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Disturbances in energy metabolism of *Daphnia magna* after exposure to tebuconazole

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ABSTRACT

This study was conducted to investigate the change of some biochemical parameters in the aquatic invertebrate *Daphnia magna* following exposure to the fungicide tebuconazole and to determine the most sensitive biomarker among the ones tested in this species. Four biochemical biomarkers (protein, glycogen, lipids and caloric content) were correlated with feeding behaviour studies of *D. magna* after fungicide exposure. Juveniles of *D. magna* were exposed to four sublethal concentrations of tebuconazole (0.41, 0.52, 0.71 and 1.14 mg L^{-1}) for 5 d. Daphnid samples were taken from each test and control group at 24, 48, 72, 96 and 120 h after the start of the experiment. Tebuconazole EC_{50} values were calculated on *D. magna* in our laboratory as 56.83 and 40.10 mg L^{-1} at 24 and 48 h, respectively. Results showed that daphnid energy content decreased as tebuconazole concentration increased, especially after 96–120 h of exposure to 0.52 mg L^{-1} and higher fungicide concentrations. The data suggest that tebuconazole is moderately toxic to *D. magna* but also that it seriously impairs the metabolic functions, resulting in alterations in biochemical constituents. In the *D. magna* feeding study, algae feeding rates were inhibited after fungicide exposure. Such findings indicate the importance of feeding studies in laboratory toxicity test as well as their relationship with others studies. The results emphasize the importance of considering different kind of biomarkers to identify and evaluate the biological effect of a fungicide in the aquatic environment. Although the biochemical biomarkers used resultated good indicators of tebuconazole toxicity, feeding rates in *D. magna* decreased after only 5 h exposure to the fungicide resulting in the most sensitive parameter of daphnid fungicide exposure.

1. Introduction

Biomarkers, can be defined as biochemical, cellular, an physiological changes caused by adverse environmental conditions (including xenobiotic exposure) that are measurable in a biological system and can be evaluated at different levels of organization, on the assumption that low toxicant levels cause biochemical responses within individual organism before those effects are observed at higher levels of biological organization (Schlenk, 1999; Mc Loughlin et al., 2000). Therefore, the utilization of integrated responses to evaluate the effects of pollutants has been suggested as a novel way to assess an organism's survival potential and to allow a better understanding of possible effects at higher levels of biological organization (Barata et al., 2007; Jemec et al., 2008).

Generally, sublethal stress induces compensatory changes in the organism's energy metabolism. Because the majority of the organism's energy budget is used for growth, reproduction, and basal metabolism, increased energy expenditure in basal metabolism to cope with the toxic stress will lead to a reduction in energy reserves. As a result of the presence of sublethal pollutant concentrations in the aquatic medium, some biochemical parameters in animal scan are altered. Within the sublethal range a wide variety of reversible and irreversible processes take place in order to maintain homeostasis. The dynamics of intermediary metabolism are greatly influenced by any sort of stressors (any sort of change altering the homeostasis of the animal).

Biochemical parameters are very sensitive to sublethal concentrations of many stress agents. Their main disadvantage is that they are often specific to special responses. It is therefore possible to observe no change in experimental results if the appropriate biochemical system is not chosen. For this reason, it is preferable to choose general parameters (e.g. glucose, glycogen, lactate) to determine a stress situation in the organisms under study.

In cladocerans, lipids and proteins are considered to be good indicators of the nutritive state and reflect the entire physiological state of the organism (Guisande et al., 1991; Printes and Callaghan,

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2003). At low food concentration, lipid reserves, mainly triacylglycerides, are metabolised while proteins are only catabolized under severe starvation (Elendt, 1989). On the other hand, the basic response of the intermediary metabolism to stress appears to consist of an enhanced mobilization and utilization of carbohydrate reserves. A central part of the organism's metabolism is the carbohydrate metabolism, which fuels the energy demanding synthetic reactions and provides necessary building blocks for various anabolic pathways (De Coen et al., 2001).

Daphnids are an important component of aquatic systems. Cladocerans, especially *Daphnia* spp., are among the most favourable test animals in aquatic toxicology. The many advantages of daphnids, e.g., sensitivity to toxicants, parthenogenetic reproduction and the short reproductive cycle and life span, can hardly be found in combination in any other species (Bodar et al., 1988).

Energy reserves as protein, glycogen and lipid content of *Daphnia magna* can be considered as indicative of its overall condition, and changes on their concentration, have been used as an indicators of exposure to stressful conditions in several researches (McKee and Knowles, 1986; Knowles et al., 1987; Bodar et al., 1988; De Coen and Janssen, 2003).

