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**Abstract**

The toxicity of trichlorfon was determined in *Macrobrachium rosenbergii* and the 24-, 48-, 72-, and 96-h LC\(_{50}\) values were 0.7739, 0.3513, 0.2697, and 0.2555 mg l\(^{-1}\), respectively. Prawns were exposed for 24 h to 0, 0.2, and 0.4 mg l\(^{-1}\) trichlorfon. Then, certain biochemical and physiological parameters, including acetylcholinesterase (AChE) activity, glucose, lactate, and glycogen contents, and the hemolymph osmolality, ions, and acid–base balance were measured. For prawns in the 0.4 mg l\(^{-1}\) trichlorfon group, decreased AChE activities of the hemolymph and hepatopancreas, decreased glycogen contents of the hepatopancreas and muscle, decreased osmolality, Cl\(^-\), Na\(^+\), pCO\(_2\), HCO\(_3^-\), and TCO\(_2\) levels of the hemolymph, increased glucose contents of the plasma, hepatopancreas, and muscle, increased lactate contents of the plasma, hepatopancreas, and muscle, and increased pH and pO\(_2\) levels of the hemolymph were observed. Similar results were also observed for the prawns exposed to 0.2 mg l\(^{-1}\) trichlorfon except for lactate in the hepatopancreas and the pH of the hemolymph. Gills of prawns exhibited hemocytic infiltration in the hemocoelic space, swelling and fusion of the lamellae, and necrotic, hyperplastic, and clavate–globate lamellae with exposure to 0.4 mg l\(^{-1}\) trichlorfon. These results suggest that the reduction in AChE activity and alteration of the histopathology of the gills in *M. rosenbergii* resulted in variations in carbohydrate metabolism, osmotic and ionic regulation, and the base–acid balance in an attempt to resist and adapt to trichlorfon stress.

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**Keywords:** *Macrobrachium rosenbergii*; Trichlorfon; Acetylcholinesterase activity; Osmolality; Ions; Base–acid balance; Carbohydrate metabolism; Histopathology

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1. Introduction

*Macrobrachium rosenbergii* is a large prawn of the family Palaemonidae indigenous to tropical freshwater and brackish-water habitats of the Indo-Pacific (George, 1969). However, it has long been commercially important worldwide as a primary inland cultured species (New, 1995).

Potentially damaging compounds are used in shrimp culture such as disinfectants, therapeutics, feed additives, algicides, pesticides, and fertilizers. These chemicals may cause biological damage at all life stages during shrimp production. Biochemical responses in penaeids to various contaminants were reviewed by Bainy (2000) and Lignot et al. (2000).

Hyperglycemia as a secondary stress response has been documented in most species of fish in response to a wide range of stresses (Pickering and Pottinger, 1995). Elevated blood glucose can result from reduced utilization of glucose or stimulation of gluconeogenesis and/or glycogenolysis. In crustaceans, elevation of hemolymph glucose is also observed in vivo when they are subjected to stressful pollutants, such as exposure to cadmium in the red swamp crayfish, *Procambarus clarkii* (Reddy et al., 1994), and in the fiddler crab, *Uca pugilator* (Reddy et al., 1996), to organic pollutants or naphthalene in *U. pugilator* (Reddy et al., 1996), and to DDT in the freshwater crab, *Barytelphusa guerini* (Fingerman et al., 1981).

Organophosphorus (OP) insecticides, used massively and repeatedly because of their rapid breakdown in the environment, are potential threats to non-target species such as fish, crab, and shrimp (Tronczynski, 1990). Elevated blood glucose can result from reduced utilization of glucose or stimulation of gluconeogenesis and/or glycogenolysis. In crustaceans, elevation of hemolymph glucose is also observed in vivo when they are subjected to stressful pollutants, such as exposure to cadmium in the red swamp crayfish, *Procambarus clarkii* (Reddy et al., 1994), and in the fiddler crab, *Uca pugilator* (Reddy et al., 1996), to organic pollutants or naphthalene in *U. pugilator* (Reddy et al., 1996), and to DDT in the freshwater crab, *Barytelphusa guerini* (Fingerman et al., 1981).

