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ORIGINAL ARTICLE

Investigation of Acute Toxicity of Two Organophosphates on Caspian Sea Gammarus (*Pontogammarus maeoticus)*

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ABSTRACT
The present study was undertaken to evaluate the acute toxicity of fenitrothion and malathion as potential dangerous organophosphate pesticides to investigate mortality effects of these organic chemicals to the Caspian gammarus (Pontogammarus maeoticus), Caspiansea, Iran. Gammarus were exposed to different concentration s of fenitrothion and malathion for 96 h and gammarus mortality was calculated with24h interval. Data obtained from organophosphate pesticides acute toxicity test were evaluated using probit analysis statistical method. Acute toxicity of fenitrothion and malathion were 11.51±0.56 and0.97±0.14pbb respectively. In this study organophosphate had adverse effects on gammarus mortality increased in related concentration with time. Also, Pontogammarus maeoticus were more sensitive to malathion than fenitrothion
Keywords: *Acute toxicity, Fenitrothion ,Malathion ,Pontogammarus maeoticus,*

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INTRODUCTION

Gammarus represent one biological group that has received considerable attention in both freshwater and marine acute toxicity testing, due to factors such as their high abundance, small size, short life cycles, and greater sensitivity to many toxins than some other common bioassay organisms such as fish [1, 4].
Gammarus is an important genus of the gammaridae family, which is distributed worldwide across a variety of aquatic ecosystems including fresh water, brackish and marine environments. They are often dominant members and play an important role in the structure and function of aquatic communities [1-5].
Pontogammarus maeoticus, which plays a key role as a carbon transfer species in the food chain, also have a good source of feeding for commercial fish such as sturgeon in the Caspian Sea [6,7].
Insecticides are commonly used in agriculture and public health for the control of harmful insects. Insecticides cause the death of target insects within a few hours by inflicting irrevocable damage on the peripheral and central nervous systems [8].
Pollutants can react with each other and develop new forms when entering the water, and they have undesirable toxic effects on living organisms, i.e., fish and arthropods [9, 10].
In recent years there has been growing concern over insecticide accumulation and persistence of pesticides in the aquatic environment, serving as a threat to biological life including human beings [11].
A vast amount of the scientific literature recognizes the adverse effect of organophosphates on aquatic habitats. Pesticides have been used in agricultural control of unwanted insects and disease vectors. The insecticides interfere with the membrane transport of sodium, potassium, calcium, or chlorine ions, inhibit selective enzyme activities, and contribute to the release and/or persistence of chemical transmitters at nerve endings [10].
Malathion, an organophosphate, is used extensively for mosquito control as well as being applied to crops. It possesses relatively high water solubility, and is therefore expected to have a low bioconcentration rate. As a consequence it has caused significant environmental pollution and exhibits moderate acute toxicity among aquatic communities [12].

Fenitrothion is an organophosphothionate insecticide, which is a contact insecticide and selective acaricide, and it also used as a vector control agent for malaria in public health programmes [13].
Fenitrothion is used as a conventional chemical insecticide to control locusts and grasshoppers, by inhibiting acetylcholinesterase [AChE] activity, thus disrupting the nervous system (14, 15).
This study was conducted to determine the acute toxicity of fenitrothion and malathion to the standard test species, Caspian gammarus (*Pontogammarus maeoticus*) using the static test system.

MATERIALS AND METHODS

Specimens of *P. maeoticus* were obtained from the southern coast of the Caspian Sea. They were kept in a glass aquarium and acclimated for two weeks under laboratory conditions. During this period, gammarus were fed daily.
Ten of the animals were then placed in 0.5 ml glass vessels containing seawater and were well aerated. Water quality parameters (particularly pH, alkalinity and hardness) were measured by standard methods. During acclimation, the animals fed on leaves brought from their original habitat. Malathion (emulsion 57%) and fenitrothion (emulsion 50%) were freshly prepared, and added to each test vessel containing seawater to achieve the required range of malathion and fenitrothion. Nominal concentrations of active ingredient tested were 0, 10, 13, 16, 19, 22 and 25 ppb for fenitrothion and 0, 0.2, 0.4, 0.7, 1.1, 1.2 and 1.6 ppb for malathion [16].
The nominal concentrations of malathion and fenitrothion estimated to result in 50% mortality of *Pontogammarus* within 24 h, 48 h, 72h and 96 h were attained by probit analysis by Finney's (1971) method [17] and using the maximum-likelihood procedure (SPSS 2002, SPSS Inc., Chicago, Illinois, USA) according to preliminary tests for determination of acute toxicity limit.
During acute toxicity experiments the temperature was 27°C. Feeding was not provided to the specimens during the assay and test media were not renewed. Gammarus were checked daily for mortality at times 0 h, 24 h, 48 h, 72 h and 96 h following pesticide exposure. Death was defined as a total lack of movement. In all experiments the distilled water and pesticides were renewed every two days. All tests were performed at least three times. LC50 values at 24 h, 48 h, 72 h and 96 h were determined using the Tox29.bas probit analysis program. After the acute toxicity test, the LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) were determined for each measured endpoint.

