Detoxification and Antioxidant Responses in Diverse Organs of *Jenynsia multidentata* Experimentally Exposed to 1,2- and 1,4-Dichlorobenzene

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ABSTRACT: We report changes in activities of detoxification and antioxidant enzymes as well as lipid peroxidation levels in liver, gills, and brain of *Jenynsia multidentata* exposed to 1,2- and 1,4-dichlorobenzene (DCB). Fish were captured at an unpolluted area, transported to the laboratory, and acclimated previous to experiments. Exposures were carried out using 1,2-DCB at 0.5, 1, 5, and 10 mg L^{-1} and 1,4-DCB at 0.05, 0.1, 1, and 5 mg L^{-1}. After 24-h exposure, fish were sacrificed and dissected separating liver, gills, and brain of each fish. Organs were used for enzyme extractions, evaluating antioxidant system through the assay of glutathione reductase, guaiacol peroxidase, glutathione peroxidase, catalase as well as detoxification system by measuring glutathione-*S*-transferase (GST) activity. Additionally, thiobarbituric acid reactive substances (TBARS) method was used to evaluate the peroxidation of lipids. No changes in GST activity were found in liver of fish exposed to DCBs but in gills and brain of exposed fish. The detoxification system was activated at lower concentrations of 1,2-DCB than 1,4-DCB. Antioxidant response is activated in liver at low DCB concentrations, followed by a drop at highest levels. We also found activation of the antioxidant system in gills and brain of exposed fish. On the other hand, we did not observe changes in TBARS concentrations in liver or gills of exposed fish with respect to controls, but in brain of fish exposed to 1,2-DCB ($\geq$0.5 mg L^{-1}) and 1,4-DCB (5 mg L^{-1}). Responses of both detoxification and antioxidant systems of *J. multidentata* suggest that 1,2-DCB is more toxic than 1,4-DCB to this specie. To the extent of our knowledge, this is the first report of oxidative stress induced by DCBs in fish. Our results evidence that the brain is the organ most severely affected by the oxidative stress caused by DCBs.
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Keywords: DCBs; fish; antioxidant; detoxification; gills; brain

INTRODUCTION

Dichlorobenzene (DCB) isomers can be introduced into the environment as solvents, deodorants, or as intermediates in the manufacture of pesticides and other chemicals (Beurskens et al., 1994). Because of their widespread use over decades, DCBs have become very common in the environment. They are found in water (He et al., 2000; Meharg

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et al., 2000), soil (Wang et al., 1995), sediment (Beck et al., 1996), sewage sludge (McPherson et al., 2002), and aquatic biota (van Wezel and Opperhuizen, 1995). The amounts of dissolved DCBs are generally within the nanograms to micrograms per liter range (Oliver and Nicol, 1982; Boutonnet et al., 2004; Malcolm et al., 2004). However, their solubility in water can reach 91 and 31 mg L^{-1} for 1,2-DCB and 1,4-DCB, respectively (Malcolm et al., 2004).

DCBs are hazardous to the health and have been ranked as priority pollutants by the USA Environmental Protection Agency (EPA), who established maximal contaminant levels in drinking water for 1,2-DCB (0.6 mg L^{-1}) and 1,4-DCB (0.075 mg L^{-1}), whereas there are no limits reported for 1,3-DCB. These compounds have high octanol–water partition coefficients (Wang and Lee, 1998), so biological accumulation can be expected in the aquatic ecosystem. Because of their acute toxicity (Belfroid et al., 1993), and potential harmfulness to the aqueous environment (Wang and Lee, 1998), most of the available information refers to acute effects of DCBs on mammals; effects of these compounds on aquatic organisms are less reported. For instance, acute exposure to nonlethal doses of DCBs induces fertility impairment on *Daphnia magna* (Le Blanc, 1980) as well as inhibition of photosynthesis on *Selenastrum capricornutum* (Calamari et al., 1983).

DCBs appear to be absorbed readily from the gastrointestinal and respiratory tracts in experimental animals after rapid distribution to highly perfused organs. Absorbed DCBs accumulate primarily in the fatty tissue, with smaller amount in the liver and other organs (Hawkins et al., 1980; Chu et al., 1987). In fish, it is possible to consider gills and intestine as possible routes of uptake as it was reported by Cazenave et al. (2005) for microcystins.

The metabolism of DCBs is mediated by microsomal oxidation and proceeds principally, either directly or through the formation of a metastable epoxide intermediate, to form the corresponding chlorophenols, which are excreted as mercapturic, glucuronic, or sulfate conjugates (Azouz et al., 1955; Hawkins et al., 1980; Lake et al., 1997). However, secondary P 450-catalized oxidation of phenols to hydroquinones, followed by their oxidation to benzoquinones, which may elicit oxidative stress to macromolecules (Hissink et al., 1997; Rietjens et al., 1997). Several studies indicate that metabolites of DCBs covalently bind to kidney and liver tissues, producing kidney tumors in male rat and liver tumors in mice (Lake et al., 1997).