Organophosphorus (OP) insecticides, used massively and repeatedly because of their rapid breakdown in the environment, are potential threats to non-target species such as fish, crab, and shrimp (Tronczynski, 1990). Metabolic changes observed in seawater and freshwater crustaceans exposed to OP insecticides create a widespread disturbance in general physiological processes such as enzymatic activities (Bhagyalakshmi et al., 1984; Repetto et al., 1988), oxidative metabolism (Bhagyalakshmi et al., 1984), oxygen consumption (Pawar and Kadtare, 1984; Cebrian et al., 1992), energy metabolism (Repetto et al., 1988), and osmoregulation (Lignot et al., 1997). In addition, histopathologic changes in the gills and epipodite cells of *Marsupenaeus japonicus* exposed to lethal and sublethal concentrations of fenitrothion (Lignot et al., 1997), and in the hepatopancreas and gills of *Macrobrachium malcolmsonii* exposed to endosulfan (Saravana Bhavan and Geraldine, 2000) were also observed. Yeh et al. (2005) indicated that long-term (8-day) trichlorfon exposure disturbs osmoregulation and the acid–base balance of *M. rosenbergii*.

The primary effect of OP on vertebrate and invertebrate organisms is the inhibition of the enzyme, acetylcholinesterase (AChE), which is responsible for terminating the transmission of nerve impulses. AChE inhibition causes accumulation of acetylcholine (ACh) at nerve synapses and disruption of the nerve function. A change in AChE activity was observed in *Litopenaeus stylirostris* treated with sublethal concentrations of fenitrothion (Lignot et al., 1998) and in *P. clarkii* treated with 0.1–15 mg l^{-1} trichlorfon (Repetto et al., 1988).

It is not uncommon to observe the intensive use of insecticides in agricultural areas adjacent to shrimp farming zones. Trichlorfon is one of the most used compounds, which is an OP pesticide of high solubility (14%) and moderate toxicity. In normal conditions of use, it is very quickly hydrolyzed to form dichlorvos (2,2-dichlorovinyl dimethyl phosphate) which is much more toxic. The objectives of our research were (1) to evaluate the acute toxicity of trichlorfon to the prawn *M. rosenbergii* by determining the 24-, 48-, and 96-h LC_{50} values; (2) to determine the biochemical and physiological parameters including AChE activity, the glucose, lactate, and glycogen contents, and the hemolymph osmolality, ions, and acid–base balance in prawns, and (3) to investigate the histoarchitecture of the gills of prawns after 24-h exposure to different concentrations of trichlorfon.

2. Materials and methods

2.1. *M. rosenbergii*

*M. rosenbergii* prawns were obtained from a commercial farm in Pingtung, southern Taiwan, and acclimated in the laboratory for 1 week before the experiments. During the acclimation period, prawns were fed twice daily with a formulated prawn diet (Shinta Feed Company, Pingtung, Taiwan). Only prawns in the intermolt stage (stage C) were used for the study. The molt stage was determined by examination of the uropoda in which partial retraction of the epidermis can be distinguished (Peebles, 1977).
For 1 day prior to the experiment, the prawns were not fed. Prawns ranged from 16.5 to 19.6 g, averaging 18.2 ± 2.1 g (mean ± S.D.) with no significant size differences among the treatment groups.

During the experiment, the water temperature was maintained at 27 ± 1 °C, pH at 7.3–7.5, total hardness at 100 mg l⁻¹, osmolality at 2 mosM kg⁻¹, Na⁺ at 0.5 mmol l⁻¹, Ca²⁺ at 0.09 mmol l⁻¹, and Mg²⁺ at 0.34 mmol l⁻¹. However, the concentrations of K⁺ and Cl⁻ were too low to be determined.