RESULTS

There was no recorded gammarus mortality during the acclimation period and control group during the acute toxicity experiment. Mean survival rates of *P. maeoticus* samples exposed to various concentrations of fenitrothion and malathion were shown in figures 1 and 2 respectively. In the treatments, the mortality of gammarus was recorded at 10 ppb after 96 h of fenitrothion and at 0.2 ppb after 72 h of malathion.

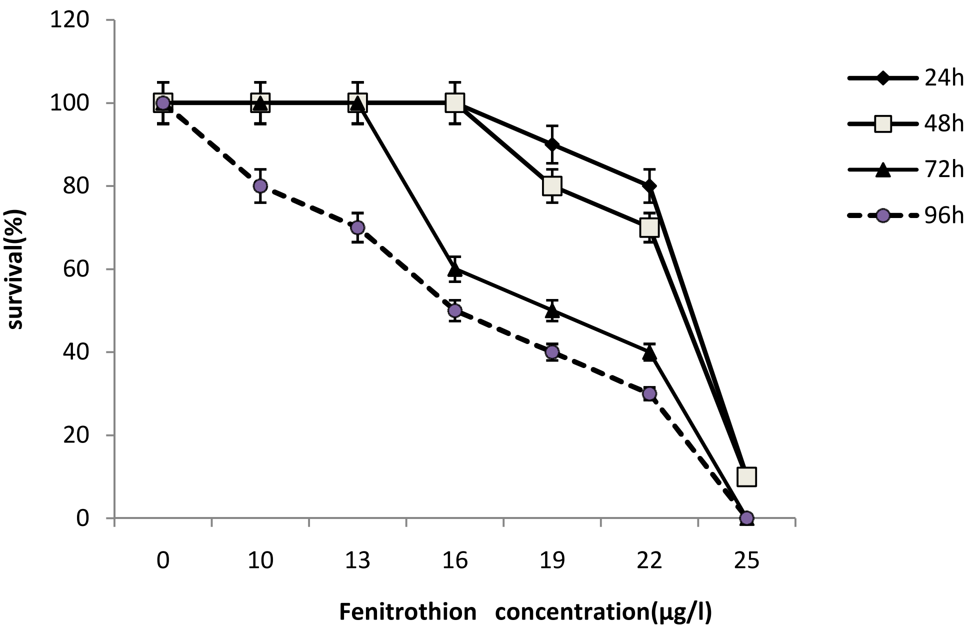

Figure1. Mean survival rates of *P.maeoticus* exposed to Fenitrothion .Each data pointrepresents the mean of three experiments

