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Midgut Pathology of Aldrin, Monocrotophos, and Carbaryl in the Freshwater Crab, *Paratelphusa masoniana* (Henderson)

N. Kaushik, S. Kumar

Department of Zoology, D. S. B. Campus, Kumaon University, Nainital 263 002, U. P., India

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The decapod crustaceans are a category of commercially valuable organisms whose existence is being threatened by a wide range of pesticides which are increasingly contaminating the aquatic environment. To assess this adversity, several studies have been undertaken but almost all of these have remained restricted to the determination of the lethal concentrations of pesticides in crabs and prawns (Eisler 1969; Omkar and Shukla 1985; Radhakrishnaiah and Renukadevi 1990). Presently, a greater emphasis is being undertaken to examine the chronically sublethal consequences of pesticide poisoning in animals. Quite often, the concentrations of pesticides which are not quickly fatal, have been found to induce serious organal pathologies that directly interfere with vital life processes (Konar 1970; Kumar and Pant 1984).

The literature thus far available does not provide any information on the histopathological effects of pesticides in crustaceans. The present paper is an endeavour to fill this gap and it describes the comparative sublethal midgut (intestinal) pathology of three commonly used pesticides, viz., aldrin (an organochlorine), monocrotophos (an) organophosphate) and carbaryl (a carbamate) in the freshwater crab *Paratelphusa masoniana*.

MATERIALS AND METHODS

The sublethal midgut pathology of the aforesaid pesticides has been studied by static bioassay experiments for a period of one month. Live, intermolt *P. masoniana* of both sexes, having a carapace width of 70±5 mm, were acclimatized to laboratory conditions for one week in dechlorinated tap water prior to their use in experiments. The presently tested concentrations of the pesticides were equivalent to ¼ (0.5975 mg l^{-1} for monocrotophos

Correspondence to: S. Kumar

and 0.2520 mg l^{-1} for carbaryl) and $^{1}/_{6}$ (34.90 mg l^{-1} for aldrin) of their 96-hr LC_{50} (median lethal concentration) values, evaluated earlier for the concerned crab (Kaushik and Kumar 1993). A relatively much lower sublethal concentration of aldrin was used in view of the fact that, during prolonged treatment, aldrin produces a greater physical stress in *P. masoniana* in comparison to the other two pesticides. The bioassay media (dechlorinated tap water) had the hardness in the range of 400-420 mg l^{-1} as $CaCO_3$ and the pH in the range of 7.62-8.90, measured according to APHA (1989). During the course of the experiments, the crabs were fed on fresh fish meat *ad libitum* for 8 hours on every alternate day. Soon after each feeding, the aquaria were cleaned and the bioassay media were replenished in order to maintain the desired concentrations of the pesticides.

For the bioassay of each pesticide, 45 crabs, divided equally into three separate test groups, were used. An equal number of crabs, grouped similarly and maintained in pure water, served as the common control animals. The bioassay media were taken in plenty so as to keep the crabs in a continuously submerged condition. At least 8 crabs from each experimental group and an equal number from the control lot were sacrificed at the end of 10, 20 and 30 days and their midguts were removed. After processing the midguts, 5-8 µm thick sections of the same were stained with hematoxylin and eosin (H&E) for microscopic examination. The midgut lesions were considered to be mild if they were less distinct and sporadic, and to be severe if the same were more distinct and found extensively in the affected tissue.

RESULTS AND DISCUSSION

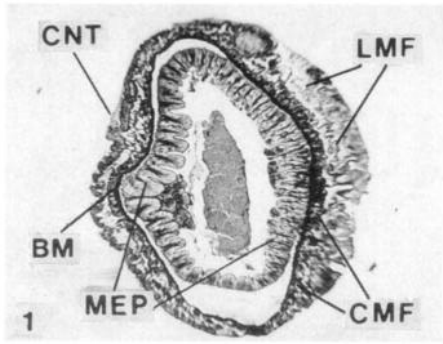
Soon after the release of the laboratory-acclimatized crabs into various pesticide solutions, they became slightly excited for 4-5 hours and attempted to evade the media by unsuccessful crawling over the walls of the aquaria. Subsequently, they settled down quietly in the bottom of the aquaria. Such a behaviour was not seen in the control crabs. Though no mortality occurred in either the pesticide-treated or the control animals during the entire course of the bioassay experiments, yet the crabs exposed to aldrin exhibited mild signs of stress like the stiffening of legs and the accumulation of a frothy mass around their bodies beyond 20 days.

