediately frozen in liquid nitrogen and stored at −80°C until biochemical determinations were carried out.

2.2 Hematological Parameters

Blood was rapidly extracted from the caudal vessel by dissection of the caudal peduncle (Reichenbach-Klinke 1980; Roberts 1981), using heparinized syringes. Red blood cells (RBC) counts were performed with a Neubauer chamber, using physiological solution for dilution. Hematocrit (Ht) values were determined by the micromethod using capillary tubes and centrifuged at 1,409×*g* for 10 min. Hemoglobin concentration (Hb) was measured by the cyanomethemoglobin method at wavelength of 546 nm on a spectrophotometer (Houston 1990). Mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated from primary indices.

A drop of freshly collected blood was smeared on clean slides to estimate the total white blood cells (WBC) counts and for determination of leukocyte frequency according to Tavares-Dias and de Moraes (2007). The air-dried blood smears were fixed in absolute methanol for 10 min and stained by May–Grünwald–Giemsa (Houston 1990). Total WBC was performed in relation to the number of erythrocytes counted in randomly selected fields and recalculated per unit volume: WBC/μL = number of WBC in blood smear × (RBC/μL/4,000 RBC counted in smear). Differential leukocytes counts were performed by identifying 100 white blood cells en each blood smear.

Additionally, plasma was separated from whole blood by centrifugation at 1,409×*g* for 10 min. Glucose and total protein concentrations were determined colorimetrically using commercial kits (Wiener Lab®).

2.3 Lipid Peroxidation

LPO in gills, intestine, liver, and brain was determined by measuring the formation of thiobarbituric reactive substances (TBARS), according to Fatima et al. (2000). Tissues were individually homogenized with 0.15 M potassium chloride solution using a glass homogenizer. Then, 1.0 mL homogenate was incubated during 1 h at 37°C with continuous shaking. Afterward, 1.0 mL of 5% trichloroacetic acid and 1.0 mL of 0.67% thiobarbituric acid were added to each sample and mixed. Then, each vial was centrifuged at 1,409×*g* for 10 min. The supernatant

was separated and placed in a boiling water bath for 10 min, cooled to room temperature, and measured spectrophotometrically at 535 nm. The rate LPO was expressed as nanomoles of TBARS formed per hour per milligram of proteins. Protein content of each sample was determined according to Bradford (1976).

2.4 Statistical Analysis

All data are reported as mean ± standard error. Data obtained for different biomarkers were first tested for normality using Shapiro–Wilks test. Variables with no normal distribution were analyzed by Kruskal–Wallis test. One-way analysis of variance, followed by a posteriori Tukey test, was performed to evaluate changes in biomarkers between treatments and control group and time periods. Differences were considered statically significant when $p<0.05$. As there were no significant differences in the studied parameters of control groups at different time periods, all control values were pooled.

3 Results

The 24-, 48-, 72-, and 96-h LC_{50} values (95% confidence limit) were 4.1 (3.7–4.5), 3.9 (3.6–4.3), 3.7 (3.3–4.1), and 3.7 (3.3–4.1) μg L^{-1}, respectively. No mortality occurred in the control and the 2.2-μg L^{-1} endosulfan-containing group. Complete mortality of the fish was observed after 72 h at the highest endosulfan concentration (5.5 μg L^{-1}).

The results showed that sublethal endosulfan concentrations produced changes in all the measured hematological parameters (Table 1). Some biomarkers, such as Ht and derived indices, had a quick response to endosulfan, showing variations after 24 and 48 h. Most of them returned to control values at the end of the experiment. Other biomarkers changed after 96 h of endosulfan exposure. A decrease in Hb, MCH, and total plasma protein level and a significant increase in WBC and plasma glucose were observed at the highest concentration (2.4 μg L^{-1}). Differential leukocytes count was also affected, showing higher proportion of thrombocytes and monocytes and lower proportion of lymphocytes and neutrophils (Fig. 1). Basophils were not found in the prepared smears and eosinophils and monocytes were the rarest white blood cells.