Xenobiotics that exert oxidative stress via redox cycling are of particular interest in toxicology (Rau et al., 2004). Thus, the metabolism of DCBs results in the formation of reactive oxygen species (ROS), which significantly contribute to their toxicity (Chovanec et al., 2003; Younis et al., 2003), evidenced in rat livers exposed to 1,2-DCB (Hoglen et al., 1998; Younis et al., 2000). Organisms can adapt to increased ROS production by upregulating antioxidant defenses (Livingstone, 2003). However, if the production of ROS overcomes antioxidant capacity, an imbalance between the generation and removal of ROS produces oxidative stress, which may provoke enzyme inactivation, protein degradation, DNA damage, lipid peroxidation (LPO) and, ultimately, cell death (Winston and Di Giulio, 1991; Rikans and Hornbrook, 1997; Barata et al., 2005). To the extent of our knowledge, effects of DCBs on antioxidant systems or LPO in fish have not been measured.

The toxicity of chemicals may be different in diverse exposed animals. Therefore, it is useful to evaluate several test species to assess their response to xenobiotics (Oruc et al., 2004). Fish species have attracted considerable interest in studies assessing biological and biochemical responses to environmental contaminants (Power, 1989), mainly considering their ecological role in the aquatic food-webs, acting as a carrier of energy from lower to higher trophic levels (van der Oost et al., 2003).

On the other hand, a better understanding on the biotransformation process in fish is necessary to improve the knowledge on mechanisms involved in toxicity of DCBs, and on the response of different organs to such compounds, considering that different organs react in diverse ways, or with different intensity, to the presence of a toxic compound (Cazenave et al., 2006). Gills provide a wide interaction surface with aquatic environment, thus, it is an important organ to evaluate toxic effects in fish (Heath, 1987). On the other hand, the liver is an important organ when considering the action of polluting chemicals on fish. It is the most important target organ for biotransformation of organic xenobiotics (Heath, 1987). The brain could be pointed out as the target of oxidative damage induced by environmental pollutants (Song et al., 2006), which could be attributed to a high content of polyunsaturated fatty acids in its membrane, and low level of antioxidant enzymes (Ozcan Oruc et al., 2004; Cazenave et al., 2006; Song et al., 2006). Additionally, the exposure route could also influence the uptake and severity of effects (Cazenave et al., 2006).

Considering the ecotoxicity of DCBs, and the relative lack of information on the effects of these compounds on fish (http://www.inchem.org/documents/ehc/ehc/ehc128.htm), we were interested in evaluating their effects on native fish. Thus, we compare changes in activities of both detoxification and antioxidant enzymes in liver, gills, and brain of *Jenynsia multidentata* experimentally exposed to 1,2- and 1,4-DCB, aiming to evaluate the response of fish to environmental relevant concentrations of these xenobiotics. Additionally, we look to assess LPO induction by these isomers, contributing to a broader understanding on the response of different fish organs exposed to DCBs.

MATERIALS AND METHODS

Reagents

DCBs (purity $>$ 99%), NADPH, glutathione (oxidized and reduced forms), 1-chloro-2,4-dinitrobenzene (CDNB),

thiobarbituric acid (TBA), and glutathione reductase (GR) are commercially available from Sigma-Aldrich (St. Louis, MO, USA).

Equipments

Enzyme extraction was performed using an ultracentrifuge (Sorvall® Ultraspeed). Enzymatic assays were carried out using a spectrophotometer (MultiSpec- 1501; Shimadzu, Tokyo, Japan), equipped with a multiple cell holder and temperature control.

Fish

J. multidentata (Pisces, Anablepidae) is a native widespread species, which was selected due to its favorable experimental properties (small size, easy to collect and maintain in the laboratory). Adult specimens (1.53 ± 0.32 g) were captured by a backpack electrofisher (Coffelt, model Mark 10) from an unpolluted site (Suquía River at La Calera – Córdoba, Argentina; Wunderlin et al., 2001; Hued and Bistoni, 2002) and transported to the laboratory within water tanks (20 L).

Fish were acclimated to laboratory conditions during 4 weeks before experiments. They were maintained in 40-L tanks containing aquarium water (distilled water containing 100 mg L^{-1} sea salt, 200 mg L^{-1} $CaCl_2$, and 103 mg L^{-1} $NaHCO_3$; Best et al., 2002). Acclimation was performed in a temperature controlled room at (21 ± 1)°C, with a light: dark cycle of 12:12 h. Fish were fed once a day with commercial fish pellets, and starved 24 h before experiment.