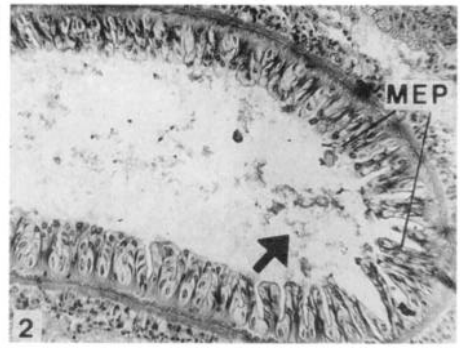
The midgut of *P. masoniana* consists of 5 layers, viz., from outside to inside (1) a connective tissue sheet; (2) a layer of longitudinal muscle fibres with a network of blood lacunae; (3) a layer of circular muscle fibres; (4) a thin basal membrane and (5) the innermost layer of mucosal epithelium, made up of columnar

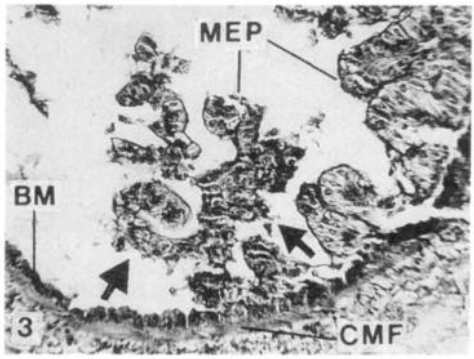
cells. Until the end of the experimental period, the histological picture of the midgut of control crabs remained the same as in a freshly caught untreated specimen (Fig. 1).

A few attempts have been made to study the influence of pesticides on the gut of certain animals but no information is so far available in this regard on crustaceans. The present investigations have shown that all the three pesticides, viz., monocrotophos, carbaryl and aldrin are greatly deleterious to the midgut of *P. masoniana* even in sublethal concentrations and induce significant histopathological alterations in it. However, it was interesting to note that the lesions which had developed during various pesticidal treatments, were restricted to the innermost mucosal epithelium layer of the midgut and no change could be detected in any of the peripheral layers at any stage. The present study has also revealed that the midgut lesions are pesticide-specific and, if initially mild, they become severe later on. Thus, monocrotophos can be considered to be a slower poison to *P. masoniana* as it could not induce any pathological alteration in the midgut of any of the crabs exposed to this pesticide over a period of 10 days. Even after 20 days of exposure, though the midguts of 75% of the examined specimens were observed to have been affected by monocrotophos, but the lesions in all such crabs remained mild and were represented merely as shrinkage and erosion in mucosal epithelium in a focal manner (Fig. 2). After a period of 30 days, however, the pesticide-induced lesions had become severe and were manifested in 100% treated crabs as extensive fragmentation and separation of mucosal epithelium from the underlying basal membrane. In addition to this, the detached epithelium in the midgut lumen showed the signs of necrobiosis in majority of the animals (Fig. 3). Separation of the mucosal layer of the stomach and intestine, along with shrinkage and necrosis in its cells, have been observed during lethal and sublethal thiodan poisoning in the fish *Gymnocorymbus ternetzi* (Amminikutty and Rege 1978).

On the other hand, in view of the severe nature of the lesions and the rapidity in their appearance in the crabs treated with carbaryl and aldrin, these pesticides seem to be stronger midgut poisons than monocrotophos to *P. masoniana*. The presently tested sublethal concentrations of both carbaryl and aldrin induced persistent hyperplasia and proliferation of mucosal epithelium, resulting in its projection as folds into the gut lumen. The process of fold formation was found to have been initiated in about 40% animals within 10 days of their exposure to carbaryl. By the end of 20 days, however, the midgut lumens of invariably all the carbaryl-treated specimens contained prominent folds along with conspicuously eroded material from





