Chemosphere 180 (2017) 65–76

Contents lists available at ScienceDirect

Chemosphere

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

Diuron and diazinon alter the behavior of zebrafish embryos and larvae in the absence of acute toxicity

Mirna Velki [a], [b], [*], Carolina Di Paolo [a], Jonas Nelles [a], Thomas-Benjamin Seiler [a], Henner Hollert [a], [c], [d], [e]

[a] *Department of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany*

[b] *Department of Biology, Josip Juraj Strossmayer University of Osijek, Cara Hadrijana 8/A, 31000 Osijek, Croatia*

[c] *College of Resources and Environmental Science, Chongqing University, 1 Tiansheng Road Beibei, Chongqing 400715, China*

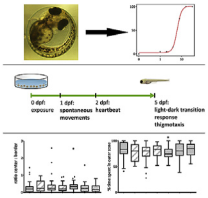
[d] *College of Environmental Science and Engineering, State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, 1239 Siping Road, Shanghai, China*

[e] *State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, China*

HIGHLIGHTS

- Acute and behavioral effects of diazinon and diuron on zebrafish early life stages.
- Moderate acute toxicity of diazinon and diuron at similar effect concentrations.
- Diuron caused reduction in embryonic spontaneous coiling movement occurrence.
- Slight anxiolytic effect of diuron on zebrafish larvae was indicated.
- Behavioral measurements can serve as sensitive indicators to pesticide exposure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 January 2017
Received in revised form
2 April 2017
Accepted 3 April 2017
Available online 4 April 2017

Handling Editor: David Volz

Keywords:
Danio rerio
Embryo toxicity
Sublethal effects
Behavior assessment
Diazinon
Diuron

ABSTRACT

The use of zebrafish for aquatic vertebrate (eco)toxicity testing allows the assessment of effects on a wide range of biological levels – from enzymes to sensory organs and behavioral endpoints. The present study investigated the effects of the insecticide diazinon and the herbicide diuron regarding the acute toxicity and behavior of zebrafish embryos and larvae. After conducting the fish embryo toxicity test, three concentrations (1, 2 and 3.5 mg L^{-1} for diazinon and 1, 2 and 3.8 mg L^{-1} for diuron) were evaluated for effects on embryonic spontaneous movement and heartbeat, larval light-dark transition response, and thigmotaxis. Although the modes-of-action are different, both pesticides proved to be moderately toxic to early life stages of zebrafish with 96 h LC_{50} of approximately 6.5 mg L^{-1} and similar EC_{50} values of approximately 4 mg L^{-1}. Changes in behavioral endpoints were detected 24 h of exposure, suggesting that behavioral measurements can serve as sensitive and early indicators of pesticide exposure. Changes in behavior, such as decrease in spontaneous coiling movements of embryos and reduction of thigmotaxis in larvae, were pronounced for diuron, indicating the usefulness of the application of behavioral endpoints to assess the effects of other herbicides. In the case of diazinon, the effects were less prominent, but the detected changes in ratios between activity in light and darkness also point to the possibility of using behavioral changes for evaluation of insecticide effects. The obtained results support the

---

[*] Corresponding author. Department of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany.

E-mail addresses: mirna.velki@gmail.com, mvelki@biologija.unios.hr (M. Velki).

http://dx.doi.org/10.1016/j.chemosphere.2017.04.017

usage of behavioral endpoints in zebrafish embryos and larvae for the detection of early effects of pesticides.

1. Introduction

The usage of pesticides is inevitable and constantly increasing. Among all groups of pesticides, herbicides and insecticides are the most commonly used, comprising of ca. 40 and 20% of the world market, respectively (Grube et al., 2011). Although pesticides have certain beneficial effects for agricultural production, their use can impact soil, terrestrial and aquatic ecosystems, and affect a great number of non-target organisms (e.g. Pereira et al., 2009; Mhadhbi and Beiras, 2012). Due to their toxic potential for aquatic organisms, the investigation of possible impacts and the identification of sensitive endpoints can enable the early detection of their adverse effects.

Diazinon, an organophosphorus pesticide, is used worldwide as an insecticide, acaricide and nematicide, and was among the 10 most commonly used organophosphate insecticide active ingredients according to 2006 and 2007 market estimates (Grube et al., 2011). Diazinon inhibits the enzyme acetylcholinesterase (AChE), leading to accumulation of neurotransmitters and altered signal transmission in chemical synapses (Whittaker, 1986; Ecobichon and Joy, 1994). It is used to control parasite and insect populations on a wide range of different fruits, vegetables and other commercially produced plants like tobacco or tea (Cycoń et al., 2009). Furthermore, diazinon had also been used in home gardening and household environments for pest control (Bailey et al., 2000) until its usage for plant protection was prohibited by the EU in 2007 (European Commission for Food Safety, 2007). Diazinon is U.S. EPA registered (Kegley et al., 2016) but only for agricultural settings (U.S. EPA I.R.E.D. for Diazinon, 2007), whereas in India it is banned for use in agriculture except for household use (Paranjape et al., 2015). As recently reviewed by Montuori et al. (2016), diazinon is found in aquatic systems all over Europe – low concentrations were measured in the North Sea (up to 0.046 ng L^{-1}) and the highest concentration was reached in the Ebro River in Spain with up to 785 ng L^{-1} diazinon. It is therefore important to investigate its effect on aquatic organisms.

