Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 99, No. 5, September 1990, pp. 397–400.

Tributyltin oxide induced alterations in exuvial weight and calcium content of the prawn, *Caridina rajadhari*

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MS received 12 February 1990

Abstract. Moulting is the predominent feature of crustaceans. Cyclic cuticle deposition and resorption of calcium from old cuticle occur in relation to the crustacean moult cycle. Prolonged exposure of the prawn, *Caridina rajadhari* to media containing sublethal doses (0·015, 0·020, 0·025 and 0·040 ppm) of tributyltin oxide led to an apparent increase in dry weight of exuvia as well as an increase in the total quantity of calcium. The increase in weight and calcium content of exuvia was not significant in the initial two moults whereas it was significant ($P < 0·05$) in the third moult when exposed to minimum (0·015 ppm) dose. However significant ($P < 0·01$) rise in both exuvial weight and calcium quantity in the first two moults and highly significant ($P < 0·001$) in the third moult exuvia were observed when exposed to maximum (0·040 ppm) dose. The observed changes in exuvia from prawns exposed to tributyltin oxide might be due to a decrease in the resorption of the old cuticle and/or due to the inhibition of chitinolytic enzymes.

Keywords. Tributyltin oxide; exuvial weight; calcium; elevation; *Caridina rajadhari*.

1. Introduction

Moulting forms the most characteristic and important metabolic event in the life cycle of crustacean animals (Highnam and Hill 1979). Cyclical cuticle deposition and resorption of calcium from old cuticle occur in relation to the crustacean moult cycle (Passano 1960). Though there has been a great deal of research on the impact of aquatic pollutants on moult cycle there have been very few studies to investigate the alterations in the exuvium (cast exoskeleton). Branon and Concklin (1978) studied the effect of sodium pentachlorophenate (NaPcP) on exoskeletal calcium in the grass shrimp, *Palaemonetes pugio*.

The above literature revealed that no attempt has been made on the impact of organotin antifouling compounds, which are extensively used in paint, agriculture and paper industries as a protective biocide. In this paper an attempt has been made to study the impact of sublethal concentrations of tributyltin oxide (TBTO) on exoskeletal weight and calcium on 3 successive moults of *Caridina rajadhari*.

2. Materials and methods

The freshwater prawns, *C. rajadhari* were procured from Kham river near Aurangabad and maintained in plastic troughs having sufficient amount of well aerated tap water. Water was changed daily and the animals were fed with green algae (Chara, Pyranimonas) twice in a week, and were acclimated to the laboratory conditions for a week prior to the commencement of the experiment.

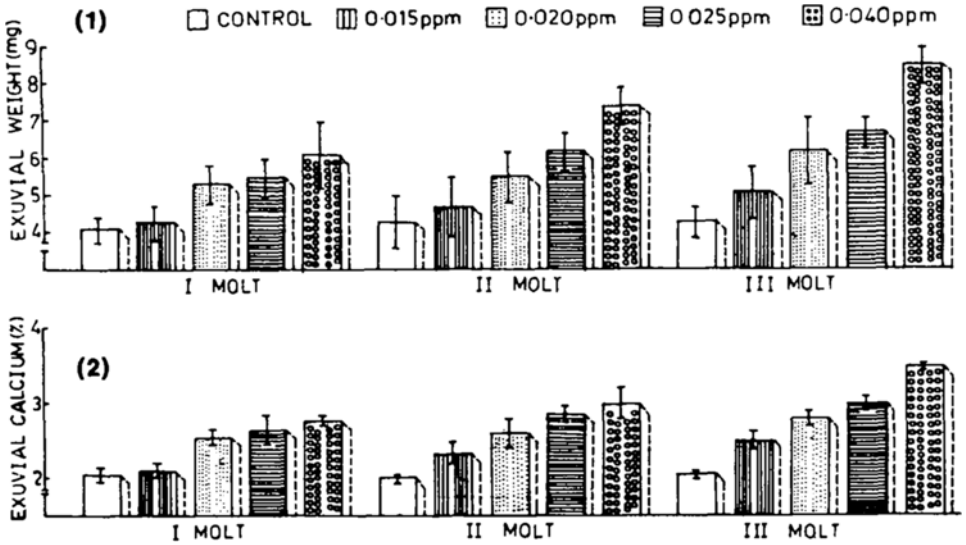
To determine the impact of sublethal concentrations of TBTO on exuvial weight and calcium quantity, prolonged experiments up to the completion of 3 moults in

the test as well as control media were performed. The solutions were not aerated but renewed every 24 h to provide fresh oxygenated media as well as to remove accumulated waste products. Healthy, mature, intermoult (stage C) laboratory acclimated prawns of same size (25 ± 3 mm length from rostrum to telson) and sex (female) were divided into 5 groups of 20 each, among which one group served as control and the remaining 4 groups exposed to 0·015, 0·020, 0·025 and 0·040 ppm of TBTO in 500 ml beakers individually. The jars were examined daily for the cast exuvia.