Experimental Design

Forty-eight fish were individually exposed to 1,2-DCB dissolved in aquarium water (1 L) at concentrations of 0.5, 1, 5, and 10 mg L^{-1} and to 1,4-DCB at 0.05, 0.1, 1, and 5 mg L^{-1} for 24 h. These concentrations were selected according to previous reports showing ecotoxic effects of these compounds to other species (Malcolm et al., 2004). Stock solutions were prepared by dissolving 500 mg of the corresponding DCB isomer in 10 mL methanol. Controls were assayed using an equivalent amount of pure methanol. Enzyme measurement and LPO assays were carried out by separate experiments (three fish for LPO and three fish for enzymatic assays for each isomer at a particular concentration). After exposure, fish were sacrificed and dissected. Gills (0.036 ± 0.019 g), liver (0.017 ± 0.009 g), and brain (0.0120 ± 0.0005 g) from control and exposed animals were separated, frozen in liquid nitrogen, and stored at −80°C until measurements.

Enzyme Extraction and Measurement

Enzyme extracts from each tissue were prepared from each individual (not pooled) according to Wiegand et al. (2000), with less modifications. Briefly, tissues were homogenized using 0.1 M potassium phosphate buffer, pH 6.5 containing 20% (v/v) glycerol, 1 mM EDTA, and 1.4 mM dithioerythritol. After removal of cell debris (10 min at 13 000 × *g*), the membrane fraction was separated by centrifugation at 105 000 × *g* for 60 min. The remaining supernatant, defined as the soluble (cytosolic) fraction, was used for enzyme measurement.

Enzyme activities were determined by colorimetry. The activity of soluble glutathione-*S*-transferase (sGST) was determined using CDNB as substrate, according to Habig et al. (1974). GR activity was assayed according to Tanaka et al. (1994). The activity of glutathione peroxidase (GPx) was determined according to Drotar et al. (1985), using H_2O_2 as substrate. The guaiacol peroxidase (POD) activity was measured using guaiacol and hydrogen peroxide (Bergmeyer, 1983). Catalase (CAT) activity was determined according to Chang and Kao (1998). The enzymatic activity was calculated in terms of the protein content of the sample (Bradford, 1976), and is reported in nano katals per milligram of protein (nkat/mg protein), where 1 kat is the conversion of 1 mol of substrate per second. Each enzymatic assay was carried out by triplicate.

Lipid Peroxidation

The TBA method described by Fatima et al. (2000) was used to evaluate the LPO in liver, gills, and brain of exposed and control fish. Briefly, 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. Afterwards, 1.0 mL of 5% trichloroacetic acid, and 1.0 mL of 0.67% TBA were added to each sample and mixed. Then, each vial was centrifuged at 3000 rpm for 10 min. The supernatant was separated and placed in a boiling water bath for 10 min, cooled to room temperature, and measured at 535 nm. The rate of LPO was expressed in nanomoles of substances reactive to TBA (TBARS) formed per hour, per milligram of proteins (nmol TBARS/mg protein). Protein content of each homogenate was determined according to Bradford (1976).

Statistics

All values are expressed as mean(s) ± standard deviation(s). Normal distribution for data was analyzed by Shapiro Willks test. One-way ANOVA were carried out to determine whether treatments were significantly different from control group ($P < 0.05$).

RESULTS

Detoxification (GST Activity)

sGST showed no significant changes in liver of fish exposed to either 1,2- or 1,4-DCB relative to control group.

