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Acute Toxicity and Oxygen Consumption in the Gills of *Procambarus clarkii* in Relation to Chlorpyrifos Exposure

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It has long been recognized that animals living in polluted waters are more commonly exposed to continuous or intermittent sublethal levels of poisons than to lethal concentrations (Abel 1980).

Dursban (Chlorpyrifos) is an organophosphorous pesticide that is currently registered, or has tolerances pending, for crops and livestock, ornamental plants, turf, household pests, and mosquito control. The most obvious threat to the aquatic environment is its use as a mosquito larvicide; fish and aquatic invertebrates can also be affected through runoff due to certain terrestial uses. Many aquatic species are extremely sensitive to Dursban (Marshall & Roberts 1978). To determine acute toxicity is the first requirement when assessing the toxicological hazards associated with a potential pollutant in the aquatic environment (Leblanc 1984).

Assessing water quality through monitoring the oxygen consumption of the living organism is a better bioassay technique than acute toxicity studies since it responds to even very low concentrations of the toxicant present in the medium (Bakthavathsalam & Reddy 1983). It is beyond doubt that respiratory changes are good indicators of the general conditions of an animal, and have been correlated to stress from factors such as temperature, salinity, starvation and pollutants.

There is much information on the effects of organochlorine insecticides on the metabolic rate and activity of insects, but such data on the comparative toxicity of organochlorine, organophosphorus, and carbamate pesticides on crayfish oxygen consumption is scarce.

It is therefore prudent to study the toxicity and the effects of chlorpyrifos and the solvent (acetone) on oxygen uptake: the basis for all physiological processes.

MATERIALS AND METHODS

The crayfish, *Procambarus clarkii*, weighing 15.0 to 30.0 g were collected from the crayfish farm in Valencia, Spain. They were kept in 300-L glass aquaria and acclimatized to laboratory conditions for, at least, 2 wk.The crayfish were fed *ad libitum* with minced pork liver. Mortality of animals was less than 1% in the preliminary testing, and they exhibited no obvious diseases or abnormal behavior during acclimatization.

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The tanks were supplied with a continuous flow of tap water (temperature 22±1°C, total hardness 250 mg/L as $CaCO_3$, pH 7.9±0.2, alkalinity 4.1 mM L^{-1}). A 12-hr photoperiod was maintained.

Stock solutions were prepared by dissolving chlorpyrifos (technical grade 99.8 %, Dow Chemical Co.) in aliquot of acetone; appropriate quantities of this solution were pipetted into glass aquaria (20 L) containing 15 L of test solution and ten crayfish. Ten more animals, used as controls, were kept in 15 L of clean water with the same concentration of acetone.

Methods used in the acute lethality test followed the static test procedures of the Committee on Methods for Toxicity Tests with Aquatic Organisms (U.S. Environmental Protection Agency 1975)

The percentages of mortality were calculated in each concentration after 24, 48, 72 and 96 hr of exposure and converted to probits (Fisher & Yates 1963). The pesticide concentration was converted to logarithms. The concentration causing 50% mortality of the test animals (LC50) and 95% confidence limits were calculated using the methods of Litchfield and Wilcoxon (1949).

For respiratory studies, the crayfish were exposed to chlorpyrifos for 96 hr. Oxygen consumption was determined at 24-hr intervals with a Gilson Differential respirometer following the techniques of Umbreit et al.(1972). Three different concentrations were studied (0.002, 0.004 and 0.021 mg L^{-1}), based on the acute toxicity test, representing a sublethal level of 1/10 th, 1/5 th and 96-hr LC50 values.

According to Dickson & Franz (1980) after 24, 48, 72 and 96 hr of exposure, the gills were placed into flasks containing 5 mL of Ringer solution at oxygen saturation. Carbon dioxide was absorbed by 0.5 mL 20% KOH on a piece of filter paper in the center well. Three groups of gills were used for each measurement: control, acetone and solvent with chlorpyrifos.

A control flask with 5 mL of Ringer solution was run simultaneously to correct for oxygen uptake by factors other than the gills and five replicates were made. Oxygen consumption was recorded every 10 min for a period of 60 min at 22°C. After measurements, the gills were dried at 80°C for 48 hr to determine dry weight. The unit of metabolism was calculated and expressed as microliters of O_2 consumed / crayfish milligram / hour.

Table 1. LC50 values,95% confidence limits in brackets.

	Chlorpyrifos LC50 (mg/L)
24 hr	0.037 (0.033-0.046)
48 hr	0.023 (0.022-0.024)
72 hr	0.022 (0.021-0.023)
96 hr	0.021 (0.020-0.022)

Data were statistically evaluated using the ANOVA test and the level of significance was taken as ($p<0.05$) and ($p<0.01$).Statistical comparisons of oxygen consumption of exposed gills were also analysed by means of Duncan's test.

RESULTS AND DISCUSSION

The acute toxicity of chlorpyrifos in experimental medium is given in Table 1, showing the 24, 48, 72 and 96-hr LC50 values with 95% confidence limits.

