Ecotoxicology and Environmental Safety 149 (2018) 248–256

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Isolated and mixed effects of diuron and its metabolites on biotransformation enzymes and oxidative stress response of Nile tilapia (*Oreochromis niloticus*)

Andréia Arantes Felício[a], Juliane Silberschmidt Freitas[a], Jéssica Bolpeti Scarin[a], Luciana de Souza Ondei[b], Fabrício Barreto Teresa[b], Daniel Schlenk[c], Eduardo Alves de Almeida[d,*]

[a] UNESP – Sao Paulo State University, Department of Chemistry and Environmental Science, Sao Jose do Rio Preto, Sao Paulo, Brazil
[b] UEG – Goias State University, University Unit of Exact and Technological Science (UnUCET), Anapolis, Goias, Brazil
[c] Department of Environmental Sciences, University of California, Riverside, USA
[d] FURB – Fundação Universidade Regional de Blumenau, Department of Natural Sciences, Blumenau, Santa Catarina, Brazil

ARTICLE INFO

*Keywords:*
Herbicide
Oxidative stress
Biotransformation
Fish

ABSTRACT

Diuron is one of the most used herbicide in the world, and its field application has been particularly increased in Brazil due to the expansion of sugarcane crops. Diuron has often been detected in freshwater ecosystems and it can be biodegraded into three main metabolites in the environment, the 3,4-dichloroaniline (DCA), 3,4-dichlorophenylurea (DCPU) and 3,4-dichlorophenyl-N-methylurea (DCPMU). Negative effects under aquatic biota are still not well established for diuron, especially when considering its presence in mixture with its different metabolites. In this study, we evaluated the effects of diuron alone or in combination with its metabolites, DCPMU, DCPU and 3,4-DCA on biochemical stress responses and biotransformation activity of the fish *Oreochromis niloticus*. Results showed that diuron and its metabolites caused significant but dispersed alterations in oxidative stress markers and biotransformation enzymes, except for ethoxyresorufin-*O*-deethylase (EROD) activity, that presented a dose-dependent increase after exposure to either diuron or its metabolites. Glutathione *S*-transferase (GST) activity was significant lower in gills after exposure to diuron metabolites, but not diuron. Diuron, DCPMU and DCA also decreased the multixenobiotic resistance (MXR) activity. Lipid peroxidation levels were increased in gill after exposure to all compounds, indicating that the original compound and diuron metabolites can induce oxidative stress in fish. The integration of all biochemical responses by the Integrated Biomarker Response (IBR) model indicated that all compounds caused significant alterations in *O. niloticus*, but DCPMU caused the higher alterations in both liver and gill. Our findings imply that diuron and its metabolites may impair the physiological response related to biotransformation and antioxidant activity in fish at field concentrations. Such alterations could interfere with the ability of aquatic animals to adapt to environments contaminated by agriculture.

## 1. Introduction

Brazil is the world's largest sugarcane producer in the world, covering this crop an area of about 10 million hectares, being the third largest area of cultivation in the country after soy and corn. In the years 2015/2016, sugarcane production reached 700,000,000 t (UNICA, 2016). However, the large-scale production means the overuse of pesticides for crops maintenance, which has generated concerns related to its environmental impacts on different ecosystems. Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is one of the most used herbicide on sugarcane crops in Brazil and it is a frequently detected pesticides in freshwater ecosystems around the world (Morin et al., 2009; Schlenk et al., 2012). Diuron is a substituted phenylurea compound with a relatively low $K_{OC}$ (418–560, according to ARSUSDA, 2004) but with long hydrolysis and aqueous photolysis half-lives, which indicates a relatively low tendency to sorb to soils and sediments, making it available to the water fraction. This herbicide has moderate to high persistence in soils, with and average field dissipation half-life of 90 days, although it can be highly variable according to soil and abiotic characteristics. According to Kidd and James (1991), diuron residues may persist for