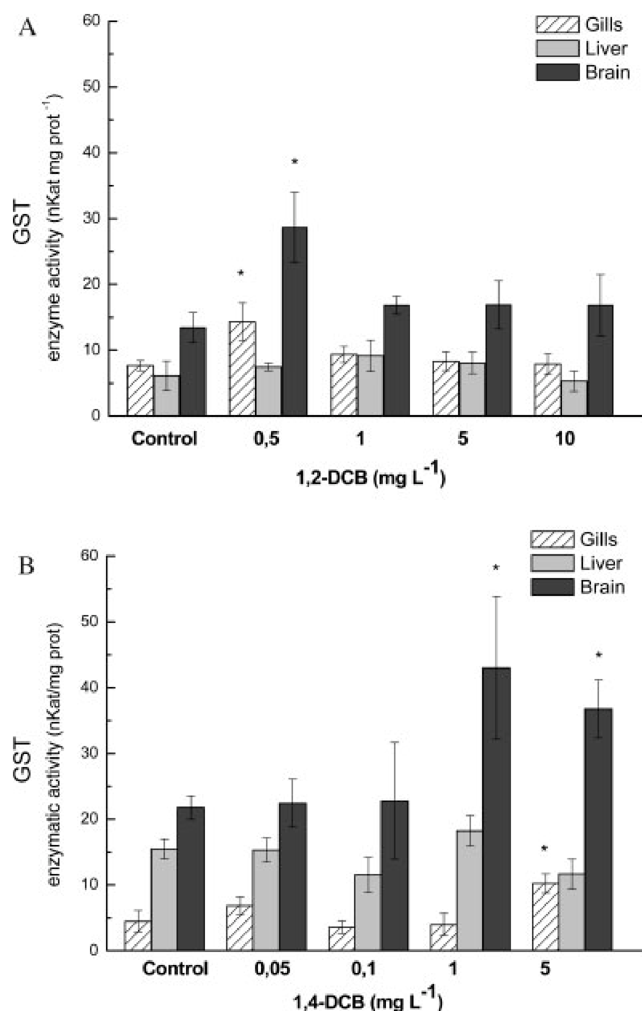

Fig. 1. Cytosolic glutathione-*S*-transferase (sGST) activities in liver (▬), gills (▨), and brain (■) of *Jenynsia multidentata* exposed to 1,2-DCB (A) and 1,4-DCB (B). Values are expressed as enzyme activity (nKat/mg protein^{-1}) and reported as mean(s) $\pm$ SD(s). (*) Significance levels observed are $P < 0.05$ in comparison with control group.

The activity of sGST in gills of fish exposed to 1,2-DCB (0.5 mg L^{-1}) and to 1,4-DCB (5 mg L^{-1}) raised 1.9- and 2.3-fold, respectively, with respect to controls. Similarly, exposure to 0.5 mg L^{-1} 1,2-DCB, and 1 mg L^{-1} 1,4-DCB raised sGST by 2.1- and 2.0-fold, respectively, in brain of exposed fish with respect to controls [Fig. 1(A,B)].

Antioxidant Enzymes

The activity of GPx in fish exposed to 1,2-DCB (1 mg L^{-1}) raised 2.6-fold in liver, with respect to controls, whereas no significant changes in gills of fish relative to control group were found. Similarly, exposure to 1,4-DCB (0.05 mg L^{-1}) raised 7.5-fold in liver and 3.9 in gills with respect to controls. We did not detect GPx activity in brain of fish [Fig. 2(A,B)].

GR showed activation in liver and gills of fish exposed to 0.5 mg L^{-1} 1,2-DCB and in brain at 1 mg L^{-1}. On the other hand, liver of fish exposed to 0.05 mg L^{-1} 1,4-DCB underwent a 4.4-fold raised GR activity, whereas gills and brain raised 3.7- and 2.4-fold, respectively, when fish were exposed to 5 mg L^{-1} 1,4-DCB [Fig. 3(A,B)].

We detected POD activity only in liver of both exposed and control fish. POD showed almost 2.8-fold increase in liver of fish exposed to 1 mg L^{-1} 1,2-DCB, and a 3.0-fold increase in liver of fish exposed to 0.05 mg L^{-1} 1,4-DCB [Fig. 4(A,B)].

We detected CAT activity only in liver of both exposed and control fish. Similarly, CAT activities showed 1.6- and 3.0-fold increase in liver of fish exposed to 0.5 mg L^{-1} 1,2-DCB and 0.05 mg L^{-1} 1,4-DCB, respectively [Fig. 4(A,B)].

