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Effects of food availability on the acute and chronic toxicity of the insecticide methomyl to *Daphnia* spp.

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Abstract

The widespread increase of pesticides application in crops threats vicinal freshwater lentic ecosystems, frequently leading to their contamination. Due to their position in the aquatic food web, the responses to these pesticide inputs of freshwater filter-feeding zooplankters, as daphnids, provide relevant information the general risk to the ecosystem of xenobiotics. Moreover, cladoceran grazers often face fluctuations in food availability due to the phytoplankton dynamics in lentic water bodies, and food acquisition naturally conditions their fitness. In this study, the responses of *Daphnia magna*, and of three genotypes within the *Daphnia longispina* complex, to acute and chronic exposures of methomyl, were assessed. In addition, we focused on whether the food level can model the *Daphnia* life-history responses to the insecticide. Results showed that methomyl was acutely and chronically toxic to both *D. magna* and the *D.* cf *longispina* populations at very low concentrations, and remarkable differences in sensitivity were noticed when comparing the responses to the toxic among *taxa*/genotypes. Furthermore, food availability conditioned the overall fitness of the species although not interacting specifically on the response to the toxicant stress.
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Keywords: *Daphnia magna*; *Daphnia* cf *longispina*; Methomyl; Food level; Life-history

1. Introduction

Pesticides are commonly used worldwide to face the need for increasing or improving agricultural production. There is a general trend to the development and preferable use of the so-called "benign pesticides" i.e. those pesticides which are selectively toxic, do not bioaccumulate, and have a relatively short persistence in the environment (Stark and Walter, 1995). In this way, insecticidal products such as carbamates have gained favour especially when compared e.g. with organochlorine compounds (Hutson and Roberts, 1985).

Methomyl [IUPAC: *S*-methyl *N*-(methylcarbamoyloxy)thioacetimidate] is a commonly used monomethyl carbamate insecticide, widely marketed since 1967 under the trade "Lannate" in certain fruit crops, vegetables, ornamentals and field crops (Clive, 2001), to control a wide range of insects and spider mites through direct contact and ingestion (WHO, 1996). It is a highly water soluble compound (25 °C: 54.7 g/L), stable in distilled water for 30 days at a 5–7 pH, and has a low octanol:water partition coefficient (Kow = 1.24); this insecticide was found to adsorb poorly even to soil organic matter and its therefore expected to be mobile in soil; and, in field conditions, methomyl does not get

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more than 20–30 cm into soil, which generally excludes ground water contamination by the chemical (WHO, 1996). Contamination of water bodies via terrestrial runoff or leaching should be a rare event; however, since application procedures involve mainly ground or aerial spraying, surface water bodies in the crop vicinities can be directly contaminated.

The contamination of the aquatic ecosystem by methomyl, as well as by other insecticides, is especially of concern when considering organisms inhabiting or having home ranges close to the surface layer of the water column: when reaching the water body, insecticides usually do not get instantaneously mixed in the water column; mixing occurs slowly, accompanied with the natural degradation and dissipation of the chemical (Van Wijngaarden et al., 2005). Moreover, the risk of such a contamination event increases when these non-target organisms have similar toxicant receptors as the target ones. WHO (1996, 2004) reports methomyl as highly hazardous, and ranges it as moderately to highly toxic to several aquatic non-target organisms, highlighting *Daphnia magna* as very sensitive to the chemical: methomyl registered a 48-h LC_{50} of 32 μg/L and a chronic maximum acceptable toxicant concentration (MATC) in the range of 1.6–3.5 μg/L for the species.

The particular mode of action of carbamates conditions their toxicity to non-target organisms such as *Daphnia*. They inhibit reversibly cholinesterase through the carbamylation of the enzyme and therefore it would be unlikely that single exposures induce long-lasting effects in surviving animals. Nevertheless, organisms inhabiting freshwater systems standing in agricultural areas often deal with frequent pesticide inputs resulting from repeated applications, and thus effects other than mortality (e.g. reproduction impairments) are likely to occur. Effects on reproduction or population growth of zooplankters, such as *Daphnia*, can be of a relevant ecological meaning since these organisms occupy a central position in the freshwater food web, and have a pivotal role as engagers of the energy transfer from producers to higher levels (Hanazato, 1998).

Amongst other natural stressors, predatory pressure, intra- and inter-specific interactions, and food availability can act singly or interactively to induce plasticity in *Daphnia* life-history traits (e.g. Gliwicz and Guisande, 1992; Burns, 2000; Pijanowska et al., 2006). Moreover, some studies have demonstrated that these natural stressors can modulate the effects of pesticides in *Daphnia*, and this joint action is likely to induce deleterious indirect effects at the population, the community and the ecosystem levels (see the reviews by Hanazato, 2001 and by Relyea and Hoverman, 2006). Within this context, food availability has a particular relevance since (i) phytoplankton densities in lentic ecosystems often fluctuate, and filter-feeding grazers need to deal with it by changing their life-history performances to optimally allocate the available resources (e.g. Gliwicz and Guisande, 1992; Guisande and Gliwicz, 1992; Boersma, 1997); (ii) coping with physiological stress (as that promoted by pesticides) is energetically costly to daphnids (Smolders et al., 2005) — often inducing *per se* compensatory changes in their energetic metabolism (Smolders et al., 2005; De Coen and Janssen, 2003) — and the nutritional state of the organism is a key factor conditioning this process (Heugens et al., 2001). Genotypic/species-specific differences were also shown to influence the *Daphnia* life-history responses to natural stressors (Kreutzer and Lampert, 1999; Burns, 2000; Antunes et al., 2003; Bernot et al., 2006; Gliwicz and Masczyk, 2007), to xenobiotics (Barata and Baird, 1998; Antunes et al., 2004; Marques et al., 2004a,b), or to their joint action (Antunes et al., 2004; Pieters and Liess, 2006; Pereira et al., in press).

Regarding the role of food availability as common natural stressor in freshwater ecosystems, and the potential ability of pesticides to harm non-target freshwater species, we intended to evaluate the combined effects of these stressors in survivorship and life-history of four different *taxa* belonging to the genus *Daphnia*. Previous studies have already reported more serious effects of a given toxic on *Daphnia* feeding in low food quantities, when compared with similar toxicant treatments having higher food supply (Folt et al., 1999; Pereira et al., in press). This study was designed in order to assess how the toxicity of the insecticide methomyl can be modelled by food, and whether this modelling pattern is consistent with those previously reported. Moreover, we focused on the relative differences in sensitiveness to acute and chronic methomyl exposures of three distinct autochthonous genotypes belonging to the *D. longispina* complex; as well, the responses of these species were compared with those of the standard species *D. magna*. This analysis is expected to produce evidence on the relevance of the use of standard species in risk evaluation procedures.

2. Materials and methods

2.1. Test organisms

The monoclonal *Daphnia* spp. bulk cultures were continuously reared in the lab, under a 16:8 h light:dark photoperiod, at a temperature of 20±2 °C, in synthetic ASTM hardwater medium (ASTM, 1980) supplied with

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