* Correspondence to: Department of Natural Sciences, Fundação Universidade Regional de Blumenau, Av. Antonio da Veiga 140, Itoupava Seca, 89030-903 Blumenau, Santa Catarina, Brazil.
*E-mail address:* eduardoalves@furb.br (E.A. de Almeida).

https://doi.org/10.1016/j.ecoenv.2017.12.009
Received 18 September 2017; Received in revised form 10 November 2017; Accepted 7 December 2017

more than one year at higher application rates. Due to its low tendency to sorb to soils and its moderate persistence, diuron is therefore prone to off-site movement in surface runoff, and migration to both surface and ground water (Troiano et al., 2001; Field et al., 2003; Giacomazzi and Cochet, 2004), posing risks to aquatic organisms.

According to World Health Organization (Abass et al., 2007), diuron is classified as a slightly hazardous pesticide (class III toxicity), and considered as moderately toxic to aquatic life (ECA, 2017). In soil, diuron can be metabolized by fungi and bacteria originating three main metabolites: 3,4-dichloroaniline (DCA), 3,4-dichlorophenylurea (DCPU) and 3,4-dichlorophenyl-N-methylurea (DCPMU) (Tixier et al., 2002; Abass et al., 2007). Spontaneous hydrolysis can also occurs in aquatic environments, generating 3,4-DCA (Salvestrini et al., 2002), the main product of diuron biodegradation and the most persistent metabolite in the environment (Tixier et al., 2000). 3,4-DCA is mentioned for causing several negative effects on aquatic organisms, such as alterations on morphological (Scheil et al., 2009; Mhadhbi and Beiras, 2012), biochemical (Sánchez-Muros et al., 2013), physiological (Miranda et al., 2008; Scheil et al., 2009; Freitas et al., 2016) and behavioral (Saglio and Trijasse, 1998) parameters. Studies have also shown that diuron metabolites (especially DCPMU and DCPU) have anti-androgenic and estrogenic effects in male and female Nile tilapia (Pereira et al., 2015, 2016; Felício et al., 2016). Although clear evidences of negative effects of diuron and its metabolites on endocrine system of fish, there are no studies regarding the effects of these compounds in biochemical parameters often used as classical pollutant biomarkers, such as the biotransformation enzymes and oxidative stress parameters, which are also important to clarify physiological aspects involved in the defense response of aquatic organisms against environmental pollutants.

The evaluation of biochemical alterations in environmental monitoring studies is a common practice to indicate the exposure of aquatic organisms to pollutants and it can be used as an early stage alert for detection of environmental contamination. Biotransformation enzymes are considered relevant biochemical parameters evaluating chemical disturbance on animal health, since they contribute to the detoxification process, minimizing the deleterious effects of the xenobiotics in the organisms. Cytochrome P450 isoforms, glutathione *S*-transferase (GST) and multixenobiotic resistance (MXR) efflux proteins are involved in the biotransformation and elimination of xenobiotics from cells and they usually act as one of the first cellular response to xenobiotic input (Van der Oost et al., 2003; Luckenbach et al., 2004; Klobučar et al., 2010). Intoxication can also increase oxygen consumption, increasing the rate of the generation of reactive oxygen species (ROS) that can lead to oxidative lesions to biomolecules and oxidative stress. In response to increased ROS production, cells alters their antioxidant defenses, which includes the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione reductase (GR), as well as glucose-6-phosphate dehydrogenase (G6PDH), which provides NADPH for the GR-catalyzed regeneration of glutathione from glutathione disulfide and for cytochrome P450-catalyzed biontransformation reactions (Stegeman et al., 1992; Lopez-Torres et al., 1993). If the generation of ROS surpasses the antioxidant capacity, an oxidative stress condition takes place, leading to the oxidation of key cellular macromolecules such as lipids, proteins, nucleic acids and carbohydrates (Van der Oost et al., 2003). One common biomarker of oxidative lesion to lipids is malondialdehyde (MDA), an aldehyde that is a by-product derived from the decomposition of lipid hydroperoxides formed by the oxidation of polyunsaturated fatty acids (Van der Oost et al., 2003; Almeida et al., 2005, 2007). MDA is a highly reactive molecule (Trivic and Leskuvac, 1994) that can react with other macromolecules (Bartels, 2001), including nucleic acids, generating mutagenic DNA adducts (Van der Oost et al., 2003; Almeida et al., 2005, 2007), and therefore must be eliminated from cells. The enzyme aldehyde dehydrogenase (ALDH) can act metabolizing MDA and other lipid peroxidation-derived aldehydes, assisting in cellular detoxification process (Nakazono et al., 2000; Kirch et al., 2001, 2004).

