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ORIGINAL ARTICLE

The acute toxicity of diazinonto the freshwater shrimp *Gammaruspulex*

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Abstract: ***The wide use of the organothiophosphate insecticide, diazinon, to control insects has led to increased levels of this pesticide in aquatic environments. However, data regarding acute toxicity of diazinon on aquatic biota are limited. The current study aimed to investigate the mortality response of the freshwater amphipod Gammaruspulex to diazinon, its metabolite diazinonoxonanda commercial diazinon in formulation using the LC_{50} test. Exposure for 24 h to all tested concentrations of the different tested forms of diazinon did not caused significant mortality of G. pulex. The 48 h LC_{50} were values 923.1 and 87.5 nM for diazinon and diazinonoxon, respectively. The 48 h LC_{50} value of the commercial formulation of diazinon could not be calculated because mortality did not exceed 50 %. At longer period of exposure, the toxicity of three tested diazinon forms was in the following order: the commercial formulation >diazinonoxon>diazinon. After 72 h of exposure, LC_{50} values not clear of the tested diazinon forms were 222, 65.3 and 16.1 nM for diazinon, diazinonoxon and the commercial formulation of diazinon, respectively. The toxicity of the tested pesticide increased with increasing exposure time. At the end of the LC_{50} test (96 h), the LC_{50} concentrations of diazinon, diazinonoxon and the commercial formulation of diazinon were 140.1, 45.7 and 10.4 nM, respectively. In conclusion, the current study provides data that can be used to examine the sublethal responses of G. pulex to diazinon.***

INTRODUCTION

Pesticides have been extensively used to control variety of agricultural pest species in a range of crops. These pesticides, however, may accidentally reach environmental components, such as water bodies and negatively affect non-target organisms [1,2] A number of methods have been employed to test the toxicity of pesticides and other chemicals towards aquatic biota [3]. These tests aim to improve knowledge regarding the harmful influences of chemicals on aquatic animals [4]. Acute toxicity tests, such as LC_{50} (a test measures the concentration of a chemical that is lethal to 50% of test organisms within the test period), is a commonly employed as an initial tool to investigate the adverse consequences of environmental pollutants [3,5]. This may be due to the ease of performing such experiments under laboratory conditions [6]. Mortality tests provide important data that is needed to estimate toxicants concentrations that can induce harmful effects on test organisms during short term exposure under controlled conditions [7,8]. Toxicological data is needed at several levels of biological organization to assess the potential risk of contaminants on aquatic resources.

Initial acute toxicity testing is less costly and clear endpoint simplifies the estimation of mortality in a short period test.

Diazinon (O,O-Diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl), formula $C_{12}H_{21}N_2O_3PS$, is an organothiophosphateinsecticideand was an acaricide developed in the 1950s [9,10,11,12]. This pesticide has been widely employed to control insects in soil and plantsin agricultural and urban surroundings [13,14]. Diazinon is also still commonly used in sheep dip to control ectoparasites[14,15,16,17].

Diazinon can rapidly dissolve in aromatic solvents, aliphatic alcohols, and ketones. Diazinon is also soluble in water to 40 mg/L.In soils, diazinon may be biologically stable for more than 6 months under conditions of low temperature, moisture and high alkalinity [18,19]. Diazinon is comparatively water-soluble, and often comes into contact with aquatic animals. 2-Isopropyl-6-methyl-4-pyrimidinol (IMP), diethyl phosphate (DEP) and diethyl thiophosphate (DETP) can be found in the environment due to the breakdown of diazinon[20].

Once inside these animals diazinon is metabolized to diazinonoxon, which is the acutely toxic form of this insecticide.

The commercial diazinon formulation contains 60% diazinon and the remaining 40% comprises excipient and trace impurities.

Numerous studies have examined the acute toxicity of diazinon to freshwater invertebrates and reported differences in sensitivity of organisms to this pesticide [21,22,23,24]. The 96 hr LC_{50} values ranged from 0.821 nM to 38 nM for the cladoceranCeriodaphniadubia, and the planarian, Dugesiatigrina, respectively.