2.2. Lethal effect of trichlorfon

Short-term median lethal concentration (LC₅₀) toxicity tests were carried out according to the methods described by the American Public Health Association et al. (1985). Prawns (stage C) were randomly sampled from holding tanks and transferred into tanks containing different test solutions. The prawns were separated into six groups (0, 0.1, 0.2, 0.4, 0.6, and 0.8 mg l⁻¹). Bioassay experiments to establish tolerance limits were conducted in 60-l glass aquaria containing 40 l of test solution. Each glass aquarium contained 10 prawns, and the solution was aerated continuously using an air-stone with a blower. Each test solution was renewed daily. Triplicate determinations were run for each test solution with a total number of 30 prawns (10 per replicate) for each test solution. During the experiments, prawns were fed a commercial diet (Shinta Feed Company, Pingtung, Taiwan) twice a day. The water temperature was maintained at 27 ± 1 °C. Observations were usually made at 24-h intervals up to 96 h. Death was assumed when prawns were immobile and showed no response when touched with a glass rod. The trichlorfon response of test prawns was determined by calculating the LC₅₀ values of trichlorfon with the computer program (Trevors and Lusty, 1985).

2.3. Effect of trichlorfon on the biochemical response of M. rosenbergii

Starved prawns were sampled from the holding tank and individually transferred to a 20-l circular plastic tank containing 10 l of each test solution. Individual tanks were aerated through an air blower attached to an aeration stone. Test solutions consisting of concentrations of 0, 0.2, and 0.4 mg l⁻¹ trichlorfon were prepared with fresh water as described above. Each test solution was evaluated using ten replicates. After a 24-h exposure, the hemolymph, muscle, and hepatopancreas were sampled and immediately analyzed.

The hemolymph (200 μl) of an individual prawn was withdrawn from the ventral sinus into a 1-ml sterile syringe (25 gauge) containing 200 μl of an anticoagulant solution (0.114 M trisodium citrate and 0.1 M sodium chloride, pH 7.45, osmolality 490 mosM kg⁻¹). The diluted hemolymph was centrifuged at 664×g at 4 °C for 15 min, the supernatant fluid was collected as a stock solution, and the AChE, glucose, and lactate levels were immediately analyzed.

One gram of tissues of muscle and hepatopancreas of a prawn was homogenized in 1 ml of ice-cold 0.1 M phosphate buffer, and then centrifuged at 9500×g at 4 °C for 30 min; the supernatant was collected as a stock solution, and the AChE, glycogen, glucose, and lactate levels were immediately analyzed.

2.3.1. Acetylcholinesterase (AChE) activity

The activity of AChE was determined using a modified version of a colorimetric technique described by Ellman et al. (1961). All samples were analyzed in triplicate at 25 °C. One hundred microliters of a hemolymph stock solution of a 5× dilution, or tissue supernatant of a 250× dilution was completely reacted with 5 μl of DTNB (dithiobisnitrobenzoic acid dissolved in phosphate buffer, pH 7.0, 0.01 M) for 10 min. Twenty microliters of acetylthiocholine iodide (0.075 M) was then added, and the optical density at 412 nm was measured each minute for 10 min using an ELISA plate reader (SPECTRA max 190, Molecular Devices, Sunnyvale, CA, USA). The increased absorbance versus the elapsed time formed a linear relationship. AChE activity was expressed as various levels of absorbance per 20 μl hemolymph, or per mg tissue supernatant.

2.3.2. Glucose quantification

Glucose concentrations were measured with a coupled glucose oxidase and peroxidase reaction using a Sigma diagnostics glucose kit (cat. no: 315-100). The optical density at 505 nm was measured using an ELISA plate reader, and glucose concentrations were calculated from a standard curve of known glucose concentrations.
2.3.3. Lactate quantification

Lactate concentrations were measured using the colorimetric lactate oxidase and peroxidase method with a Sigma diagnostics lactate kit (cat. no: 735-10). The optical density at 540 nm was measured using an ELISA plate reader, and lactate concentrations were calculated from a standard curve of known lactate concentrations.

2.3.4. Glycogen quantification

Muscle and hepatopancreas glycogen levels were determined by an enzymatic method using amyloglucosidase as described by Murat and Serfaty (1974). Briefly, 1 g of muscle or hepatopancreas tissue was homogenized in 100 ml citrate buffer, and then centrifuged at 9500 × g at 4 °C for 30 min, after which the supernatants were collected and analyzed. The glucose concentration of 20 μl of supernatant was determined and named value A. Fifty microliters of supernatant was incubated with 950 μl of an amyloglucosidase solution (0.01 g amyloglucosidase in 10 ml H2O) at 37 °C for 2 h, then centrifuged at 9500 × g at 4 °C for 30 min. The supernatant was collected, and the glucose concentration was determined and named value B. The glycogen concentration (GC) was expressed as follows:

\[
GC = \frac{20}{A}B - A.
\]