Table 1 Hematological parameters of *P. lineatus* exposed to sublethal concentrations of endosulfan at different time periods

Parameter	Control group	1.2 μg L^{-1}			2.4 μg L^{-1}		
		24 h	48 h	96 h	24 h	48 h	96 h
RBC (10^6 μL^{-1})	1.64±0.04	1.84±0.11	2.26±0.12*	1.92±0.12*	1.65±0.11	1.71±0.07	1.63±0.04
Ht (%)	34.52±1.11	24.12±1.89*	31.18±0.99	34.70±1.11	23.08±2.47*	30.94±1.10	31.60±1.97
Hb (g dL^{-1})	6.56±0.27	8.04±0.49*	8.90±0.28*	7.46±0.35	7.46±0.58	7.50±0.35	5.35±0.18*
MCH (pg)	38.53±1.19	43.90±2.68	39.64±1.70	39.42±1.08	41.38±4.57	43.96±2.06	32.88±0.88*
MCV (μm^3)	200.61±6.88	133.19±14.45*	151.26±13.29*	185.07±7.26	154.39±16.79	181.30±6.63*	194.54±12.29
MCHC (%)	18.51±0.65	33.98±2.90*	26.62±1.31*	21.42±0.46*	28.35±4.52*	24.27±0.87*	17.40±0.94
WBC (μL)	27,401±3,965	19,749±5,409	34,378±8,777	32,520±5,480	15,444±3,227	34,892±5,905	54,656±9,004*
Glucose (g L^{-1})	0.56±0.10	0.76±0.05	0.70±0.06	0.72±0.04	1.02±0.06*	0.73±0.11	0.96±0.07*
Total protein (g dL^{-1})	2.60±0.06	2.35±0.19	2.34±0.11	2.26±0.11*	2.17±0.19*	2.17±0.07*	1.84±0.06*

The values are expressed as means ± SE

*$p<0.05$, the significance levels observed in comparison to control group

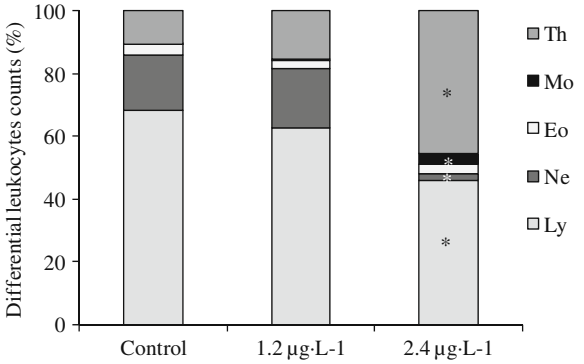

Fig. 1 Differential leukocytes count of *P. lineatus* exposed to sublethal concentrations of endosulfan for 96 h (*Th* thrombocytes, *Mo* monocytes, *Eo* eosinophils, *Ne* neutrophils, *Ly* lymphocytes). *$p<0.05$, the significance levels observed in comparison to control group

In the highest concentration, a significant increase in liver, intestine, and brain LPO was found at 96 h. Increased LPO was only registered at 24 and 48 h, in the liver and brain of 1.2 µg L^{-1} exposed fish (Fig. 2). There were no significant differences in gills LPO levels between control and endosulfan exposed fish.

4 Discussion

The endosulfan 96-h LC_{50} value reported in the present study falls in the range of previously published studies (Table 2). The Asian swamp eel (*Monopterus albus*), the neotropical fish *Jenynsia multidentata*, and the estuarine fish *Chanos chanos* seem to be the most sensitive species, with LC_{50} values lower than those of *P. lineatus*. On the other hand, Pandey et al. (2006) and Tellez-Bañuelos et al. (2009) found higher values in *C. punctatus* and the Nile tilapia (*O. niloticus*), recording values of 7.7 and 12.8 µg L^{-1}, respectively. These differences in endosulfan toxicity could be due to several factors, such as differences in the sensitivity of target site, detoxification systems, lipid content, and fish size. The metabolic and dispositional influences will determine how much active toxicant is available for interaction with its targets (Chambers and Carr 1995). Ballesteros et al. (2007) found differences in endosulfan toxicity between males and females of *J. multidentata*. According to the authors, the lower lipid content of male explained the differences in endosulfan sensitivity between sexes. Other factors