Cast exuvia were collected following ecdysis and preserved in individual vials after rinsing with deionized, glass distilled water. Precaution was taken not to include exuvia which had been partially eaten by molted shrimp. The exuvia were dried at 110°C for 48 h after which dry weights was measured. After obtaining dry weights, exuvia were processed for calcium analysis as described by Hawk *et al* (1965) with a little modified method by Sarojini *et al* (1983). The calcium content is expressed in percentage.

3. Results

Alterations in exuvial weight and calcium quantity of *C. rajadhari* are shown in figures 1 and 2. The observed data showed that control prawns did not exhibit any change in weight and calcium of exuvia in the 3 moults, whereas it was altered when introduced to TBTO contaminated media. The increase in dry weight and calcium content of exuvia was not significant in the initial two moults, when exposed to minimum (0·015 ppm) dose whereas it was highly significant ($P < 0·01$) when it was exposed to maximum (0·040 ppm) dose. Even the exuvia from prawns exposed to 0·015 ppm TBTO, which underwent third ecdysis were heavier and had significantly greater ($P < 0·05$) weight and higher amount of calcium than control.



Figures 1 and 2. Exuvial weight (1) and calcium content (2) of 3 consecutive moults of the prawn, *C. rajadhari* exposed to TBTO.

Thus the elevation in both weight and calcium content of exoskeleton is dose and exposure period dependent.

4. Discussion

In the present investigation increase in dry weight and calcium quantity of exuvia of prawn administered with different sublethal concentrations of TBTO from initial moult to 3 subsequent moults was noticed. A decrease in the resorption from the old exoskeleton after exposure prior to moult may be one of the reasons for the shedding of heavier exoskeleton. Similar observations and suggestions were reported by Branon and Concklin (1978) in the prawn, *P. pugio* exposed to NaPcP. Sarojini *et al* (1983) observed increase in weight and calcium quantity of exuvia of *C. rajadhari* after exposure to NaPcP and suggested that NaPcP might be preventing the resorption of calcium from the old exoskeleton. These results were also supported by the findings of Sarojini and Reddy (1984), who reported rise in exuvial weight of *M. kistnensis* exposed to sevimol.

Further causative factor for rise in calcium and weight of the exuvia might be due to the activity of chitinolytic enzymes. The passing of minerals from the exuvial space is via the already intercalated new epi- and exocuticle via the epidermis and the basement membrane into the haemolymph. Presumably controlled by the moult hormone, an ecdysteroid (Buchholz 1989) chitinase activity rises sharply at the onset of the premolt (D_0 stage). This coincides with the opening of the exuvial cleft and an increase of glucosamine in the haemolymph may be responsible for apolysis and the initiation of cuticular material resorption (Buchholz and Buchholz 1988). The activity of N-acetyl-β-D-glucosaminase was high when the endoenzyme chitinase reaches its maximum in late precdysis (stage D_3-D_4) (Buchholz 1989). The relatively high chitinase activity in D_1-D_2 stage in Antarctic krill, *Euphausta ruperba* and maximum glucosamine concentration in the haemolymph during D_3-D_4 stage indicates some enzymatic digestion of the old cuticle (Buchholz and Buchholz 1989). For the rapid decomposition of cuticular chitin in D_3-D_4 stages the above mentioned processes should be effectively operative without any interruption.

Malley and Chang (1985) reported the inhibition of calcium uptake by aluminium at pH 5·5 by the crayfish, *Orconectes virilis*. Patil (1989) observed the rise in dry weight and calcium content of the exuvia when the prawn, *M. lamerrii* exposed to benzene and suggested that this might be due to the prevention of reabsorption of calcium from the old exoskeleton.

By considering the above reports it can be suggested that TBTO might inhibit the resorption of calcium and or inhibits the chitinolytic enzymes resulting in the shedding of heavier exoskeleton with higher quantity of calcium. Further research is necessary to show in which way TBTO acts in the processes of exoskeleton digestion and resorption.

Acknowledgement

One of us (PSR) is thankful to PL-480 authorities for financial assistance.

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