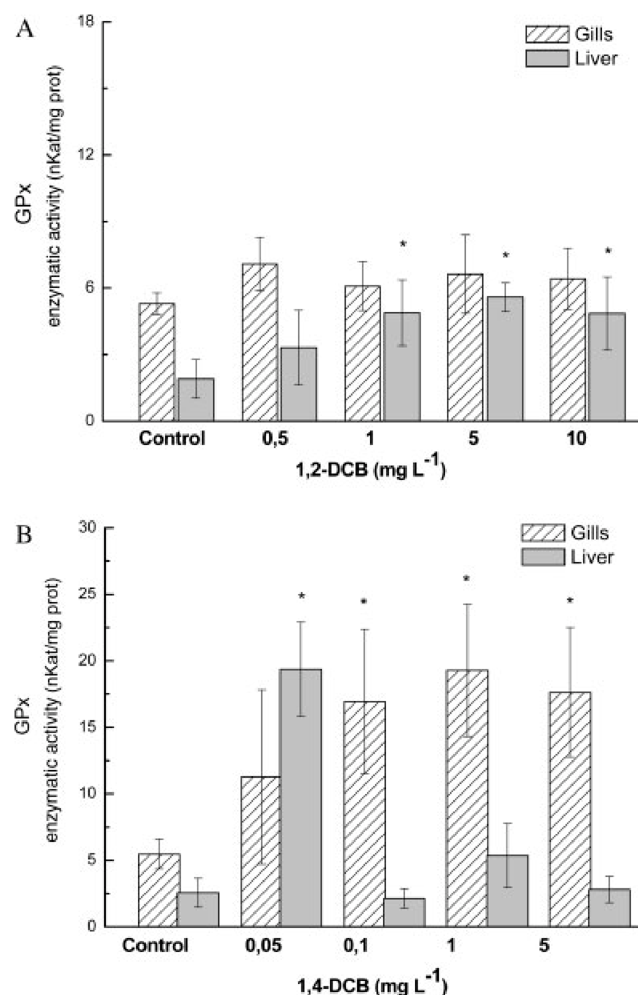

Fig. 2. Glutathione peroxidase (Gpx) activities in liver (▬) and gills (▨) of *Jenynsia multidentata* exposed to 1,2-DCB (A) and 1,4-DCB (B). Values are expressed as enzyme activity (nKat/mg protein^{-1}) and reported as mean(s) $\pm$ SD(s). (*) Significance levels observed are $P < 0.05$ in comparison with control group.

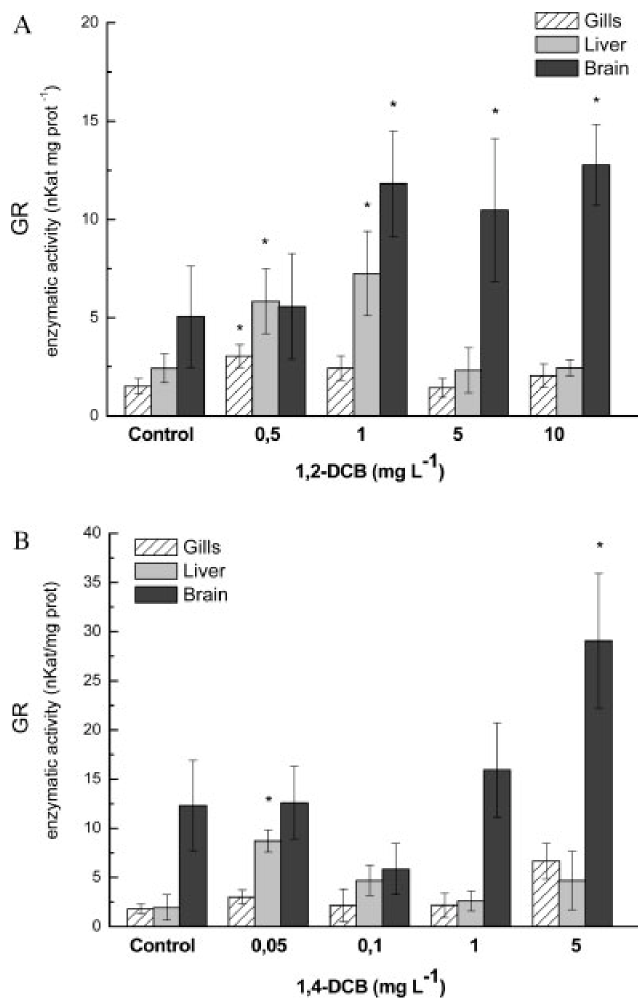

Fig. 3. Glutathione reductase (GR) activities in liver (▬), gills (▨), and brain (■) of *Jenynsia multidentata* exposed to 1,2-DCB (A) and 1,4-DCB (B). Values are expressed as enzyme activity (nKat/mg protein^{-1}) and reported as mean(s) $\pm$ SD(s). (*) Significance levels observed are $P <$ 0.05 in comparison with control group.

Lipid Peroxidation

LPO, measured as TBARS, showed no significant changes in liver and gills of exposed fish to 1,2-DCB, but a significant drop in gills of fish exposed to 1,4-DCB was detected. LPO was enhanced in brain of fish exposed to concentrations $\geq$ 0.5 mg L^{-1} 1,2-DCB and at 5 mg L^{-1} 1,4-DCB [Fig. 5(A,B)].

DISCUSSION

Detoxification (GST Activity)

Enzymatic biotransformation is an important process responsible for the detoxification of xenobiotic and endogenous compounds. An increase in the activity of detoxification enzymes can arise from its activation, and/or from *de novo* enzyme synthesis upon exposure to different toxics (natural or xenobiotics; Sijm and Opperhuizen, 1989). Thus, levels and activities of detoxification enzymes can be used as sensitive biomarkers of exposure to environmental toxics (van der Oost et al., 2003).