The LC50 values are similar or identical for 48, 72 and 96 hr. The results suggest that the greatest effect of pesticide is between 24 and 48 hr. These data are in agreement with Canyurt (1984) and Ferrando et al.(1987)

The results presented in this paper are different from values shown by Cebrián (1988) in *P. clarkii*, with other pesticides. These results show the following decreasing order of toxicity: (96-hr LC50 at 22°C) fenitrothion 0.009 (0.010-0.080), chlorpyrifos 0.021 (0.020-0.022), endosulfan 0.120 (0.090-0.150) lindane 0.190 (0.120-0.280), methidathion 0.280 (0.230-0.330), thichlorfon 0.990 (0.770-1.190) mg/L.

Different estimated acute values were reported by Schimmel et al.(1983) for estuarine animals: *M.bahia* 0.330 mg/L (0.260-0.410), *C.variegatus* 1.370 mg/L (1.350-1.380), *F. similis* 0.004 mg/L (0.002-0.006), *M. menidia* 0.001 mg/L (0.001-0.002), *M. cephalus* 0.005 mg/L (0.004-0.006).

Jarvinen et al. (1988) reported the 96-hr LC50 value for continuously exposed larval fathead minnows was 0.122 mg/L. These resuls show that the *P. clarkii*, exposed to chlorpyrifos, present an intermediate sensitivity.

Application of ANOVA to the lowest concentration (1/10 th LC50), revealed at 72 hr ($p<0.05$) Table 2. The same results were obtained by Bakthavathsalam & Reddy (1985) in *A. testudineus* when the fish was exposed to disyston and acetone.

Bansal et al. (1979) have demonstrated a small decrease in the rate of oxygen consumption at 72 hr with no significant differences between 1/12 th TL50 (chlordane) and control in *L. rohita*. In 1/5th LC50 concentration statistical differences were observed at 48 hr between control and pesticide ($p<0.05$). However, Bansal et al. (1979) found that when *L. rohita* were exposed to 1/4 LC50 chlordane, metasystox or sevin, the oxygen uptake values did not significantly differ.

LC50 concentration produces a significant decrease ($p< 0.05$) in the oxygen consumption rate between control-solvent and control-pesticide at 24 hr. These results are similar to values shown by Prasada et al.(1985) in *T. mossambica* when exposed to 1/3 LC50-48 hr of methylparathion. Bakthavathsalam & Reddy (1983) also found insignificant differences in the oxygen consumption of *A. testudineus* between solvent (acetone) and disyston.

LC50 concentration produces a significant increase after 48 hr ($p<0.05$) in the oxygen consumption rate between control-solvent and control-pesticide. However, at 72 hr a significant increase in the oxygen consumption was found in pesticide.

Table 2 shows that oxygen consumption is not uniform throughout the experiment. There are no changes in oxygen consumption in low concentrations of chlorpyrifos, but animal physiology is affected by acetone and there are significant differences after 24 hr.

Table 2.Oxygen consumption rate in the crayfish gills of *Procambarus clarkii.*

Treatments	Time (h)	Acetone[a]	Pesticide[a]
	0	1.10 ± 0.09	1.35 ± 0.22
		[b]-29	[b]-13
	24	1.68 ± 0.23	1.62 ± 0.22
		+16	+12
1/10 LC50	48	1.52 ± 0.47	1.52 ± 0.37
(0.002 mg/L)		+17	+17
	72	1.80 ± 0.05	1.35 ± 0.26
		+20##	&&-10
	96	1.56 ± 0.09	1.54 ± 0.42
		+20	+18
F Snedecor values		4.71 **	0.59
	0	1.74 ± 0.19	1.46 ± 0.28
		+39	+17
	24	1.05 ± 0.26	1.28 ± 0.19
		-20	0
1/5 LC50	48	1.33 ± 0.25	1.86 ± 0.63
(0.004 mg/L)		+20	+67#
	72	1.15 ± 0.31	1.04 ± 0.09
		-8	-16
	96	1.30 ± 0.1	1.06 ±0.16
		+16	-5
F Snedecor values		2.25	4.20**
	0	0.84 ± 0.34	1.09 ± 0.30
		-26	-4
	24	1.04 ± 0.19	1.13 ± 0.14
		-27##	-21##
LC50	48	1.34 ± 0.22	1.51 ± 0.10
(0.021 mg/L)		+22##	+37##
	72	1.44 ± 0.34	2.23 ± 0.46
		+16	&&+80##
	96	1.15 ± 0.22	1.28± 0.22
		+20	+33
F Snedecor values		3.09*	11.81**

a Mean ± SD of 5 individual observations (values expressed in $\mu L\ O_2$ consumed. $mg^{-1}.\ hr^{-1}$)
b + or - indicates % increase or decrease with the control.
* $P < 0.05$ ** $P < 0.01$
# $P < 0.05$ ## $P < 0.01$ compared with the control
& $P < 0.05$ && $P < 0.01$ compared with the acetone

In medium concentrations (1/5 th LC50) the organism treated with pesticide at 72 hr and 96 hr shows a significant decrease in oxygen consumption but there are no differences in acetone.

In high concentrations the oxygen consumption is altered by both acetone and pesticide, although much more significantly in organisms treated with the pesticide only.

These results show that oxygen consumption is changed by high concentrations of chlorpyrifos (LC50). Throughout the experiment, however, differences caused by the pesticide are only found after 72 hr.

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