2.4. Effect of trichlorfon on the physiological response

The starved prawns were sampled and treated as described above. Hemolymph was sampled individually after 24 h of exposure to different concentrations of trichlorfon. Hemolymph samples were carefully withdrawn from the ventral sinus of each prawn into a 1-ml (25-G) syringe. Hemolymph pH, \(pO_2\), \(pCO_2\), HCO\(_3^−\), and TCO\(_2\) levels were immediately determined by injecting hemolymph (85 μl) into a pH/blood analyzer (model ABL-5; Radiometer, Copenhagen, Denmark). Details of the measurement were previously described (Chen and Kou, 1998).

Hemolymph osmolality and medium osmolality were measured by injecting a 20-μl sample into a micro-osmometer (model 3300; Advance Instruments, Norwood, MA, USA). To determine Na\(^+\), K\(^+\), and Cl\(^−\) levels, 100 μl of the hemolymph sample was immediately injected into an Ion-Selective Electrode Analyzer (Medica EasyLyte PLUS, Bedford, MA, USA).

2.5. Effect of trichlorfon on gill histological changes of M. rosenbergii

Gill tissue of representative prawns from each test and control group were dissected out and fixed in Davidson’s solution. The fixed specimens were embedded in paraffin wax and cut into 5-μm sections, stained with modified Mayer’s hematoxylin and phloxine eosin stain before being viewed under a light microscope (Bell and Lightner, 1988).

2.6. Statistical analysis

Effects of trichlorfon on lactate, glucose, AChE activity, and glycogen, and hemolymph osmolality, ions, pH, \(pO_2\), \(pCO_2\), HCO\(_3^−\), and TCO\(_2\) levels were statistically analyzed using Duncan’s multiple range test in version 8.2 SAS (Statistical Analysis System) (SAS, 2001).

3. Results

3.1. Lethal effect of trichlorfon

All prawns survived in 0 mg l\(^−1\) trichlorfon for all exposure times. However, all prawns exposed to 0.6 mg l\(^−1\) trichlorfon after 96 h, and those exposed to 0.8 mg l\(^−1\) trichlorfon after 72 h died. The 24-, 48-, 72- and 96-h LC\(_{50}\) values and their 95% confidence limits in mg l\(^−1\) of trichlorfon for M. rosenbergii were 0.7739 (0.5869, 1.0205), 0.3513 (0.3222, 0.3830), 0.2697 (0.2461, 0.2954), and 0.2555 (0.2331, 0.2790), respectively (Fig. 1).

3.2. Effect of trichlorfon on the biochemical parameters of M. rosenbergii

3.2.1. AChE activity

Hemolymph AChE activity of prawns following 24 h of exposure to 0.2 and 0.4 mg l\(^−1\) trichlorfon had significantly decreased compared to prawns exposed to the control solution (0 mg l\(^−1\)). Hemolymph AChE activity had decreased by 32.4% and 36.4% for prawns following 24 h of exposure to 0.2
and 0.4 mg l⁻¹, respectively, compared to prawns held in the control solution. Hepatopancreas AChe activity of prawns exposed to 0.4 mg l⁻¹ trichlorfon had significantly decreased by 46.3% compared to prawns held in the control solution after 24 h. Although, no significant difference in muscle AChe activity was noted among the prawns exposed to trichlorfon from 0 to 0.4 mg l⁻¹, the activity slightly decreased with an increase in the trichlorfon concentration (Fig. 2).

### 3.2.2. Glucose concentration

Hemolymph glucose of prawns directly increased with ambient trichlorfon in the range of from 0 to 0.4 mg l⁻¹ after 24 h. Hemolymph glucose of prawns had significantly increased by 50.1% and 100.8% following 24 h of exposure to 0.2 and 0.4 mg l⁻¹ trichlorfon, respectively, compared to prawns kept in the control solution (Fig. 3A). Hepatopancreas and muscle glucose levels of prawns following 24 h of exposure to 0.2 and 0.4 mg l⁻¹ trichlorfon were higher than those of prawns held in the control solution. Hepatopancreas glucose of prawns had significantly increased by 82.8% and 92.0%, and muscle glucose had significantly increased by 434.4% and 421.1% in prawns following 24 h of exposure to 0.2 and 0.4 mg l⁻¹ trichlorfon, respectively, compared to prawns kept in the control solution (Fig. 3B).