Behaviour changes have been used successfully as rapid and sensitive indicator of toxic stress. Several researchers (Flickinger et al., 1982; Day and Kaushik, 1987) have suggested that the feeding behaviour of zooplankton is a physiological function and could be considered as and important factor in studies dealing with the toxicity of pollutants to aquatic organisms. Thus, the impairment of biochemical and physiological functions of an organism may be taken as the first indication showing the effect of an environmental perturbation. This impairment could result in measurable changes in the behaviour of the organism. These changes may be used as rapid and sensitive indicators of toxic stress and may help to explain other observed changes in the energy reserves and the cycle of the organism. Furthermore, a toxicant-induced reduction in feeding rate is relevant from the ecological point of view as it could be related to reductions in an organism energy assimilation, which, in turn, could lead to a reduction in energy reserves and finally translate into effects at the population level (Maltby and Naylor, 1990; Maltby, 1994; Maltby et al., 2001; Irving et al., 2003).

Aquatic filter-feeders tend to be primary consumers occupying a key role at the base of the food chain within aquatic ecosystems. Alterations in their feeding behaviour and energy reserves could have significant consequences for ecosystem structure and function, including alteration of the population size of both invertebrate and vertebrate predators (Taylor et al., 1998).

Current-used pesticides (CUPs) can be defined as those modern pesticides that are currently registered for use, generally developed from chemical synthesis, and typically used in the agriculture or lawn care sector (Konwick et al., 2006). Tebuconazole is widely used as fungicide in paddy fields from Eastern Spain among the Spanish Mediterranean wetlands to avoid and treat rice blast disease caused by *Pyricularia oryzae*. Tebuconazole (commercial name FolicurR) is classified as a moderately toxic material to aquatic organisms that may cause long-term adverse effects in the aquatic environment (Bayer CropScience Limited, 2005). It is important to understand the toxicity of such fungicides to non-target aquatic organism because of the amounts of pesticides field application and the risk of mixture with water exiting fields, furthermore no information is available about the effects on energy metabolism of *D. magna*.

In the present study, the effects of sublethal tebuconazole exposure on the energy content of the crustacean *D. magna* were studied and compare with feeding behaviour as physiological and ecological parameter.

2. Materials and methods

2.1. Test organisms

D. magna were obtained from continuous culture maintained in the laboratory in 6 L aquaria at a constant temperature of 22 ± 1 °C, in dechlorinated tap water (total hardness, 240 mg L^{-1} as $CaCO_3$; pH 7.9 ± 0.2; alkalinity, 4.1 mmol L^{-1}), 12 h:12 h light:dark photoperiod and a density of below 50 animals L^{-1}. The medium was renewed two times each week (Ferrando et al., 1992).

The daphnids were fed daily with the algae *Nannochloris oculata* (5×10^5 cells mL^{-1}). This algae was also continuously cultivated in our laboratory using a nutrient medium (Bischoff and Bold, 1983).

2.2. Test chemical

Tebuconazole fungicide used in the experiments was 96.5% pure (Bayer Crop Science Limited, Germany). Stock solutions were prepared by dissolving the toxicant in acetone prior to each experiment. The octanol–water partition-coefficient ($\log P_{ow}$) for tebuconazole is 3.7 (Turesson et al., 2007). Solid-phase extraction (SPE) followed by Gas-chromatography/Mass-Spectrophotometry techniques (GC-MS/MS) confirmed the presence of tebuconazole in the water at the desired concentration (nominal concentrations) over the entire exposure periods. Half-life of tebuconazole in the experimental laboratory tap water was 22 d as determined by Andreu et al. (2008).

2.3. Acute toxicity test

Preliminary acute toxicity test were conducted in our laboratory in order to calculate tebuconazole EC_{50} data. All experiments were performed according to the OECD standard procedure (OECD, 1984) for determining the 24- and 48-h-EC_{50} for *D. magna*. Tebuconazole concentrations used in the experiment were 30, 40, 50, 60 and 70 mg L^{-1} plus the blank control and the acetone control containing the highest concentration of acetone used to solve tebuconazole in our experiment (70 μL L^{-1}). Ten neonates (<24 h old) from a designated brood were placed in 30 mL glass beakers each of them containing 25 mL for each test concentration and controls. Test organisms were not fed during the testing period. Observations were made at 24 and 48 h, and results recorded. All experiments were done in triplicate.

2.4. Sublethal toxicity study

The sublethal test concentrations used in the present study were based on the 24-h-EC_{50} (56.83 mg L^{-1}) of tebuconazole for *D. magna*. In a short-term exposure experiment, daphnids were exposed during 5 d to the following sublethal fungicide concentrations: 0.41 (1/1140 EC_{50} −24 h), 0.52 (1/110 EC_{50} −24 h), 0.71 (1/80 EC_{50} −24 h) and 1.14 (1/50 EC_{50} −24 h) and blank control, respectively. Since acetone was required as a carrier, an acetone control with the highest concentration of acetone used to solve tebuconazole in our experiment (57 μL L^{-1}) was also included.

Neonates (<24 h old) of *D. magna* were exposed to tebuconazole in 60 mL glass beakers at each of the selected pesticide concentrations, plus the control. Twenty daphnids were randomly assigned to each of the beakers, and five replicates of each were done (for each fungicide concentration and time exposure). This was necessary for obtaining the minimum amount of sample for carrying out the analyses.

Water quality characteristics were constantly maintained by transferring the cladocerans to fresh test solutions or control water every day. The cladocerans were exposed to a wide-spectrum light