US EPA [25] reported that, amongst the invertebrates, the Class Crustacea has the highest sensitivity to diazinon. The 96 h LC50 values of diazinon in three species of amphipods (G. faciatus, G. pseudolimneaus and Hyallelaazteca) ranged from 6.7 to 55.3 nM.

It has been shown that chemical toxicity is vary between species and within class sizes [26]. They also reported that chemicals toxicity is affected by the life stage of test organisms. The authors found that in the freshwater shrimp Caridinalaevis adults are less sensitive than juvenile to diazinon (the 96 h LC_{50} values were 0.005 and 0.002nM, respectively) [26].

In the present study, acute toxicity tests were conducted to determine the 96 h LC_{50} values for diazinon, diazinonoxon and the diazinon in the commercial formulation in the freshwater Gammaruspulex. Such information is needed to investigate chemical concentrations used in short term exposure, sublethal and sub-chronic toxicity tests.

MATERIAL AND METHODS

1.Collection and maintenance of animals

Adult G. pulex of 25–30 mg wet weight were collected using a hand net from a single population in a freshwater slow-running unpolluted stream located at the Creswell Crags nature reserve, Derbyshire (Ordnance Survey grid reference SK533741). After field collection animals were transported to the laboratory within one hour in water-filled plastic aquaria. Only undamaged and active organisms were collected using a net and placed in plastic aquaria filled with continuously aerated de-chlorinated tap water (pH 8.5; conductivity 561 micro-Siemens; hardness 231 mg/L $CaCO_3$) (50 animals in 6 L of water) under a 12 h light/dark photoperiod at 15°C. Animals were acclimated to laboratory conditions for a minimum of 7 days prior to their use in the experiments and fed to excess with wheat germ. To reduce metabolic activity and the consequential fouling of the test solutions with metabolic waste (e.g. faeces), experimental animals were starved for 24h prior to tests.

2.Preparation of glassware

To reduce potential contamination, glassware were

washed with detergent and then rinsed and filled with 5% nitric acid for 24 h prior to each experiment. The tanks were then washed and filled with de-chlorinated tap water.

3.LC50 toxicity tests

A semi-static experiment, in which test organisms were subjected to periodic renewal of tank water with or without added pesticides, was designed to determine the 24, 48, 72 and 96 h LC_{50} values of diazinon, diazinonoxonandthe commercial diazinon formulation. Diazinon and diazinonoxon were prepared by dissolving in 0.01% v/v DMSO, whilst the commercial diazinon formulation was prepared in distilled water. Serial dilutions of stock solutions were prepared when required.

Small aliquots of the appropriate dilution were transferred to 1 L volumetric flasks and made up to 1L with de-chlorinated tap water. The pesticide solutions were replaced every 24 h with freshly prepared solutions of the same pesticide concentration. All controls and treatments received the same concentration of DMSO (0.01% v/v). This concentration of DMSO had previously been shown to have no effect on G. pulex. A semi-static acute toxicity bioassay was performed to determine the 96 h LC_{50} values for diazinon, diazinonoxon and the commercial diazinon formulation. Three replicates were used for each of the pesticide and control solutions. Exposure conditions were 10 adult intermoult G. pulex 500 ml of the appropriate control or diazinon solution per glass container. During the experiment G. pulex were not fed and diazinon solutions were changed daily.The level of mortality was recorded and dead animals removed after 24, 48, 72 and 96 h of exposure.

4.Statistical analysis

The data were analysed with the use of the Trimmed Spearman-Karber method to estimate LC_{50} values and 95% confidence intervals (available from the US Environmental Protection Agency at www.epa.gov/nerleerd/stat2.htm). The trimmed Spearman-Karber method is suitable for actual and hypothetical bioassay and has good statistical properties. It is easy to use, and is recommended for accurate and precise calculation of LC_{50} values and their 95% confidence interval end points .

The Trimmed Spearman-Karber method is a nonparametric statistical test, which is less susceptible to artefacts when compared to the parametric logistic and probit analysis alternative methods.