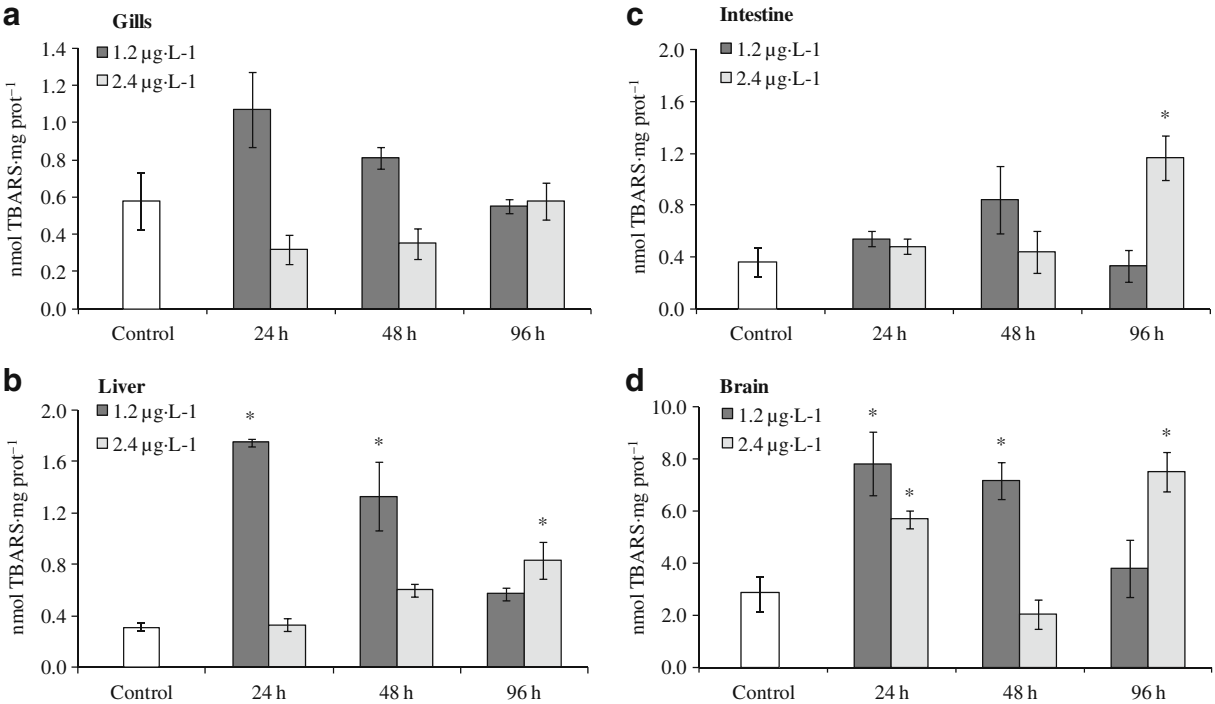

Fig. 2 LPO in **a** gills, **b** liver, **c** intestine, and **d** brain of *P. lineatus* exposed to sublethal concentrations of endosulfan at different time periods. The values are expressed as means ± SE. *$p<0.05$, the significance levels observed in comparison to control group

Table 2 Median lethal concentration of endosulfan in some fish species

Species	Duration (h)	Purity (%)	System	LC_{50} ($\mu g\ L^{-1}$)	References
Monopterus albus	96	33	Static	0.4	Hii et al. (2007)
Chanos chanos	96	35	Static renewal	0.6	Magesh and Kumaraguru (2006)
Jenynsia multidentata ♂	96	35	Static	0.7	Ballesteros et al. (2007)
Jenynsia multidentata ♀	96	35	Static	1.3	Ballesteros et al. (2007)
Oncorhynchus mykiss	96	33	Static renewal	1.7	Capkin et al. (2006)
Prochilodus lineatus	96	35	Static	3.7	Present study
Channa punctatus	96	35	Flow-through	7.7	Pandey et al. (2006)
Oreochromis niloticus	96	35	Static	12.8	Tellez-Bañuelos et al. (2009)
Hyphessobrycon bifasciatus	24	97	Static renewal	2.6	Jonsson and Toledo (1993)
Brachydanio rerio	24	97	Static renewal	1.6	Jonsson and Toledo (1993)

that are known to affect endosulfan toxicity to aquatic life include temperature, salinity, life cycle stage and bioassay procedure (Naqvi and Vaishnavi 1993).

