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Effects of chlordecone on 20-hydroxyecdysone concentration and chitobiase activity in a decapod crustacean, *Macrobrachium rosenbergii*

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ABSTRACT

Chlordecone (CLD) is an organochlorine insecticide abundant in aquatic environment of the French West Indies. However, few studies have investigated its impact on freshwater invertebrates. Whereas CLD is suspected of inducing endocrine disruption, this work aimed to study the effects of environmentally relevant concentrations of CLD on the 20-hydroxyecdysone (20-HE) hormone concentration and on the chitobiase activity, both having key roles in the molting process of crustaceans. In addition, the bioaccumulation of CLD was measured in the muscle tissue of *Macrobrachium rosenbergii* to underline potential dose-response relationship. The results have shown that CLD was bioaccumulated in exposed organisms according to a trend to a dose-response relationship. Moreover, it was observed that CLD decreased the 20-HE concentration in exposed prawns when compared to control, whatever the duration of exposure, as well as it inhibited the chitobiase activity after 30 days of exposure. The present study indicates that CLD could interfere with molting process of *M. rosenbergii* by disturbing the 20-HE concentration and the activity of chitobiase, suggesting consequences at the long term on the shrimp development. This study also confirmed that CLD could be an endocrine disruptor in decapod crustaceans, as it was already observed in vertebrates.

1. Introduction

Endocrine disrupting compounds (EDCs) are exogenous substances or mixtures able to interfere with the endocrine system of exposed organisms. They could affect the hormonal signaling pathways through several mechanisms, for example by inhibiting the synthesis of hormones or decreasing the hormone release by endocrine cells (Craig et al., 2011; Tabb and Blumberg, 2006; Rodríguez et al., 2007). EDCs can also disrupt hormone-receptor interactions and act as an agonist or antagonist by binding to the hormone-receptor complex (Rodríguez et al., 2007; Tabb and Blumberg, 2006). Aquatic environments are considered the main sink for contaminants (Kloas et al., 2009; Meyer-Reil and Köster, 2000), and thus aquatic organisms are therefore major potential targets for EDCs (Kloas et al., 2009).

Several studies on the biological effects of EDCs in aquatic vertebrates have led to the development of biomarkers in order to measure the biological responses towards anthropogenic pollutions, such as changes in the reproductive function. For example, Jobling and Sumpter (1993) introduced the concept that the production of vitellogenin (Vg) in male fish indicates an exposure

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to estrogenic compound and Vg is now a widely used biomarker of xenoestrogen exposure in vertebrates. Although EDC effects have been extensively described in aquatic vertebrates (Kortenkamp et al., 2011; LeBlanc, 2007), only few studies have examined the effects of EDCs in invertebrates, although they represent the major part of the animal kingdom (DeFur et al., 1999). This lack could be partially due to the fact that the endocrine system of invertebrates is still obscure (Tillmann et al., 2001). It has nevertheless been observed that EDCs can also disrupt invertebrate physiology. For example, Giusti et al. (2013) have shown that tributyltin affected the production of yolk ferritin (i.e. Vg equivalent) in *Lymnaea stagnalis*, and Xuereb et al. (2011) observed that nonylphenol (estrogenic compound) impacted the expression of a vitellogenin-like gene in the amphipod *Gammarus fossarum*. It appears that vitellogenin-like proteins in invertebrates could serve as biomarkers of EDC exposure, but mechanisms involved in Vg production in invertebrates are still unclear (e.g. hormonal receptor).