### 3.2.3. Lactate concentration

Hemolymph lactate levels of prawns directly increased with ambient trichlorfon in the range of 0 to 0.4 mg l⁻¹ after 24 h. Hemolymph lactate levels of prawns had significantly increased by 50.1% and 100.8% following 24 h of exposure to 0.2 and 0.4 mg l⁻¹ trichlorfon, respectively, compared to prawns kept in the control solution (Fig. 3A). Hepatopancreas and muscle glucose levels of prawns following 24 h of exposure to 0.2 and 0.4 mg l⁻¹ trichlorfon were higher than those of prawns held in the control solution. Hepatopancreas glucose of prawns had significantly increased by 82.8% and 92.0%, and muscle glucose had significantly increased by 434.4% and 421.1% in prawns following 24 h of exposure to 0.2 and 0.4 mg l⁻¹ trichlorfon, respectively, compared to prawns kept in the control solution (Fig. 3B).
prawns had significantly increased by 165.4% and 325.8% for prawns following 24 h of exposure to 0.2 and 0.4 mg l\(^{-1}\) trichlorfon, respectively, compared to prawns kept in the control solution (Fig. 4A). Hepatopancreas and muscle lactate levels of prawns following 24 h of exposure to 0.2 and 0.4 mg l\(^{-1}\) trichlorfon were higher than those of prawns reared in the control solution. The hepatopancreas lactate level of prawns following 24 h of exposure to 0.4 mg l\(^{-1}\) trichlorfon had significantly increased by 178.3% compared to that of prawns kept in the control solution (Fig. 4B). The muscle lactate levels had significantly increased by 38.4% and 48.0% for prawns following 24 h of exposure to 0.2 and 0.4 mg l\(^{-1}\) trichlorfon, respectively, compared to prawns kept in the control solution (Fig. 4B).

3.2.4. Glycogen concentration

Hepatopancreas glycogen levels of prawns following 24 h of exposure to 0.2 and 0.4 mg l\(^{-1}\) trichlorfon had significantly decreased by 75.9% and 68.3%, respectively, compared to that of prawns kept in the control solution. The muscle glycogen level of prawns had significantly decreased following 24 h of exposure to 0.4 mg l\(^{-1}\) trichlorfon as compared to those of prawns held in 0.2 mg l\(^{-1}\) and the control solution. Muscle glycogen levels had decreased by 6.5% and 36.9% in prawns fol-
Fig. 4. Lactate contents in the hemolymph (A), hepatopancreas, and muscle (B) of *M. rosenbergii* exposed to 0, 0.2, and 0.4 mg l\(^{-1}\) trichlorfon for 24 h. Statistical descriptions are the same as those in Fig. 2.

Fig. 5. Glycogen contents in the hepatopancreas and muscle of *M. rosenbergii* exposed to 0, 0.2, and 0.4 mg l\(^{-1}\) trichlorfon for 24 h. Statistical descriptions are the same as those in Fig. 2.
lowing 24 h of exposure to 0.2 and 0.4 mg l\(^{-1}\) trichlorfon, respectively, compared to that of prawns kept in the control solution (Fig. 5).

3.3. Effect of trichlorfon on physiological responses

3.3.1. Osmotic and electrolyte concentrations

Hemolymph osmolality of prawns following 24-h exposure to 0.4 mg l\(^{-1}\) trichlorfon had significantly decreased by 2.4% compared to prawns kept in the control solution (0 mg l\(^{-1}\)) (Fig. 6A).

Hemolymph chloride and sodium levels of prawns exposed to 0.2 and 0.4 mg l\(^{-1}\) were significantly lower than those exposed to the control solution after 24 h. Hemolymph chloride levels had significantly decreased by 15.4% and 15.4%, and hemolymph sodium levels had significantly decreased by 5.7% and 6.5% in prawns following 24 h of exposure to 0.2 and 0.4 mg l\(^{-1}\) trichlorfon, respectively, compared to those of prawns held in the control solution (Fig. 6B,C). However, hemolymph potassium levels did not significantly differ among prawns exposed to 0 to 0.4 mg l\(^{-1}\), and were in the range of 4.0–4.2 mmol l\(^{-1}\) (Fig. 6D).

