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SHORT COMMUNICATION

Toxicity of the organophosphate pesticide diazinon to crayfish of differing age

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Abstract Diazinon is a widely applied agricultural pesticide whose effect importantly on the environment and the possible contamination of surface waters has led to increased interest in toxicological studies. Crayfish, as an ecologically important benthic macroinvertebrate, seems to be an appropriate model organism for such assessments. Acute toxicity tests were carried out on three crayfish age groups: young-of-the-year (total length = 25.0 ± 4.9 mm), juvenile (total length = 56.5 ± 3.8 mm) and adult (total length = 83.5 ± 5.7 mm). Young-of-the-year crayfish were found to be the most sensitive to diazinon (96 h LC50 = 0.15 mg L^{-1}), followed by juvenile crayfish (96 h LC50 = 0.27 mg L^{-1}), and adults (96 h LC50 = 0.51 mg L^{-1}). Crayfish were highly sensitive to diazinon. A delayed effect of Diazinon 60EC on adults was detected (144 h LC50 = 0.44 mg L^{-1}) suggests functional damage from the use of sublethal concentrations.

Keywords Decapod · Developmental stages · Environment · Pesticide · Mortality

Introduction

Chemical pollutants are common in natural environments, and a great deal of research has been done on predicting the effects of these contaminants (Amato et al. 1992; Relyea and Hoverman 2006; Mirbagheri and Hashemi Monfared 2009). The most common type, especially in aquatic environments, is the pesticide. Aquatic habitats throughout the world are contaminated with at least one pesticide, and commonly with complex mixtures including, e.g., atrazine, chlorpyrifos, diazinon, and malathion (Battaglin et al. 2003; Daly et al. 2007; Banaee et al. 2011).

Diazinon is an organophosphorus pesticide widely used on agricultural crops. It is an active ingredient in many commercial repellents (Burkepile et al. 2000), is used to control agricultural soil-dwelling insects, and as a sheep dip to control ectoparasites (Tomlin 1997; Virtue and Clayton 1997). Many river catchments throughout the world are seriously polluted by diazinon, especially in areas of intensive agriculture or extensive sheep rearing (Banaee et al. 2011; Scholz et al. 2000; Adedeji et al. 2009; Arjmandi et al. 2010). Recent studies cite a wide range of direct and indirect negative impacts of diazinon on aquatic biota, including dramatic reduction of zooplankton (Giddings et al. 1996); possible lethal effects on fish (Máchová et al. 2007; El-Sherif et al. 2009) and amphibians (Sparling and Fellers 2007); disruption of behavior patterns (Scholz et al. 2000); reduction in phytoplankton and periphyton (Relyea 2009); sublethal effects on olfactory functions (Moore and Waring 1996); changes in the erythocyte and leucocyte profile in fish probably due to the disruption of hematopoiesis or a decrease in non-specific immunity (Adedeji et al. 2009); cytotoxicity, endocrine disruption (Bisson and Hontela 2002); and indirect effects on growth and metamorphosis of tadpoles due to direct effects on zooplankton, phytoplankton, and periphyton (Relyea 2009). In addition, diazinon is listed as potentially carcinogenic (Beane Freeman et al. 2005).

Crayfish, as representatives of large crustaceans, are ecologically important benthic macroinvertebrates, and are often key species in both still and running waters (Momot 1995). Despite this, and the fact that the most sensitive

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organisms to diazinon were shown to be Crustacea (draft ambient aquatic life water quality criteria diazinon), relatively little is known about the lethal effects of diazinon on crayfish and their different developmental stages.

The main aim of the present study was to assess the effects of diazinon on crayfish, an ecologically important aquatic organism of benthic niches, in particular to describe the sensitivity of crayfish at different developmental stages [young-of-the-year (YOY), juveniles, and adults] to diazinon. The research work was conducted in research facilities of South Bohemian University, Faculty of Fisheries and Protection of Waters in Vodňany, Czech Republic in the summer 2009.

Materials and methods

Tested preparation

Diazinon [(O,O-diethyl-0-2-isopropyl-4-methylpyrimidinyl-4-yl)-thiophosphate] stock solutions were prepared using Diazinon 60EC preparation (Nippon Kayaku Co., Ltd., Japan) at a concentration of 600 g L^{-1}.

Animals

The organisms tested were selected with consideration of the status of crayfish in Europe. Autochthonous crayfish are endangered, and their sources are limited, making their use for toxicity tests unethical. Thus non-native spiny-cheek crayfish (Orconectes limosus) and signal crayfish (Pacifastacus leniusculus) from own cultures were used. Three age groups were tested: YOY c. 100 days' old spiny-cheek crayfish [total length (TL) mean ± SD = 25.0 ± 4.9 mm]; and signal crayfish juveniles, age 1 + (TL = 56.5 ± 3.8 mm); and adults >2 years' old (TL = 85.6 ± 5.7 mm). Tested animals were fed by fresh and frozen zooplankton (YOY) and frozen chironomid larvae (juveniles, adults) before the tests conducted.