Considering the importance of the biotransformation enzymes and oxidative stress parameters as indicators of health status of aquatic animals in natural systems, we are interested in investigating how diuron and its metabolites can impair physiological mechanisms of fish at environmental relevant concentrations. As mentioned before, diuron has relatively high persistence in the environment, which is likely to occur in combination with other degradation products, such as its metabolites DCPMU, DCPU and 3,4-DCA. Thus, this study evaluated the effects of diuron and its metabolites alone, or in mixture, on the activities of enzymes related to biotransformation process (ethoxyresorufin-*O*-deethylase, EROD, benzyloxyresorufin-*O*-deethylase, BROD, and pentoxyresorufin-*O*-deethylase, PROD, GST and MXR) and oxidative stress response (activities of SOD, CAT, GPx, GR, G6PDH, ALDH and MDA levels) in gills and livers of Nile tilapia. Biological responses triggered by chemical exposure were assessed based on individual biomarkers and on an integrative index, the integrated biomarker response (IBR, Beliaeff and Burgeot, 2002). We hypothesize that diuron and its metabolites may increase detoxification activity of biotransformation enzymes and Mxr levels as consequence of intoxication process caused by chemical exposure. We also suggest that antioxidant response and lipid peroxidation will be associated to biotransformation performance, since ROS production can be rised by intoxication process, which would stimulate antioxidant enzymes and on the opposite way, increase lipid peroxidation as consequence of damage of cellular components. The information brought by this study is relevant to better clarify how aquatic animals are dealing with pesticides presence in their environments.

## 2. Material and methods

### 2.1. Chemical

All chemicals were ordered from Sidma-Aldrich Chemical Co (ST. Louis, Mo), including Diuron, DCPMU, DCPU and DCA (> 98% pure).

### 2.2. Test organisms

Male Nile tilapias (*Oreochromis niloticus*) were obtained from the Aquiculture Center of the Sao Paulo State University - UNESP. The weight and length of tilapias used in this study were, 77.81 ± 12.98 g and 13.68 ± 0.83 cm (mean ± standard deviation), respectively. Before exposures, the animals were acclimatized and maintained in the laboratory under ideal conditions of temperature, pH and oxygen (28 °C, pH 7.5–8.0) during 7 days, and fed to satiation with commercial fish food (commercial pellets for tropical fish, 32% crude protein - Guabi-Pira/Brazil). Our experiments had permission from Ethics Committee from Animal Use in research of the Sao Paulo State University (CEUA-IBILCE/UNESP) (71/2013).

### 2.3. Exposure experiments

After acclimatization, 66 fish were separated in eleven groups, with six fish per group (N = 6). The animals were maintained in 17 L individual aquariums (one fish per aquarium) with dechlorinated water and controlled temperature (25 ± 1 °C), constantly aerated and kept in a 12:12 h light-dark cycle. For isolated exposures, the animals were exposed to each individual compound – diuron, DCPMU, DCU or 3,4-DCA - at two different concentrations of 40 and 200 ng $L^{-1}$, totalizing eight individual exposures. For the mixtures, fish were exposed to a mix containing diuron and its three metabolites (diuron + DCPMU + DCPU + DCA) at concentrations of 10 ng $L^{-1}$ and 50 ng $L^{-1}$ each, and totalizing two combined exposures. The concentrations were chosen based on mean diuron values found in contaminated aquatic environments (up to 160 ng/L) (Köck-Schulmeyera et al., 2013; Masiá et al., 2015), and based on previous studies done by our research group on the effects