3.3.2. Acid–base balance

Hemolymph pH of prawns had increased significantly following 24 h of exposure to 0.2 and 0.4 mg l\(^{-1}\) trichlorfon as compared to that of prawns in the control group (Fig. 7A). Hemolymph pCO\(_2\), HCO\(_3\)\(^{-}\), and TCO\(_2\) had significantly decreased in prawns following 24 h of exposure to trichlorfon at 0.2 and 0.4 mg l\(^{-1}\). Hemolymph pCO\(_2\) had de-

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**Fig. 6.** Hemolymph osmolality (A), Cl\(^{-}\) (B), Na\(^+\) (C), and K\(^+\) (D) of *M. rosenbergii* exposed to 0, 0.2, and 0.4 mg l\(^{-1}\) trichlorfon for 24 h. Statistical descriptions are the same as those in Fig. 2.
creased by 32.8% and 34.3%, HCO$_3^-$ by 17.1% and 18.5%, and hemolymph TCO$_2$ by 18.2% and 19.7% in prawns following 24 h of exposure to 0.2 and 0.4 mg l$^{-1}$ trichlorfon, respectively, compared to those of prawns kept in the control solution (Fig. 7C–E). On the contrary, hemolymph $pO_2$ of
Fig. 8. Cross-sections of gill lamellae of *M. rosenbergii* after 24 h of exposure to a control solution and 0.4 mg l\(^{-1}\) trichlorfon. (A and B) Control prawn showing normal lamellae (L) with uniform interlamellar spaces (ILS), the lamellar sinus (LS), and pillar cell (PC) and hemocyte (HC) in the lamella. (C–F) Trichlorfon-exposed prawn showing hemocytic infiltration (HI), swollen (SL) and fused (FL) lamellae, enlargement of the lamellar sinuses (ES) and hyper-mucus (HM) in the interlamellar spaces, necrosis (N), hyperplasia (HY), and clavate–globate (CG) tip of lamellae. H&E stain, scale bar= 80 μm.
prawns following exposure to 0.4 mg l\(^{-1}\) trichlorfon had significantly increased by 27.1\% compared to that of prawns in the control group (Fig. 7B).

3.4. Effect of trichlorfon on the histoarchitecture of gills of \(M.\) rosenbergii

The gills of control prawns showed uniform arrangement of lamellae with uniform interlamellar spaces. No structural abnormalities or abnormal gill lesions were observed in the gills of control prawns. Pillar cells, which are specialized epithelial cells, were found to extend into the lamellar sinus at intervals and to abut similar cells extending from the opposite surface (Fig. 8A,B).

Gills of prawns following 24-h exposure to 0.4 mg l\(^{-1}\) trichlorfon exhibited hemocytic infiltration in the hemocoelic space, swelling of the gill lamellae, lifting of the lamellar epithelium, and fusion of the lamellae (Fig. 8C,D). While the tips of the gill lamellae appeared abnormal, peculiar malformations, necrosis, and hyperplasia resulted in the formation of clavate–globate (clubbing) lamellae (Fig. 8E,F).

4. Discussion

The lethal concentration has been widely used to evaluate the toxicity of pollutants in various aquatic organisms. The sensitivity of aquatic organisms to pollutants is affected by both intrinsic and extrinsic factors, which result from alterations of the biochemistry, physiology, and histology of the animal and changing parameters of water quality (Lignot et al., 1997, 1998). In this study, the same water source and water temperature range, the same approximate size, and the same molt stage (stage C) of prawns were used to minimize intrinsic and extrinsic variations.