EDCs can come from different sources such as synthetic hormones (e.g. ethinylestradiol), synthetic substances (e.g. plasticizers such as phthalates, herbicides) and pesticides often used to eradicate insects. In the French West Indies (FWI), the tropical climate promotes the rapid development of pests which exerts significant pressure on crops leading to the use of considerable amounts of pesticides in these regions (Bocquené and Franco, 2005). The use of organochlorine pesticides first started in the 1950's and led to widespread contamination of the environment (Coat et al., 2006). Because of their physicochemical properties, organochlorine pesticides are persistent in the environment and are known to be accumulated along food chains, resulting in pronounced ecotoxicological damage (Cailleaud et al., 2009; Matsumura, 1975). The most worrying organochlorine pesticide residue in Guadeloupe (FWI) is chlordecone (CLD) (Coat et al., 2011). CLD is an insecticide that was commonly employed to control the banana weevil *Cosmopolites sordidus* from 1972 to 1993 under the trade name Kepone® or Curlone® (Cabidoche et al., 2006, 2009). A few years after the use of CLD, widespread pollution of soils, rivers, wild animals and aquatic organisms was reported (Cavelier, 1980; Snegaroff, 1977), which led to the prohibition of the use of this pesticide in Guadeloupe in 1993. Moreover, in 2009, CLD was included in the Stockholm Convention on POPs (Persistent Organic Pollutants) and its production and use were banned worldwide (Zaldívar and Baraibar, 2011). Nowadays, CLD is still present in soils, especially in the densely cultivated areas from south of Basse-Terre (Guadeloupe) (Cabidoche et al., 2009). Although, Fernández-Bayo et al. (2013) showed the existence of CLD degrading organisms in a tropical soil (andosol) microcosm under aerobic conditions, CLD undergoes no significant or quick biotic or abiotic degradation (Dolfing et al., 2012; Levillain et al., 2012). Driven by the water cycle, CLD in soils is progressively transferred to aquatic ecosystems (Coat et al., 2011) and also in the food web because of its high K_{oc} (Soil Organic Carbon Water Partitioning Coefficient) (15849 L/Kg), K_{ow} (Octanol-Water Partition Coefficient) (4.5–6.0) and its affinity for lipids (Cabidoche and Lesueur-Jannoyer, 2012; Clostre et al., 2013; Sterrett and Boss, 1977; UNEP, 2005; US-EPA, 2008). Human contamination has also been detected in FWI population mainly resulting from consumption of contaminated food, seafood and root vegetables (Dubuisson et al., 2007; Gaume et al., 2014; Guldner et al., 2010). Fishing became prohibited when most aquatic species have exceeded the European legal maximal residual limit (LMR) of 20 μg of CLD per kg wet weight (determined by National ordinance, Anon., 2008). Until 2008, one of the most important aquaculture resources in Guadeloupe was the farms of the tropical giant freshwater prawn *Macrobrachium rosenbergii*. Several studies have underlined effects of pesticides on this species (Revathi and Munuswamy, 2010; Satapornvanit et al., 2009), but very few investigations were carried on the CLD impacts on *M. rosenbergii*. However, Gaume et al. (2014) have observed that the CLD exposure caused the induction of genes involved in defense mechanisms against oxidative stress (e.g. catalase and glutathione peroxidase), or involved in the biotransformation process (i.e. cytochrome P450 and glutathione-S-transferase).

As CLD is suspected of being an endocrine disruptor (Newhouse et al., 2009) and as *M. rosenbergii* molting is hormonally controlled, the present study aims to investigate the CLD impact on the molting process by investigating the effects of CLD on the concentration of the 20-Hydroxyecdysone (20-HE) hormone and on chitobiase activity in tissues of *M. rosenbergii*. The 20-HE hormone is an ecdysteroid hormone, secreted by the Y-organ (Mykles, 2011), which initiates many physiological processes, such as ovarian maturation, growth, molting, and reproduction in crustaceans (LeBlanc, 2007). A few studies have been designed to investigate the effects of exposure to environmental EDCs on the endocrine system of crustaceans through ecdysteroid concentration, or to investigate the endocrine system of invertebrates (Oberdörster et al., 1999; Palma et al., 2009; Soetaert et al., 2007), but no studies have used the 20-HE concentration to investigate the effects of chlordecone.

Chitobiase is a chitinolytic enzyme involved in the exoskeleton degradation in arthropods and thus plays an important role in the molting and growth of crustaceans (Duchet et al., 2011; Zou and Fingerman, 1999a). Several studies have demonstrated that chitinolytic enzymes are induced by the 20-HE hormone (Zou and Fingerman, 1999b). However, many pollutants have been also shown to induce the chitobiase enzyme. Indeed, Zou and Fingerman (1999c) observed that some estrogenic agents, such as Aroclor 1242, diethylstilbestrol or endosulfan, inhibited chitobiase activity of the crustacean *Uca pugilator*. Similarly, Gismondi and Thomé (2014) noted that some pollutants, suspected of being EDCs (i.e. polybromodiphenyl ethers), could disturb the chitobiase activity of the amphipod *Gammarus pulex*.

Finally, in parallel with these two parameters, the bioaccumulation of CLD in *M. rosenbergii* was evaluated to underline potential concentration-response relationships between the CLD concentration in tissues, the 20-HE concentration and the chitobiase activity. These measures ensure assessing the molting disruption, called the invisible endocrine disruption, because of the disruption of the crustacean molting which is not readily seen in the wild (Zou, 2005).

2. Materials and methods

2.1. Tested organisms

The 3-month-old post-larvae of *M. rosenbergii* (approximately 2 g, 7 cm length and sexually undifferentiated) coming from the same berried female, were provided by an aquaculture farm (OCEAN-SA) located at Pointe-Noire (Guadeloupe, FWI) in a geographic area free of CLD contamination. Pretests have previously been carried out to evaluate the presence or absence of CLD in tissues of prawns from Pointe-Noire and results have shown no contamination (concentrations below detection limit) (data not shown). Prawns were then transferred to the laboratory (DYNECAR, University of the French West Indies and Guiana, Guadeloupe), and acclimated for one week in glass aquaria filled with 28 L of tap water prefiltered on activated carbon. Aquaria were under constant aeration with a 12 h light/dark photoperiod. During acclimation, prawns were fed once daily with artificial shrimp pellets (complete food for rearing, Le Gouessant, France) at one pellet per individual. A constant water temperature of 27.6 ± 0.2 °C was maintained, and pH remained at 7.57 ± 0.03 throughout the experiment. These values are in accordance with optimal water temperature and pH commonly used in prawn farms (New, 2002). Survival was