Acute toxicity tests

Acute toxicity tests were performed in compliance with the International Organization for Standardization (1984) with one modification; the bath was not changed during the test, since the active substance, diazinon, decomposes to toxic diazoxon, and test results would have been affected by bath exchanges. For all tested groups, artificially prepared adulterating water was used according to the above standard. All tests were conducted in triplicate. Preliminary tests were conducted to determine the optimal concentration range for acute toxicity tests. In accordance to preliminary tests, diazinon concentrations of 0, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mg L^{-1} were used for all groups. Temperature, oxygen level, and pH were measured daily (Table 1). Young of year were placed into small circular glass tanks (150 mm in diameter) containing 300 mL of bath. Juveniles and adults were kept in aquaria with a 10,000 mL bath. Each trial comprised ten specimens. The light regime was natural ambient. Crayfish were not fed during the tests.

Test groups were monitored three times per day, and dead specimens were removed. Acute toxicity tests lasted for 96 h in all groups with the exception of a prolonged mortality observation (144 h) in the adult group. Results were processed by probit analysis, 96 h LC50 and confidence limits were calculated (The EKOTOX 5.1 software (INGEO Liberec, Czech Republic).

Results and discussion

Young-of-the-year

Excited behavior (fast and chaotic movement outside the shelters, specimens attempting to "escape") was observed at the three highest concentrations (0.3, 0.4, and 0.5 μg L^{-1}) directly after stocking. No extraordinary behavior was seen at other concentrations. Greatest mortality occurred during the first 24 h. Mortality after 96 h is shown in the Table 2. Calculated 96 h LC50 values with confidence intervals are given in Table 3. Mortality in the control group was zero.

Juveniles

Reaction to test preparations was slower than in YOY. The highest mortality occurred 72 h after initial exposure. A lower mortality rate was found at lower concentrations (Table 2) and, therefore lower 96 h LC50 values (Table 3). Mortality in the control group was zero.

Adults

Reaction to test preparations was slower than in juveniles. The highest mortality occurred as long as 72 h after initial exposure and was relatively constant until the end of treatment. Generally, adults showed lower mortality rates than did YOY and juveniles (Table 2). Table 3 presents 96 h LC50 and 144 h LC50 values for adults. Mortality in the control group was zero.

Table 1 Water parameters during acute toxicity tests for all tested groups of crayfish

Crayfish	Temperature (°C)	Oxygen level (mg L^{-1})	pH
YOY	17.8 ± 0.16	9.0 ± 0.10	7.3 ± 0.19
Juveniles	17.8 ± 0.64	8.8 ± 0.15	7.7 ± 0.03
Adults	17.0 ± 0.34	8.6 ± 0.31	7.6 ± 0.08

Data are presented as mean ± SD

Table 2 Mortality of groups of crayfish at various concentrations of diazinon

Diazinon concentration mg L^{-1}	Mortality			
	YOY (96 h) (%)	Juveniles (96 h) (%)	Adults (96 h) (%)	Adults (144 h) (%)
0.05	3.3 ± 4.71	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
0.1	20.0 ± 14.14	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
0.2	73.3 ± 4.71	5.1 ± 3.63	3.3 ± 4.71	3.3 ± 4.71
0.3	83.3 ± 9.43	12.81 ± 3.63	0.0 ± 0.00	13.3 ± 4.71
0.4	86.7 ± 9.43	100.0 ± 0.00	23.3 ± 4.71	53.3 ± 12.47
0.5	96.7 ± 4.71	100.0 ± 0.00	33.3 ± 12.47	63.3 ± 18.86
Control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

The adult group includes mortality values at 96 and 144 h. Data are presented as mean ± SD

Table 3 96 h LC50 and confidence intervals for all test groups of crayfish and 144 h LC50 for the adult group

Crayfish group	96 h LC_{50} (mg L^{-1})	Confidence interval	144 h LC_{50} (mg L^{-1})	Confidence interval
YOY	0.15	−0.02; +0.02	–	–
Juveniles	0.27	−0.37; +0.15	–	–
Adults	0.51	−1.58; +0.39	0.44	−0.12; +0.09