An increase in temperature is known to increase fenitrothion toxicity among crustaceans (Johnston and Corbett, 1986). Lignot et al. (1997) indicated that the 24-h LC\(_{50}\) for \(P.\) japonicus larvae exposed to fenitrothion at 28 \(^\circ\)C was 100 times lower than that of larvae exposed to fenitrothion at 25 \(^\circ\)C as published by Rompas et al. (1989). Repetto et al. (1988) indicated that the approximate LC\(_{50}\) concentration of trichlorfon in red crayfish, \(P.\) clarkii, weighing 21.8 \(\pm\) 3.4 g, at 96 h of exposure was 5 mg l\(^{-1}\) at 20 \(^\circ\)C. In the present study, the 24-, 48-, 72- and 96-h LC\(_{50}\) values of trichlorfon for \(M.\) rosenbergii (18.2 \(\pm\) 2.1 g) were 0.77, 0.35, 0.27, and 0.26 mg l\(^{-1}\), respectively, at 27 \(\pm\) 1 \(^\circ\)C. These facts suggest that the tolerance of crustaceans to trichlorfon is related to temperature and species.

Direct energy costs of resisting a toxicant include avoidance, exclusion, and removal abilities of organisms; moreover, they may need energy for repair mechanisms and the eventual pathological effects. To meet the heightened energy demands of stressed animals, glycogen, due to its easy availability for energy production, may be rapidly catabolized resulting in losses of those reserves in tissues; consequently, a significant elevation in blood glucose levels results (Ferrando and Andreu, 1992). In the present study, a remarkable reduction in the glycogen reserves in the hepatopancreas and muscle tissues, and notable elevation in glucose in tissues and plasma occurred in \(M.\) rosenbergii exposed to trichlorfon at more than 0.2 mg l\(^{-1}\). The plasma glucose increased with an increase in trichlorfon and was dose-dependent. These facts suggest that the depletion of glycogen stores should be accompanied by an increase in glucose content after exposure of \(M.\) rosenbergii to trichlorfon.

Increased energetic requirements during toxic stress necessitate an increase in ATP through the operation of the reaction sequence normally leading to the formation of excess pyruvate. Under hypoxic conditions, the impaired oxidation of pyruvate in the mitochondria leads to lactic acid formation. Conversion of lactic acid accounts for only a minor fraction of the total lactic acid removal and there is a steady accumulation of lactic acid in all tissues. A large proportion of lactic acid can diffuse into the blood like carbon dioxide, and increases the concentration of lactic acid in the blood (Karlsson and Jacobs, 1982). In the present study, the high lactate levels found in various tissues and hemolymph, and the elevated \(pO_2\) found in the hemolymph are suggestive of the emphasis placed on anaerobic glycolysis in tissues of \(M.\) rosenbergii under trichlorfon stress.

Gills of aquatic organisms are a vital organ; they play an important role in transportation of respiratory gases and regulation of osmotic and ionic balance. Toxic substances may damage gill tissues, thereby reducing the oxygen consumption and disrupting the
osmoregulatory function of aquatic organisms (Ghate and Mulherkar, 1979). Lignot et al. (1997) indicated that fenitrothion decreased the osmoregulatory capacity of *M. japonicus* juveniles at both lethal and sub-lethal concentrations, and the effect was dose-dependent. They also observed histopathological changes in the epipodites and in gill lamellae at lethal concentrations of fenitrothion. In addition, OP compounds are also capable of inducing programmed cell death (apoptosis) by multifunctional pathways (Carlson et al., 2000).

In our previous study, following 8 days of exposure of *M. rosenbergii* to 0.3 mg l\(^{-1}\) trichlorfon, hemolymph osmolality of prawns significantly decreased as compared to prawns kept in 0 mg l\(^{-1}\), and the hemolymph chloride level of prawns was significantly lower than those of prawns exposed to 0.2, 0.1, and 0 mg l\(^{-1}\). However, hemolymph sodium, potassium, calcium, and magnesium levels did not significantly differ among prawns exposed to 0 to 0.3 mg l\(^{-1}\) trichlorfon (Yeh et al., 2005). In the present study, significantly decreased hemolymph osmolality following 24 h of exposure of prawns to 0.4 mg l\(^{-1}\) trichlorfon and notably reduced hemolymph Cl\(^{-}\) and Na\(^{+}\) levels following 24 h of exposure to 0.2 and 0.4 mg l\(^{-1}\) trichlorfon were observed. However, hemolymph potassium levels did not significantly differ among the prawns exposed to 0 to 0.4 mg l\(^{-1}\). Those data suggest that under long-term exposure to lower than 0.2 mg l\(^{-1}\) trichlorfon, *M. rosenbergii* maintains adaptive regulation of osmolality and ions, but that above 0.3 mg l\(^{-1}\) trichlorfon, this regulation breaks down. However, the physiological parameters of prawns following 24 h exposure to trichlorfon are still unstable.