Diuron is a phenylurea herbicide that blocks the electron transfer to the photosystem II within the thylakoid membrane of chloroplasts, which inhibits photosynthetic oxygen and energy production in plants and algae (Wessels and Van der Veen, 1956). It is currently used for agricultural purposes in the EU to control weeds and mosses in crop areas (European Commission for Food Safety, 2008) and also for non-agricultural purposes such as home gardening or on railway lines (Giacomazzi and Cochet, 2004). Diuron is also U.S. EPA registered (Kegley et al., 2016) and was among the 25 most commonly used conventional pesticide active ingredients in agricultural market sector in the U.S. in 2007 (Grube et al., 2011). The highest concentrations measured in European waters were up to 2000 ng L^{-1} in the Ebre Delta in Spain and up to 6742 ng L^{-1} in the Southampton water estuary in United Kingdom (Konstantinou and Albanis, 2004). In addition, the product of hydrolysis and photodegradation of diuron is 3,4-dichloraniline (3,4-DCA), which has been reported to be highly toxic (Giacomazzi and Cochet, 2004) also after biotransformation (Xiao et al., 2016). Generally, herbicides are considered to be less toxic to fish and aquatic life than insecticides, however some can be highly toxic to aquatic organisms. Consequently, it is important to assess the toxicity of commonly used herbicides like diuron to aquatic organisms and investigate the mechanism of action in animal organisms.

Pollutant-induced changes in fish behavior are among the most sensitive indicators and earliest warning signals of aquatic stress conditions (e.g. Giattina et al., 1982; Beitinger, 1990; Scott and Sloman, 2004; Sloman et al., 2006). Therefore, the quantitative analysis of fish behavioral responses to pollutant exposure is useful to explore the effects of environmental stressors (Eissa et al., 2010). Even though the measurement of behavioral endpoints in toxicity testing in different fish species has been utilized for quite some time, in zebrafish the assessment of behavioral endpoints can be considered as an upcoming approach. Namely, zebrafish has gained increasing popularity as a model organism for aquatic vertebrate toxicity testing over the two last decades. The acute toxicity of a variety of chemicals on embryonic life stages can be determined by conducting the Fish Embryo Toxicity (FET) test (OECD, 2013). Additionally, the utilization of zebrafish as a test organism also allows for the investigation of effects on a wide range of biological levels, from enzymes (Yen et al., 2011) to sensory organs such as the lateral line (Buck et al., 2012) and, as already stated, behavioral endpoints (Vignet et al., 2013). Behavioral effects can be assessed at early stages, with zebrafish larvae performing complex swimming (Ali et al., 2011; Budick and O'Malley, 2000). In addition, anxiety-like behavioral responses such as thigmotaxis can be evoked in zebrafish confronted to novel environments (Champagne et al., 2010). The expression of thigmotaxis can be modified by stressful and anxiety-provoking contexts (Sharma et al., 2009) and it is known that some pollutants can affect the thigmotactic response of larval and adult zebrafish (e.g. Richendrfer et al., 2012; Schmidel et al., 2014; Gonzalez et al., 2016). Furthermore, behavior studies can help to bridge the gap between high throughput *in vitro* assays that focus on lower biological level and whole-organism assays that investigate complex endpoints (Selderslaghs et al., 2010). Zebrafish behavioral assessment recently started to be used for the investigation of effects of a wide variety of chemicals, including the effects of pesticides (e.g. Jin et al., 2016; Nüßer et al., 2016; Wang et al., 2016). Few studies to date have investigated the effects of diazinon and diuron on fish behavior. Since diazinon is an AChE inhibitor it is likely that it would cause neurotoxic and thus behavioral effects. Yen et al. (2011) indeed demonstrated that diazinon lowers larval swimming activity and inhibits AChE activity in zebrafish. In contrast, diuron is an inhibitor of photosystem II and although it was not probable that it would affect behavior it was shown that short-term exposure of goldfish (*Carassius auratus*) influenced swimming and social behaviors such as burst swimming activity and grouping, respectively (Saglio and Trijasse, 1997). The present study aimed to investigate and compare the toxicities of two commonly used pesticides with completely different mode of actions – diazinon as a representative of insecticides and diuron as a representative of herbicides. An additional goal was to verify and identify zebrafish behavior as a useful biomarker to pesticide exposure. In order to achieve these goals, the acute toxicity and behaviors of zebrafish embryo and larvae were assessed after exposures to diazinon and diuron. After determination of respective acute toxicity effect concentration values, three exposure concentrations ranging between half of the NOEC and reaching up to the