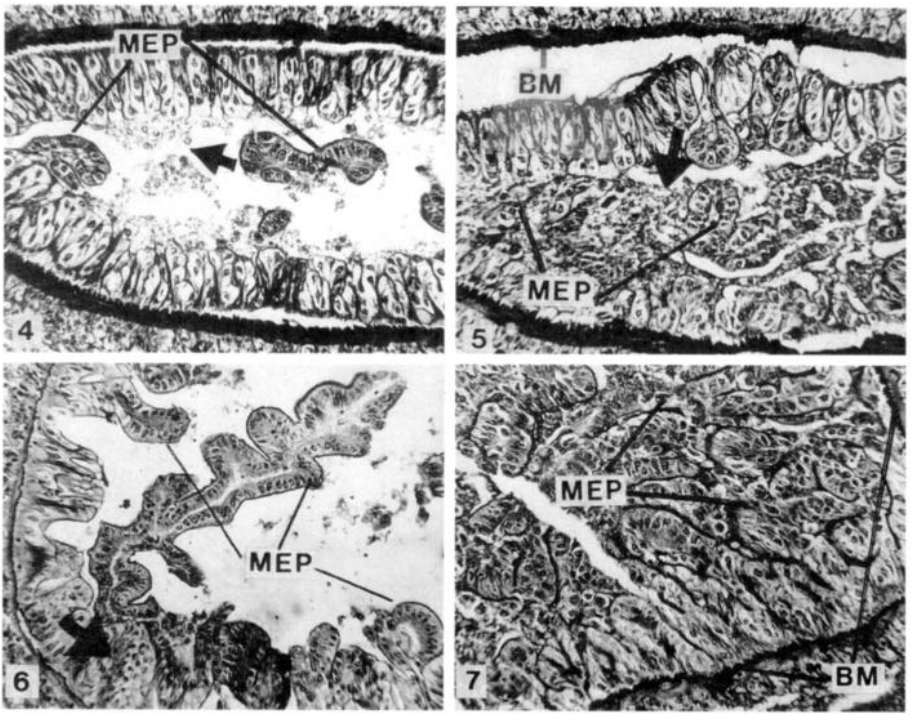


T.S. midgut of *P. masoniana,* chronically exposed to monocrotophos (0.5975 mg l^{-1}). H&E.

Figure 1. Midgut of control crab, after 30 days. Connective tissue (CNT), longitudinal muscle fibres (LMF), circular muscle fibres (CMF), basal membrane (BM) and mucosal epithelium (MEP) X 150.

Figure 2. After 20 days, showing shrinkage and erosion in mucosal epithelium. Arrow indicates the eroded material. X 300.

Figure 3. After 30 days, showing fragmentation and separation of mucosal epithelium from the basal membrane. Arrows show necrobiosis in separated epithelium. X 350.



T.S. midgut *of P. masoniana,* chronically exposed to carbaryl (0.2520 mg l^{-1}) and aldrin (34.90 mg l^{-1}). H&E.

Figure 4. After 20 days (in carbaryl) showing conspicuously eroded mucosal epithelium (arrow) along with its proliferation as folds (MEP) into the midgut lumen. x 300.

Figure 5. After 30 days (in carbaryl), showing obliteration of midgut lumen by the folds of mucosal epithelium. Arrow indicates necrobiosis in the folds. X 300.

Figure 6. After 10 days (in aldrin), showing a long and many small folds of mucosal epithelium being proliferated. Arrow shows an area of active cell division. X 350.

Figure 7. After 30 days (in aldrin) showing proliferated mucosal epithelium filling the midgut lumen. X 350.

the mucosal epithelium (Fig. 4). The hyperplastic proliferation of mucosal epithelium was a much faster process under the influence of aldrin, as was evidenced by the presence of a few very large folds in the gut lumens of 90% specimens within 10 days of their exposure to this pesticide (Fig. 6). The new mucosal folds so formed occupied the central space and by the end of the experimental period, they had filled the midgut lumens almost completely in 100% of carbaryl-treated as well as aldrin-treated crabs (Figs. 5 & 7). The development of such a lesion may be alarming because the folds may obstruct the passage of food through the midgut and cause serious complications in the digestive activity in *P. masoniana*. It may be added that carbaryl, besides inducing mucosal hyperplasia, also induced necrobiosis in the cells of the mucosal folds within 30 days of the exposure of crabs to this pesticide (Fig. 5). A somewhat comparable alteration has been observed in the midgut of *Peripianeta americana* following its treatment with dieldrin. In this insect, an outgrowth, resulting from hyperplasia and the infiltration of cells, was circumscribed around the periphery of the midgut and it projected into the haemocoel (Saxena 1992).

According to Weisburger and Williams (1980), the chlorinated compounds may induce tumor formation by bringing about certain endogenous changes in the tissues. Pesticides like lindane and chlordane have been shown to act as promoters to enhance the carcinogenic expression of genotoxic agents (Trushimoto *et al.* 1983). Though the exact nature of the proliferated mucosal folds in the midgut of the pesticide-treated *P. masoniana* could not be ascertained, yet in view of their formation in such a large number over a short period of time (say 30 days), their appearance through the carcinogenic activity of carbaryl and aldrin cannot be ruled out.

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