GST is a multigene family of isoenzymes that catalyze the conjugation of electrophilic compounds to GSH. GST exhibits broad specificities and the ability to conjugate substrates to GSH. The diversity of substrates accommodated by GST is a result of both the relatively nonspecific nature of the binding site for hydrophobic substrates, and the existence of numerous isoforms as well (Whitbread et al., 2005). GST metabolized carcinogens, environmental pollutants, drugs, natural toxins, and a broad spectrum of other xenobiotics (Wiegand et al., 2001; Pietsch et al., 2001; Padrós et al., 2003; Ahmad et al., 2005; Cazenave et al., 2006). Experiments carried out with rats and rabbits

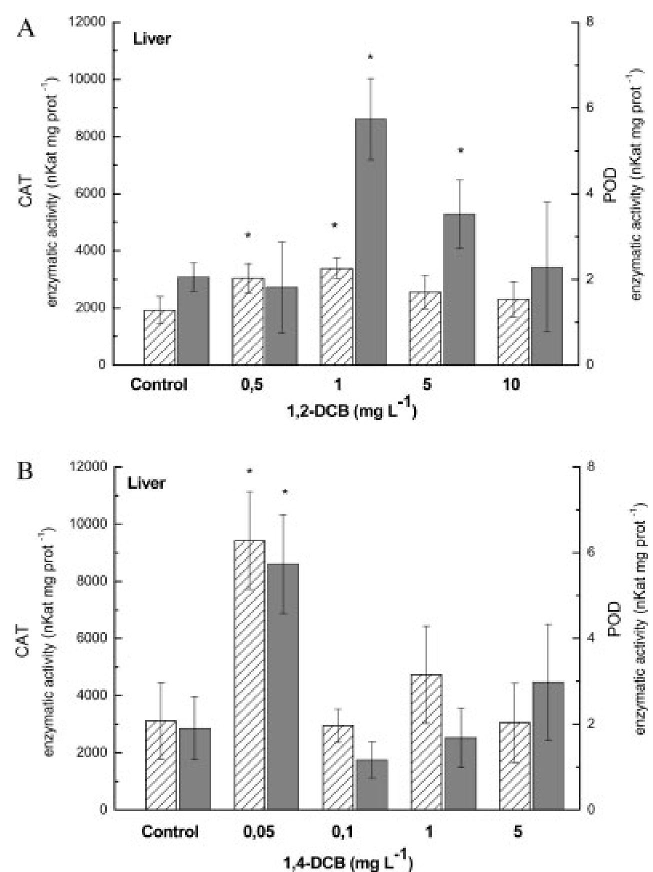

Fig. 4. Cytosolic catalase (CAT ▨) and guaiacol peroxidase (POD ▬) activities in liver of *Jenynsia multidentata* exposed to 1,2-DCB (A) and 1,4-DCB (B). Values are expressed as enzyme activity (nKat/mg protein^{-1}) and reported as mean(s) $\pm$ SD(s). (*) Significance levels observed are $P < 0.05$ in comparison with control group.

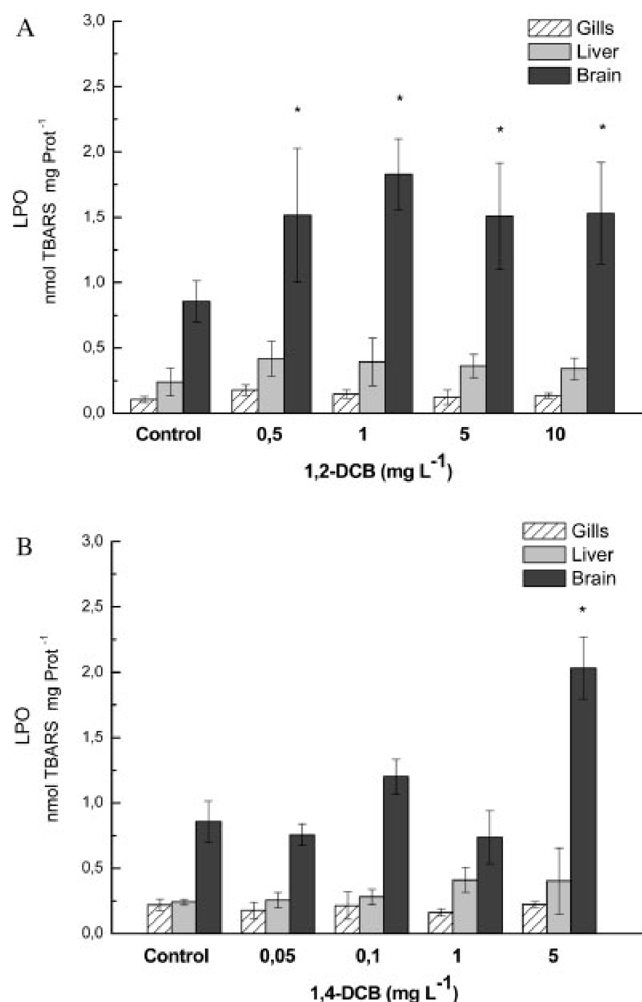

Fig. 5. Lipid peroxidation (LPO) values in liver (▬), gills (▨), and brain (■) of *Jenynsia multidentata* exposed to 1,2- (A) and 1,4-DCB (B). LPO values are expressed as nanomoles of TBARS released during 1 h of incubation, and are reported as mean(s) ± SD(s). (*) Significance levels observed are $P < 0.05$ in comparison with control group.