RESULTS

1. The effect of diazinon on G. pulex survival

As it can be seen in Figure 1, diazinon did not cause mortality to G. pulex over 24 h of exposure at a concentration of ≤ 10 nM. Exposure to higher diazinon concentrations (75,100,250 and 1000 nM) for 24 h caused less than 4% mortality in test organisms. After 96 h of exposure, the survival rate of organisms exposed to ≤ 10 nMdiazinon was higher that 80%. The mortality rates of test organisms exposed to 75, 100 and 250 nMdiazinon for 96 h were 27%, 43% and 84% respectively. The highest tested diazinon concentration (1000 nM), caused a dramatic increase in the mortality of exposed G. pulex with increase exposure period. The 96 h LC_{50} values of diazinon on G. pulex are presented in Table 1.

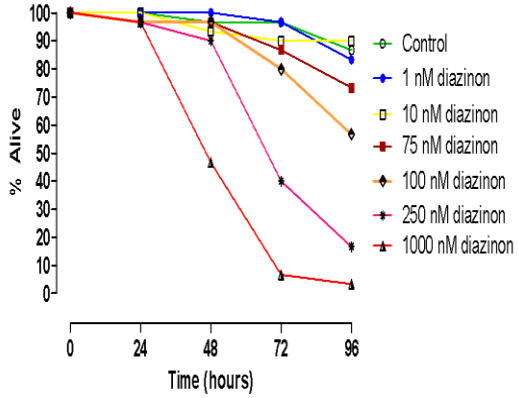

Figure 1 The time dependent survival ofG.pulexexposed to different concentrations of iazinon (n=30

2.The effect of oxondiazinon on G. pulex survival

The survival of G. pulex exposed to diazinonoxon is shown in Figure 2. As it can be seen, 13% of organisms exposed to de-chlorinated tap water (control) died by 96 h. After 96 h of exposure, mortality of exposed organisms was 60%, 93% and 100% at 50, 75 and 100 nMdiazinonoxon, respectively. Table 1 shows the LC50 values of diazinonoxon for G. pulex

Exposure time	Diazinon	Diazinonoxon	Diazinon in the commercial formulation

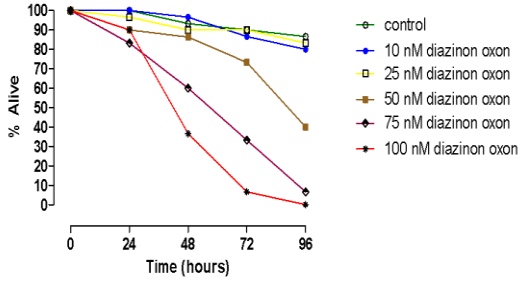

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Figure 2 The time dependent survival pulex exposed to different concentrations of diazinonoxon (n=30)toxicity data

Table 1 Trimmed Spearman-Karber analysis of Trimmed Spearman-Karber analysis toxicity data

Statistics	48h	72h	96h	48h	72h	96h	48h	72h	96h
LC50 nM	923. 1	222.6	140.1	87.5	65.3	45.7	-	16.1	10.4
Upper 95% Confidence Interval	1585.4	300.4	183.2	101.4	73.1	51.6	-	20.7	14.1
Lower 95% Confidence Interval	537.5	160.7	107.1	75.5	58.3	40.5	-	12.5	10.4

3.The effect of the commercial diazinon formulation on the survival of G. pulex

The effect of the commercial diazinon formulation on the survival of G. pulex can be seen Figure 3. After 96 h of exposure, the survival rate of control group was 80%. At the same period of exposure to 7.5 nM the survival rate of test organisms was 70%. The toxicity of diazinon in the commercial formulation to G. pulex was both concentration and time dependent (Figure 3).After 96 h of exposure, the survival of test organisms was 43%, 21% and 0% at 10, 25 and 50 nM, respectively. The LC50 of diazinon in the commercial formulation are given in Table 1. The 24 h LC50 and 48h LC_{50} values could not be calculated because mortality did not exceed 50%.