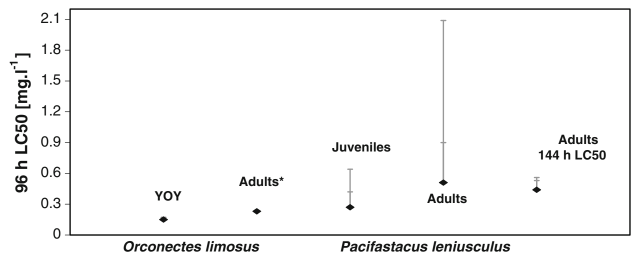

Fig. 1 Comparison of 96 h LC50 in young-of-the-year (YOY) and adult spiny-cheek crayfish, juvenile and adult signal crayfish, and 144 h LC50 in adult signal crayfish. The data for adult spiny-cheek crayfish (marked by *asterisk*) originated from work of Kozák et al. (2006). Data are presented with confidence intervals at $\alpha = 0.05$

Potential biological effects of diazinon, including disruption of behavior patterns and olfactory function, cytotoxicity, and endocrine effects, as well as direct toxicity have been reported (Scholz et al. 2000; Moore and Waring 1996; Bisson and Hontela 2002), but comparable data for crayfish are lacking, despite the fact that crayfish are recommended test organisms according to the reference/standard methods of the Organization for Economic Co/operation and Development (OECD) (1992). There is reported the first study investigating comparative toxicity of diazinon to several age groups of crayfish.

In general, acute toxicity of diazinon differs widely among aquatic organisms such as fish [Cyprinus carpio larvae 96 h LC50 = 1.53 mg L^{-1} (Aydın and Köprücü 2005), Poecilia reticulata 96 h LC50 = 3 mg L^{-1} (Máchová et al. 2007)]; cladocerans [Daphnia magna 48 h LC50 = 0.0024 mg L^{-1} (Burkepile et al. 2000)]; algae [Desmodesmus subspicatus 72 h IC50 = 10.2 mg L^{-1} (Kozák et al. 2006)]; insect larvae [Chironomus tentans 48 h LC50 = 0.052 mg L^{-1} (Burkepile et al. 2000)]; and amphibians [Rana clamitans tadpoles 96 h LC50 = 0.0028–0.005 mg L^{-1} (Harris et al. 1998)]. The results (see Table 2) of the present study (96 h LC50 ranging between 0.15 and 0.51 mg L^{-1}), showed that crayfish are, compared with other aquatic organisms, moderately sensitive to diazinon. However, the results show that diazinon is highly toxic to crayfish, confirming previous results using adult spiny-cheek crayfish (48 h LC50 = 0.23 mg L^{-1}) (Kozák et al. 2006) (Fig. 1). Signal crayfish seem to be less sensitive than spiny-cheek crayfish (Fig. 1). The results obtained fits well to the usual view of increased mortality with increasing concentration of tested chemicals. The only one disproportion was observed when adult crayfish were tested. Mortality occurred at 0.2 mg L^{-1} diazinon, but no mortality was obtained at 0.3 mg L^{-1} (Table 2). That could be explained by a different physiological state of one crayfish (unexpected health disproportion of individual leading to higher susceptibility to diazinon) in the lower concentration. The disturbing effect is namely caused only this one crayfish. However, if the prolonged observation data (144 h) are taken into account (Table 2), the above mentioned disproportion disappeared.

Sublethal effects of diazinon such as olfactory inhibition and impairment of homing behavior occur at much lower concentrations (0.001–0.01 mg L^{-1}) in fish (Scholz et al. 2000), which are less sensitive (acute toxicity) than crayfish. Lower diazinon concentrations may influence some physiological and behavioral cues in crayfish. Juveniles, in particular, may be affected by extremely low concentrations, due to their higher sensitivity compared to adults (Fig. 1). The higher sensitivity of juveniles to diazinon confirms the general statement of their higher vulnerability to pesticides or other environmental chemical factors (Evans and Edgerton 2002).

A small difference was observed between acute toxicity in YOY spiny-cheek crayfish and previous findings for adults (Kozák et al. 2006). Despite the expected sublethal effects on crayfish, no unusual behavior changes at lower, non-lethal, concentrations were observed. The possible physiological or behavioral reactions to sublethal doses of diazinon should be investigated.

Conclusion

The results of the present study provide evidence of toxicological impacts of diazinon. The observed 96 h LC50 values were much higher than, e.g., the recommended effective concentration for a treatment in carp pond culture (Máchová et al. 2007). Nevertheless, possible sublethal effects of diazinon were suggested. The impact on aquatic biota is therefore too great to use diazinon for carp pond culture, or any other purpose. In addition, the representative of European native astacofauna, the noble crayfish (Astacus astacus), is more sensitive to chemical pollutants (Füreder et al. 2006; Holdich et al. 2006), and may be expected to exhibit even lower toxicity thresholds than explored in presented study. There should be the limitation of environmental contamination by diazinon and restriction of its use worldwide.

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