demonstrated that DCBs are metabolized by microsomal oxidation, mediated by cytochrome P-450, to form the corresponding chlorophenol. These chlorophenols are primarily excreted as sulfate (50% of total eliminated), and glucuronide conjugates (32%) in urine, while glutathione conjugates represent minor metabolites (10%) (Hawkins et al., 1980; http://www.inchem.org/documents/ehc/ehc/ehc128.htm).

Our results suggest that DCBs are conjugated with GSH in brain and gills of *J. multidentata* to facilitate its excretion, although no significant changes in GST activities were found in liver of fish exposed to 1,2- or 1,4-DCB [Fig. 1(A,B)]. Our results contrast with those reported by Qian et al. (2004), who reported increased GST activity in liver of crucian carp (*Carassius auratus*) injected with chlorobenzenes. Differences between our results and those reported by Qian et al. (2004) might be attributed to experimental conditions (i.e., exposition routes, levels of toxic, exposure time, etc.), and also to the fact that different species present differential responses to the same toxic (Fisher et al., 1995). On the other hand, it is likely to think that *J. multidentata* could detoxify DCBs through a pathway similar to that observed with rats and rabbits; thus, forming either sulfate or glucuronide instead of glutathione conjugates (Hawkins et al., 1980; http://www.inchem.org/documents/ehc/ehc/ehc128.htm).

Antioxidant Enzymes

Several studies have demonstrated that DCBs induce production of ROS (Hissink et al., 1997; Rietjens et al., 1997; Hoglen et al., 1998; Younis et al., 2000, 2003), which may be scavenged by the antioxidant defense system. The activity of enzymes such as GPx, GR, POD, SOD, and CAT prevents from adverse effects of oxidative stress to cells. Oxidative stress will occur whenever the activity of this antioxidant system decreases, or the production of ROS is increased (Moreno et al., 2005).

The production of ROS in cells may cause an elevation of peroxidases (POD, GPx), which catalyze the reduction of peroxides levels. The activity of POD prevents damage to cell membranes, proteins, and DNA (Di Giulio et al., 1989). GPx catalyzes the reduction of hydrogen peroxide (H_2O_2) to water (and lipid or hydroperoxides to alcohols), with the concomitant oxidation of GSH to GSSG (Winston and Di Giulio, 1991).

We observed induction of GPx activity in liver of fish exposed to 1,2-DCB ($\geq$1 mg L^{-1}) or 1,4-DCB (0.05 mg L^{-1}). We also observed induction of GPx activity in gills of fish exposed to 0.05 mg L^{-1} of 1,4-DCB [Fig. 2(A,B)]. These results agree with previous reports pointing out an increase in the oxidation of glutathione (GSH) to glutathione disulfide (GSSG) after 1-h exposure of a primary culture of rats hepatocytes to 1,2-DCB (Younis et al., 2003). On the other hand, we did not observe significant changes on GPx activity in gills of fish exposed to 1,2-DCB with respect to controls [Fig. 2(A)].

Our results also show that both POD and CAT activities were increased in liver of fish exposed to 1,2-DCB (1 and 5 mg L^{-1}) and 1,4-DCB (0.05 mg L^{-1}), followed by a drop at higher DCBs concentrations [Figs. 4(A,B) and 5(A,B)]. These results are consistent with those reported by Palace et al. (1996), who proposed that chlorinated hydrocarbons could inhibit the activities of protective enzymatic antioxidants. A separate study also demonstrated that xenobiotic-metabolizing enzymes activities are increased in *Mytilus galloprovincialis* at low concentrations

of PCBs, but significantly depressed at highest levels (Michel et al., 1993).

GR also plays an important role in cellular antioxidant protection, catalyzing the reduction of glutathione disulfide (GSSG) to reduced glutathione (GSH); thus, providing regulation of metabolic pathways. Our results show enhanced activity of GR in liver, gills, and brain of fish exposed to DCBs [Fig. 3(A,B)]. These results agree with previous reports showing an increase in antioxidant enzymes (GPx) in liver of *Perna viridis* transplanted from a reference site to various sites polluted with chlorinated hydrocarbons (Cheung et al., 2002).