In the present study, exposure of prawns to 0.4 mg l\(^{-1}\) trichlorfon for 24 h resulted in notable structural alterations in the lamellae of gills including necrotic, hyperplastic, and clavate–globate lamellae, as well as swelling, fusion, and increased mucus secretion. Similar lesions have been reported to have occurred in the prawns *Macrobrachium kistensis* (Ghate and Mulherkar, 1979) and *M. malcolmsonii* exposed to endosulfan (Saravana Bhavan and Geraldine, 2000).

In general, OP compounds produce specific inhibition of AChE, which in some cases is accompanied by the inhibition of neuro-target esterase (NTE). The modification of NTE activity (esterase activity at pH 8) is responsible for the appearance of the syndrome of delayed neurotoxicity induced by some OP compounds (Johnson, 1977; Repetto et al., 1988). Repetto et al. (1988) reported that NTE activity was inhibited as the time of exposure to 0.1 μg ml\(^{-1}\) trichlorfon progressed, which shows the typical effect of some organophosphates. In the present study, hemolymph and hepatopancreatic AChE activities of prawns following 24-h exposure to more than 0.2, and 0.4 mg l\(^{-1}\) trichlorfon, respectively, had significantly decreased compared to those of prawns exposed to the control solution. However, muscle AChE activity slightly decreased with an increase in the trichlorfon concentration. These facts suggest that trichlorfon might have been acting to reduce ventilation of the gills of *M. rosenbergii* by causing an accumulation of ACh at neuromuscular junctions.

In our previous study, hemolymph pH, HCO\(_3^{-}\), and TCO\(_2\) of prawns had decreased with an increase in trichlorfon concentration at 8 days. Hemolymph pCO\(_2\) had, on the contrary, increased with an increase in concentration of trichlorfon treatment. Although no significant difference in hemolymph pO\(_2\) was found among prawns exposed to 0 to 0.3 mg l\(^{-1}\) trichlorfon treatments, hemolymph pO\(_2\) slightly decreased with an increased concentration of trichlorfon (Yeh et al., 2005). In the present study, there were decreased hemolymph pCO\(_2\), HCO\(_3^{-}\), and TCO\(_2\) levels and increased hemolymph pH and pO\(_2\) of *M. rosenbergii* exposed to 0.2 to 0.4 mg l\(^{-1}\) trichlorfon for 24 h. These results suggest that short-term trichlorfon stress induces compensatory hyperventilation resulting in respiratory alkalosis. However, long-term trichlorfon stress causing metabolic acidosis (accumulation of lactate) in *M. rosenbergii* could be attributed to impairment of gill activity resulting from apoptosis, and to hypoventilation resulting from prevention of the hydrolysis of ACh through the inhibition of AChE activity.

Trichlorfon has been used as an agriculture pesticide, as a human medicine to combat internal parasites, and as an ectoparasiticide in the livestock and aquaculture industries (WHO, 1992). The application rate of trichlorfon varies from 0.1 to 1.0 mg l\(^{-1}\) in ponds to eradicate ectoparasites (Herwig,
1979). However, farmers often apply excess amounts of trichlorfon in fish and agriculture farm management, and the trichlorfon-polluted water from the livestock and aquaculture industries occasionally contaminates prawn ponds in Taiwan.

In conclusion, short-term trichlorfon stress in *M. rosenbergii* reduced the acetylcholinesterase activity of tissue and changed the histoarchitecture of the gills resulting in respiratory alkalosis by compensatory hyperventilation and decreased osmoregulatory capacity; increased energetic requirements and hypoxia in vital organs promoted glycolysis of tissues resulting in increased glucose levels and lactate production. It may be possible to reduce or prevent trichlorfon contamination in prawn ponds and thus improve growth performance and energy efficiency.

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**References**