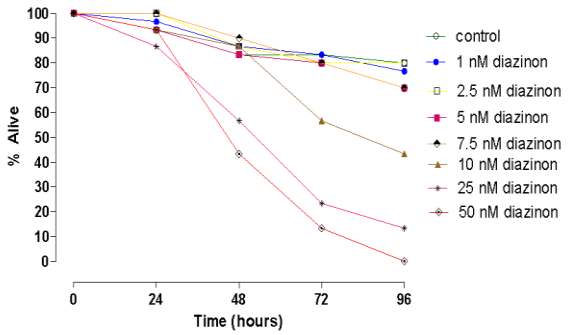

Figure 3 The time dependent survival of G. pulexexposed to different concentrations ofdiazinonin the commercial formulation(n=30)

DISCUSSION

Despite the environmental importance of G. pulex, data regarding the toxicity of organophosphate pesticides towards this organism is limited. The results of the current study show that the toxicity of diazinon in the commercial diazinon formulation, diazinonoxon and diazinon on G. pulex was both concentration and time dependent

(Figure 1, 2 and 3 and Table 1). As it is shown in Table1, the 96 h LC50 of diazinon in thecommercialformulation was 4.4 and 13.5 time more toxicthan diazinonoxon and diazinon,inrespectively. Higher toxicity of diazinon inthe formulation may be a consequence tothe presence ofthe presence of other compounds such as sulfate in the commercial diazinon formulation. These compounds may be more toxic to test organisms than diazinon itself.

In the comparison with the result of the present study, published data have shown different sensitivity of G. pulex to diazinon exposure. For instance, Ashauer et al. [27] reported that LC50 values for diazinon on G. pulex were 27.8, 18.7 and 13.6 nM for 48 h, 72 h and 96 h of exposure time, respectively.

Different of response of G. pulex to diazinon exposure can be, at least partly, attributed to differences between sampling locations, season, size of containers, and/or exposure conditions. Chemical toxicity toward aquatic biota can be also affected by dissolved organic matter (DOM).

Kim et al.[28] reported that the toxicity of copper to Ceriodaphniadubia has a negative relationship with copper–DOM reaction time.

The sensitivity of organisms to diazinon may also differ between related species. For example, the acute toxicity of diazinon to G. pseudolimnaeus was 13 fold higher than that to G. fasciatus (the 96 h LC_{50} values were 0.54 and 7 nM, respectively [29,30]. In another study, it was reported that the 96 h LC_{50} of diazinon on the tropical freshwater shrimp Caridinalaevis were 0.02 nM[26]. Burkepile et al.

[10] reported that the 48 LC_{50} values were 3 and 7.9 nMon D. magna and Ceriodaphniadubia, respectively. There are also differences in sensitivity of G. pulex to other organophosphate pesticides when compared with other gammarids. For instance, the 96 h LC_{50} concentrations ofmethyl parathion and fenitrothion on G. pulex were 12.2 and 19.8 nM, respectively[31]. These concentrations are higher than those observed on *G. fossarum by* Kuhn and Streit [32]. They reported that the 96 h LC50 values on test organisms were 9.6 and 10.4 nM for methyl parathion and fenitrothion, respectively.

In conclusion, the present study shows that, the toxicity of three tested diazinon forms was in the following order the commercial formulation >diazinonoxon>diazinon. The toxicity of diazinon to test organisms has a positive relationship with the pesticide concentration and the period of exposure. This study provides important data regarding the acute toxicity of diazinon to G. pulex. These concentrations are needed to design sublethal experiments to investigate the behavioural and biochemical responses of G. pulex to diazinon. Previous reports have indicated that short exposure of high levels of diazenon can cause severe harmful effects to human health, therefore, it is mandatory to investigate and develop new formulations of diazenon or its derivatives which may cause insignificant damage to human health.

The methodology of present study can be utilized for attempting such efforts for the development of new formulation of potentially effective insecticides pesticides preventing ill effects to human health and protect environment.

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