Thus, the antioxidant response in several organs of *J. multidentata* exposed to DCBs demonstrated that these xenobiotics are inducing oxidative stress in this fish, which is in good agreement with previous reports for other animals (rat and mouse; Hoglen et al., 1998; Younis et al., 2000).

Lipid Peroxidation

Failure of antioxidant defenses to remove exogenous ROS will disrupt the balance between antioxidant and prooxidant systems within a given organism, leading to oxidative damage (Barata et al., 2005). Diverse behavior of the antioxidant system among different organs of *J. multidentata* suggests that the efficiency of this system to remove ROS, and to prevent oxidative stress, vary across different organs within the same species (Avci et al., 2005; Cazenave et al., 2006). So, we decided to evaluate the oxidative damage at different organs by determining LPO, expressed as TBARS.

Our results show that the activity of antioxidant enzymes are increased in liver of *J. multidentata* exposed to either 1 mg L^{-1} 1,2-DCB, or 0.05 mg L^{-1} 1,4-DCB. However, we only observed a slight stimulation of LPO, but no significant changes, in the liver of exposed fish [Fig. 5(A,B)]. We did not observe significant changes in LPO of gills exposed to 1,2-DCB, but a significant drop at the highest concentrations of 1,4-DCB [Fig. 5(A,B)]. Low levels or lack of LPO in tissues reflect the protective effects of antioxidant enzymes. Our present results are similar to those reported by Ozcan Oruc et al. (2004) in kidney and brain of *Cyprinus carpio* exposed to pesticides. Also, Song et al. (2006) found that GPx is involved in the removal of hexachlorobenzene-induced LPO.

On the contrary, we observed that DCBs induced an increase in LPO levels in brain of exposed fish. This last result is in good agreement with that reported by Song et al. (2006) for brain of common carp exposed to hexachlorobenzene. These findings indicate that the brain has poor antioxidant capacity in relation to other organs, which is coincident with previous reports indicating that brain is the target for oxidative damage of xenobiotics and natural toxins (Ozcan Oruc et al., 2004; Cazenave et al., 2006). The main factors that contribute to the vulnerability of brain include high content of polyunsaturated fatty acids in brain membrane, and low level of enzymatic and nonenzymatic antioxidant (Pérez-Campo et al., 1993; Fatima et al., 2000). Thus, the brain of *J. multidentata* appears as the organ most severely affected by oxidative stress induced by DCBs.

Overall Discussion

Gills and brain of *J. multidentata* are more sensitive to 1,2-DCB than to 1,4-DCB, since their detoxification system (measured through sGST) is activated at lower concentrations with the first isomer. On the other hand, our results suggest that DCBs are not conjugated to glutathione in the liver of *J. multidentata* at the concentrations evaluated during this job. Additional work is necessary to confirm the presence of P-450 oxidation products in fish as well as their sulfates or glucuronide conjugates as reported for rats and rabbits (Hawkins et al., 1980; http://www.inchem.org/documents/ehc/ehc/ehc128.htm).

The increased activity of antioxidant enzymes in *J. multidentata* indicates the formation of ROS as well as ongoing detoxification (Pflugmacher, 2004). Considering our present results, it is clear that the exposition to DCBs caused a general activation of the antioxidant system in various organs of *J. multidentata*, which suggests that the defense system of affected organs (mainly liver and gills) could be playing important roles in scavenging ROS, regeneration of GSH, helping to protect cells from adverse oxidative effects.

If we consider that LPO is one of the main manifestations of oxidative damage, being in a big part responsible for the toxicity of DCBs to *J. multidentata*, we could conclude that the brain is the most affected organ, since it presented increased LPO levels and poor antioxidant activity in exposed fish.

Additionally, considering that 1,2-DCB induced LPO at a lower concentration than 1,4-DCB (0.5 and 5 mg L^{-1}, respectively), we could suggest that 1,2-DCB is more toxic than 1,4-DCB to *J. multidentata*. This last result is in good agreement with previous studies showing that 1,2-DCB was more toxic to rat and mouse liver, and to rat kidney, than 1,4-DCB (Allis et al., 1992; Valentovic et al., 1993; Fischer et al., 1995; Umemura et al., 1996). However, our results contrast with the limits established by the USA EPA for DCBs in drinking water: 0.6 mg L^{-1} for 1,2-DCB, and 0.075 mg L^{-1} for 1,4-DCB. Thus, an accurate revision of toxicity of DCBs to aquatic biota is necessary to establish which isomer is more toxic to different species.

Our present results demonstrate that exposition of a native fish (*J. multidentata*) to realistic environmental concentrations of DCBs could result in oxidative stress to native fish, the brain being one of the most severely affected organs. These findings raise questions on the need to regulate more restrictive limits of these compounds in rivers and lakes to protect the aquatic biota.

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