Metabolism may be an important determinant of pesticide toxicity. The technical grade endosulfan is a mixture of isomers α-endosulfan and β-endosulfan in a ratio of 2:1–7:3. Biotransformation of α- and β-endosulfan occurs in the liver, through oxidation of cytochrome P450 (Lee et al. 2006). This metabolic pathway leads to the formation of endosulfan sulfate, the main biotransformation product of both isomers in aquatic organisms (Wan et al. 2005). Of all the metabolites of endosulfan, the sulfate appears to be the one that accumulates, predominantly in the liver and kidneys. Berntssen et al. (2008) found a relatively low formation of endosulfan sulfate and higher proportion of both isomers in muscle of *Salmo salar* exposed to dietary endosulfan for 92 days. Carriger et al. (2009) conducted flow-through toxicity test in two indigenous fish from South Florida exposed to endosulfan sulfate. They found 96-h LC_{50} values of 2.1 and 2.7 $\mu g\ L^{-1}$ for *Heterandria formosa* and *Gambusia affinis*, respectively, showing lethal concentrations similar to those reported for technical endosulfan.

Hematological parameters in control fish were within the normal values range previously reported by Parma de Croux (1994). Sublethal concentrations of endosulfan had adverse effects on hematological biomarkers of *P. lineatus*, mainly at the highest concentration tested. After 24 h of exposure, the fish showed a decrease in Ht and MCV and an increase in MCHC, returning to control values at the end of the experiment. Similarly, both a rise and an eventual decrease in Ht were recorded in *M. albus* by Hii et al. (2007). Because of unchanged RBC, the decrease in Ht after 24 h was likely due to cell size adjustments (decreased MCV), but the Hb content remains the same, which led to an increase in MCHC. This is a reaction that occurs in vivo in the blood due to the ability to regulate cell size or volume of erythrocytes, a characteristic widely documented in fish (Soivio and Nikinmaa 1981), and can be associated with stress response (Lecklin et al. 2000). At the end of our experiment, Hb content and MCH were significantly lower in exposed fish than in control ones. In *P. lineatus*, Parma et al. (2007) and Simonato et al. (2008) reported similar results after intoxication with cypermethrin and diesel oil, respectively. Conversely, Petri et al. (2006) found an increase in Hb and MCH in *S. salar* exposed to sublethal concentrations of endosulfan for 96 h.

Endosulfan effects on immune response of fish have been previously studied. For instance, exposure to sublethal concentrations of this pesticide significantly diminished the phagocytic activity in Nile tilapia (Girón-Pérez et al. 2008) and leukocytes count in *M. albus* (Hii et al. 2007). By contrast, we observed a significant increase in WBC of endosulfan exposed *P. lineatus*, which evidences immunostimulation. A similar immune response was found by Tellez-Bañuelos et al. (2009), who registered an activation of spleen macrophages in endosulfan exposed fish. The leukocytosis reported in this study may be attributed to an increased leukocyte mobilization to protect the body against infections in damaged tissues.

Differential leukocytes count showed that lymphocytes and neutrophils were the most frequent white blood cells in control *P. lineatus*, in according with results founded by other authors (Páliková et al. 1999; Ranzani-Paiva et al. 2003; Velisek et al. 2009). Both white blood cells diminished significantly in endosulfan exposed fish. On the contrary, there was an increase in the percentage of monocytes and thrombocytes, while eosinophils remained the same. These results are in agreement with those found by Shafiq-ur-Rehman (2006) in mirror carp (*Cyprinus carpiovar specularis*) exposed to endosulfan. Mazon et al. (2002) registered a significant decrease in lymphocytes and an increase in monocytes percentages of *Prochilodus scrofa* exposed to copper, but thrombocytes remained unchanged. Thrombocytes are comparable to mammalian blood platelets and function in blood clotting, and monocytes are the precursors and function as macrophages (Takashima and Hibiya 1995). It may be suggested that pesticides trigger a rapid mobilization of the hemopoietic system and fish adjust their blood clotting time by increasing the amount of circulating thrombocytes (Agrahari et al. 2006). A high number of these white blood cells reduce the clotting time which can prevent blood loss from hemorrhaging (Mazon et al. 2002). Monocytes are active phagocytes and ingest not only foreign materials, such as bacteria, but also dead cells. It may be suggested that monocytes migration in endosulfan exposed is due to inflammatory processes in damage tissues.