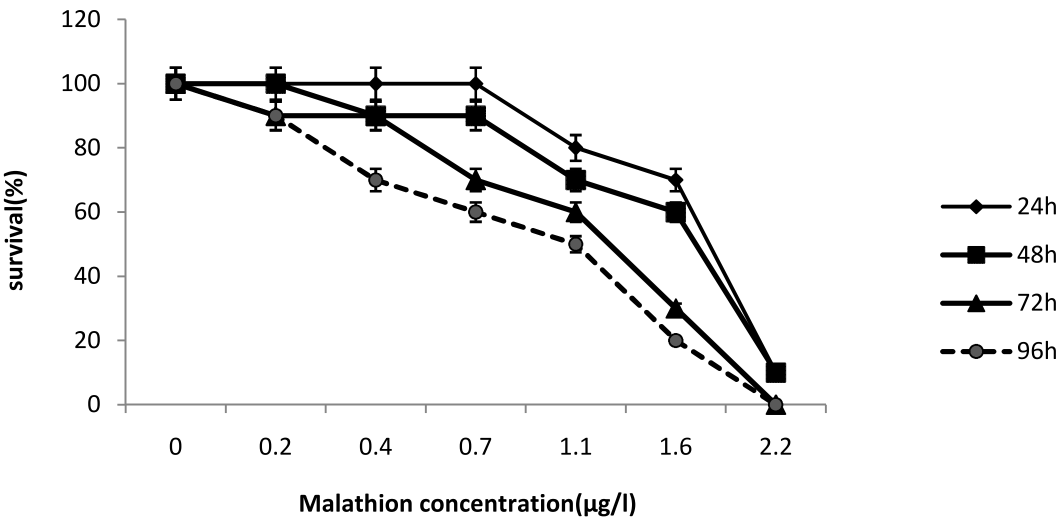

Figure2. Mean survival rates of *P.maeoticus* exposed to Malathion .Each data point represents the mean of three experiments

Median lethal concentration LC10, LC50 and LC90 of fenitrothion and malathion are presented in tables 1 and 2, respectively. Table 1. Lethal concentrations of Malathion during different times after exposure in *Pontogammarus maeoticus*

Table 1. Lethal concentrations of Malathion during different times after exposure in *Pontogammarus maeoticus*

Point	concentration of Malathion (ppb)			
	24h	48h	72h	96h
LC_{10}	0.85±0.16	0.34±0.13	0.31±0.22	0.31±0.14
LC_{50}	12.2±0.16	3.81±0.13	0.97±0.22	0.97±0.14
LC_{90}	85.92±0.16	22.25±0.13	4.88±0.22	3.05±0.14

Table 2. Lethal concentrations of Fenitrothion during different times after exposure in *Pontogammarus maeoticus*

Point	Concentration of Fintrothion (ppb)			
	24h	48h	72h	96h
LC_{10}	11.9 ± 0.37	10.77±0.43	9.34±0.14	4.77±0.72
LC_{50}	88.65±0.31	49.05±0.37	20.21±0.26	11.51±0.66
LC_{90}	234.1±0.43	49.2±0.17	43.71±0.51	27.74±0.56

The results of LOEC (lowest observed effect concentration) values and NOEC (non observed effect concentration) of fenitrothion and malathion are indicated in figures 3 and 4, respectively. LOEC values of fenitrothion and malathion for *P. maeoticus* were 16 ppb and 0.4 ppb, respectively. NOEC values for fenitrothion and malathion were 13 ppb and 0.2 ppb, respectively.

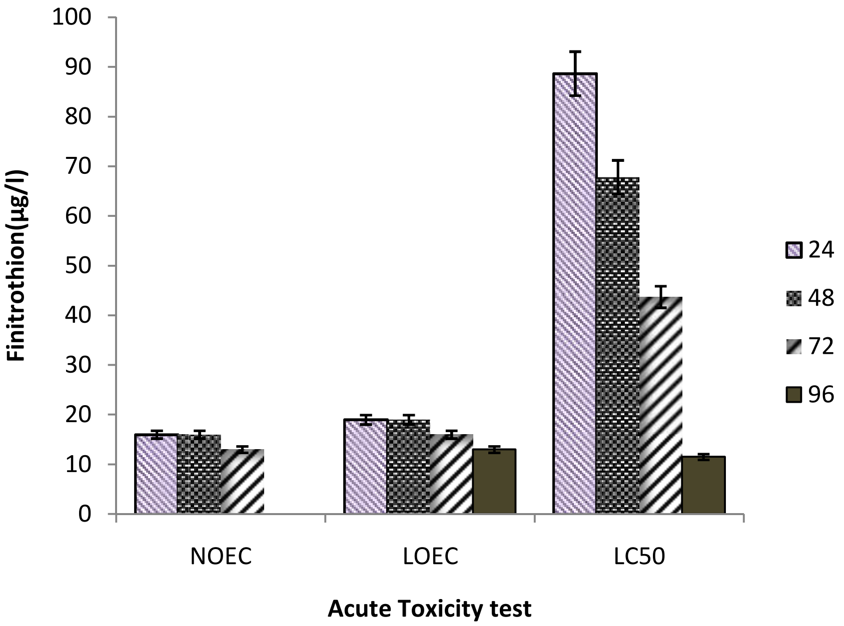

Figure 3. Acute toxicity testing statistical endpoints of *Pontogammarusmoeticus* during different times after exposed to various concentration of Fenitrothion

