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Non-target effects of the insecticide methoprene on molting in the estuarine crustacean *Neomysis integer* (Crustacea: Mysidacea)

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Abstract

Ecdysteroids, the molting hormones in crustaceans and other arthropods, play a crucial role in the control of growth, reproduction and embryogenesis of these organisms. Insecticides are often designed to target specific endocrine-regulated functions such as molting and larval development such as methoprene, a juvenile hormone analogue.

The aim of this study was to examine the effects of methoprene on molting in a non-target species, the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea). Mysids have been proposed as standard test organisms for evaluating the endocrine disruptive effect of chemicals. Juveniles (<24 h) were exposed for 3 weeks to the nominal concentrations 0.01, 1 and 100 μg methoprene/l. Daily, present molts were checked and stored in 4% formaldehyde for subsequent growth measurements. Methoprene significantly delayed molting at 100 μg/l by decreasing the growth rate and increasing the intermolt period. This resulted in a decreased wet weight of the organism. The anti-ecdysteroidal properties of methoprene on mysid molting were also evaluated by determining the ability of exogenously administered 20-hydroxyecdysone, the active ecdysteroid in crustaceans, to protect against the observed methoprene effects. Co-exposure to 20-hydroxyecdysone did not mitigate methoprene effects on mysid molting. This study demonstrates the need for incorporating invertebrate-specific hormone-regulated endpoints in regulatory screening and testing programs for the detection of endocrine disruption caused by man-made chemicals.

Keywords: Endocrine disruption; Juvenile hormone analog; Molting; Methoprene; *Neomysis integer*

1. Introduction

It is increasingly recognized that for the assessment of the ecological impact of potential endocrine disrupters relevant hormonal mechanisms for both invertebrates and vertebrates need to be considered. Invertebrates account for roughly 95% of all animals (Barnes, 1980), yet surprisingly little effort has been invested to understand their value in signaling potential environmental endocrine disruption. Since the hormones produced and used in invertebrates are not directly analogous to those of vertebrates, it is essential to incorporate invertebrate-specific hormone-regulated endpoints in studies aimed at evaluating potential endocrine disruption.

Mysid crustaceans have been traditionally used in standard marine/estuarine toxicity testing because of their ecological importance, wide geographic distribution, year-round availability in the field, ease of transportation, ability to be cultured in the laboratory, and sensitivity to contaminants. In addition, mysids have been proposed as potential test organisms for the regu-

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latory screening and testing of endocrine disruptors by several agencies such as USEPA, OECD and the Ministry of the Environment of Japan (Verslycke et al., 2004a).

Molting is regulated by a multihormonal system, but is under the immediate control of molt-promoting steroid hormones, the ecdysteroids, secreted by the Y-organ (Fig. 1). The Y-organ 69 secretes ecdysone, which upon release in the hemolymph, is converted into active 20-hydroxyecdysone. Ecdysteroids also play a fundamental role in the control of reproduction and embryogenesis (Subramoniam, 2000). One major advantage of using ecdysteroid metabolism as an endpoint is that it provides a means of evaluating the impact of environmental chemicals on crustaceans (and potentially other arthropods); chemicals which may not necessarily affect vertebrates (Verslycke et al., 2004a). Juvenile hormones regulate metamorphosis and reproduction in insects. With the discovery of the chemical structure of insect juvenile hormone in 1967 (Roller et al., 1967), attempts were made to produce synthetic analogs for use as "third generation" insecticides (Williams, 1956). Methoprene is such an insecticide which acts as a juvenile hormone analog and disrupts normal development of insects by inhibiting developing pupae from molting and passing into the adult stage. Methoprene is one of the most widely used and successful insect growth regulators. One of the main applications of methoprene is mosquito control. Methoprene can enter estuarine environments by either direct application for controlling aquatic-borne pests (such as mosquitoes) or indirectly through land-drainage or erosion from adjacent pesticide-treated agricultural lands (Dhadialla et al., 1998; Retnakaran et al., 1985). Methoprene degrades rapidly in sunlight (Quistad et al., 1975) and in water (Schaefer and Dupras, 1973). Methoprene may have broken down during the bioassay, but methoprene breakdown products are also known to be bioactive (Harmon et al., 1995; LaClair et al., 1998). It was beyond the scope of this study to determine whether the effects observed were mediated by methoprene itself or by its breakdown products such as methoprenic acid. The use of methoprene at recommended application rates is expected to result in environmental concentrations of ~10 μg/l (Ingersoll et al., 1999). Methoprene concentrations in natural water of the US ranged from 0.39 to 8.8 μg/l (Knuth, 1989), which is in the concentration range where laboratory effects were observed on endocrine regulated processes in crustaceans (McKenney and Celestrial, 1996; McKenney and Matthews, 1990; Peterson et al., 2001). However, US-EPA has not reported any specific ecological effects indicating a significant risk associated with methoprene and considers methoprene use (US EPA, 2001).

Similarities between the endocrinology of molting in crustaceans and insects led to the discovery of a crusta-

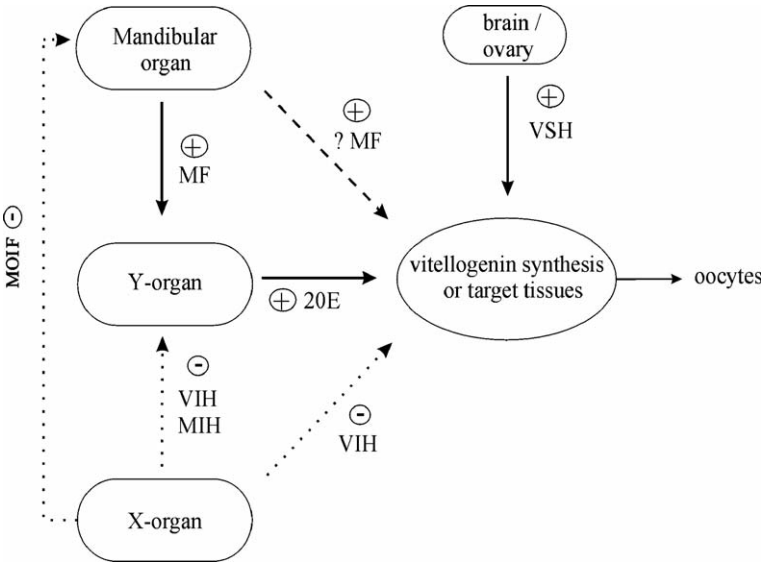

Fig. 1. Schematic representation of the hormonal control of the crustacean molt cycle and vitellogenesis. Adapted from Defur et al., 1999; Meusy and Payen, 1988; Oberdörster and Cheek, 2000. Interrupted arrows (−) represent inhibition and full arrows (+) stimulation. The following hormones play an important role in regulating crustacean molting and vitellogenesis: 20E, 20-hydroxyecdysone, the active molting hormone; MF, methyl farnesoate; MOIF, mandibular organ-inhibiting factor; VIH, vitellogenesis-inhibiting hormone; MIH, molt-inhibiting hormone; VSH, vitellogenesis-stimulating hormone.