A rise in blood glucose reflects a generalized stress response to a variety of environmental conditions. This has been also observed in *P. lineatus* exposed to pesticides (Winkaler et al. 2007; Langiano and Martinez 2008; Pereira Maduenho and Martinez 2008) and complex field conditions (Cazenave et al. 2009). Sastry and Siddiqui (1983) have reported lower levels of glucose in *C. punctatus* after 30 days of endosulfan exposure. It is likely that an increase in time of exposure leads to changes in carbohydrates metabolism or the use of another kind of substrates for energy demands. The hyperglycemia is mostly mediated by the action of cortisol which stimulates liver gluconeogenesis and also halts peripheral sugar uptake (Jobling 1995). However, studies *in vivo* have demonstrated a decreased in plasma cortisol levels of fish exposed to sublethal endosulfan concentrations (Thangavel et al. 2010). In addition, Dorval et al. (2003) observed the same results in head kidney cells of endosulfan exposed rainbow trout. However, there have been observed increases in blood glucose even as cortisol secretion is impaired. Trenzado et al. (2003) suggest that an increase in glucose may be attributed to a different mechanism not linked to cortisol. To this respect, it has been demonstrated that catecholamines itself can increase sugar levels (Wagner et al. 2003). Therefore, more studies are needed in order to explain the mechanism that leads to an increase of glucose levels induced by endosulfan.

In our study, plasma proteins fell significantly in both endosulfan treatments, decreasing with increasing concentrations and exposure time. Similar results have been reported in fish exposed to other agrochemicals (i.e., Glusczak et al. 2006; Sweilum 2006; John 2007).

Chemical toxic pollutants are important sources of ROS in biological systems (Valavanidis et al. 2006). It has been shown that pesticides, in particular, alter the cellular redox balance by different mechanisms (Franco et al. 2009). Some authors suggest that endosulfan induces oxidative damage via generation of ROS. When *Saccharomyces cerevisiae* cells were exposed to 250 μM of endosulfan, ROS generation was increased to 410% compared with untreated control, resulting in an increased of LPO (Sohn et al. 2004). In addition, Tellez-Bañuelos et al. (2009) reported an increase in ROS production, phagocytosis, and LPO in macrophages of Nile tilapia exposed to endosulfan. The excess of ROS is scavenging by antioxidant enzymes to avoid oxidative stress and cell injury. However, the impairment of the antioxidant system, observed in adrenocortical cells of rainbow trout exposed *in vitro* to endosulfan, is likely to affect the capacity of cells to defend themselves and respond to endosulfan-induced oxidative stress (Dorval et al. 2003).

Significant increases in LPO were observed in liver, intestine, and brain of *P. lineatus* exposed to sublethal concentrations of endosulfan, while there were no changes in gill LPO levels. Similarly, Ballesteros et al. (2009b) found a LPO elevation in the liver and brain of *J. multidentata* exposed to this pesticide. Unlike our findings, they did not observe changes in intestine LPO levels. *P. lineatus* is a detritivorous fish with a complex digestive system (Sverlij et al. 1993), which makes it a possible target organ susceptible to oxidative stress. According to Matés (2000), brain is especially sensitive to oxidative

damage. In spite of the high rate of ROS production, owing to high rate of oxidative metabolism and abundance of polyunsaturated fatty acids in cell membrane, brain has a relatively low antioxidant defense system. On the other hand, exposure to endosulfan for 24 h increased LPO levels in liver, kidney, and gills of *C. punctatus* (Pandey et al. 2001; Atif et al. 2005). A similar response was observed in liver of *P. lineatus* at 24 and 48 h of endosulfan exposure but only in the lower concentration tested. An analogous trend was found in gills, but without significant differences. The different LPO response between both concentrations may be related to the metabolical pathways and time needed to activate the antioxidant system under sublethal pesticide stress. However, more research is required to understand the mechanisms involved in endosulfan oxidative stress in *P. lineatus*.

In conclusion, sublethal endosulfan concentrations alter hematological and oxidative stress parameters in *P. lineatus*. Our results showed that there were changes in blood composition mainly related to the immunologic system and oxidative stress damage in vital organs such as the liver and brain. Although these changes do not lead to lethal outcome, it might compromise fish ability to handle changing environmental situations and infection agents. Despite the fact that the parameters analyzed are nonspecific in their response to stressors, our results reinforced their use as biomarkers of chemical exposure. Summarizing, the present study on the acute and sublethal toxicity in *P. lineatus* is expected to be a useful tool for assessment of possible risks to endosulfan and determination of water quality criteria for control policies and conservation strategies in neotropical regions.

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