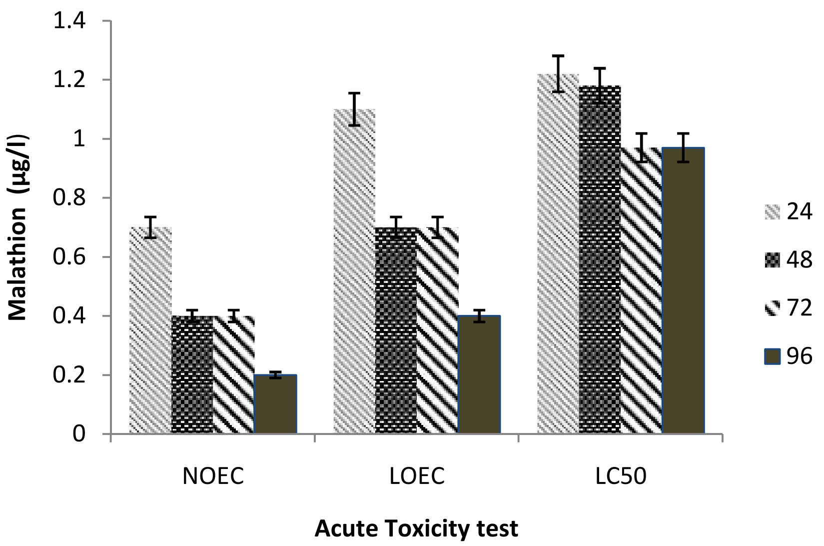

Figure 4. Acute toxicity testing statistical endpoints of *Pontogammarusmoeticus* during diffrent times after exposed to various concentration of Malathion

DISCUSSION

Amphipods are often used as sentinel organisms for ecotoxicological studies because they play an important role in the processing of detritus in streams and naturally present in lotic waters [18,19].

The results of median lethal concentration (LC_{50}) for fenitrothion and malathion in the present study were 18.2 ppb and 1.22 ppb at 96 h respectively. The toxicity of both organophosphate pesticides increases with increasing concentration and exposure time. As the LC50 values of fenitrothion at 96 h were more than malathion, malathion was more toxic to *P. maeoticus* than fenitrothion.

Most previous studies of acute toxicity of organophosphate pesticides have been focused on fish species and *G. pulex* from the gammaridae family [18, 20]. However, there is limited information about the acute toxicity of metals on *P. maeoticus*.

G. pulex proved to be well suited for toxicity tests. The LC50 values of malathion at 24 h, 48 h, 72 h and 96 h for *G. pulex* were 3.84, 3.45, 1.68 and 1.01 nmol/l respectively [20, 21]. *G. pulex* was more sensitive to malathion compared to *P. maeoeticus*. The laboratory toxicity data indicated that *G. pulex* could serve as a useful bioindicator of contaminant stress pollutants in streams [22]. Other studies on *Gammaruspalustris* demonstrated less sensitivity to malathion exposure than to *P. maeoicus* with LC50= 4.65 ppb at 96 h [23]. Various factors have been considered to cause different sensitivity levels among fish and crustaceans to organophosphate pesticides including absorption, inhibition of acetyl cholinesterase, and different detoxification mechanisms [19,20, 21].

Fenitrothion and malathion generally have been shown to be moderately toxic to *P. maeoeticus*. However, in this study *P. maeoeticus* showed less sensitive to fenitrothion compared to malathion. Further research into the sensitivity of this organism to other pesticides and studies of the behavior, physiology and histology effects of these chemicals should be undertaken.

CONCLUSION

Fenitrothion and malathion generally has been shown to be moderate toxicity to *P.maeoeticus* the. However,in this study *P. maeoeticus* was showed less sensitive to fenitrothion compared to malathion. It needs to do further researches about the sensitivity of this organism to the other pesticides and study physiology and histology effects of those chemicals.

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