THE EFFECT OF DIAZINON AND GLYPHOSATE (PESTICIDES) ON OXYGEN CONSUMPTION OF THE BOX MUSSEL *SEPTIFER BILOCULARIS* L.

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ABSTRACT

Oxygen consumption of box mussel *Septifer bilocularis* L. (0.17-0.18 g d.w.) was monitored for one hour during exposure to diazinon and glyphosate pesticides. Depletion of dissolved oxygen was also monitored at 10 min intervals for 2 h. There were no significant differences ($p>0.05$) between the control and the treatments at low concentrations. At concentrations of 0.6, 6, and 30 ppm diazinon, the oxygen consumption rates were [mean ± standard error (SE)] 193.46 ± 38.84, 239.77 ± 40.36, and 208.05 ± 38.57 ml O_2 h^{-1} g^{-1} respectively. In sublethal concentrations of 480, 720, and 960 ppm glyphosate, the rates were 195.26 ± 43.06, 252.28 ± 36.06, 225.43 ± 22.40 ml O_2 h^{-1} g^{-1} respectively (157.27 ± 34.10 ml O_2 h^{-1} g^{-1} in the control). Concentrations of 6 and 30 ppm diazinon, and 720 and 960 ppm glyphosate were required to show a statistically significant ($p<0.05$) effect on the oxygen consumption. In low concentrations, both pesticides tended to increase oxygen consumption of the mussels, but oxygen consumption decreased if the concentrations increased.

INTRODUCTION

Oxygen consumption, pumping rate, and filtration rate have been widely studied in terms of the effects of metals on marine invertebrates (*eg* Abel 1976; Howell *et al.* 1984; Redpath & Davenport 1988; Zanders & Rojas 1992). The effect of pesticides on marine organisms has been studied by Hooftman & Vink (1980); Rompas *et al.* (1989); Kobayashi *et al.* (1990); Monserrat *et al.* (1991); Rodriguez & Monserrat (1991); Rodriguez & Pisanò (1993); Lasut (1996); Kaligis & Lasut (1997). The effect on the oxygen consumption, however, has not been investigated. Oxygen consumption is an important physiological parameter, because it represents a measure of the energy required to support and sustain life (Bayne *et al.* 1985). It has commonly been used as an indicator of the metabolic rate and damage on organisms exposed to contaminants (Rodriguez & Monserrat 1991). Mussels have been widely used as test organisms (Granmo 1995).

Pesticides (especially insecticides) inactivate the enzyme cholinesterase (ChE) and break down the neurotransmitter acetylcholine (Ach) in synapses of the nervous system, thereby disrupting the nervous coordination. They may further cause deleterious effects by affecting the human body (Gallo & Lawryk 1991), increasing mortality, and inhibiting growth and reproduction in marine invertebrates (Connel & Miller 1984, p. 199; Persoone *et al.* 1985; Rompas *et al.* 1989; Kobayashi *et al.* 1990; Monserrat *et al.* 1991; Rodriquez & Pisanò 1993; Lasut 1996; Kaligis & Lasut 1997). In sublethal concentrations, the chemicals affect growth and reproduction of the marine polychaete *Ophryotrocha diadema* (Lasut 1996). In high concentration they cause mortality in the abalone *Haliotis varia* (Kaligis & Lasut 1997). Glyphosate acts as a glycine mimic and becomes accepted into peptides where it blocks normal development (Alloway & Ayres 1993).

The aim of this study is to demonstrate the effect of pesticides (diazinon and glyphosate) in sublethal concentrations on the box mussel *Septifer bilocularis* L. The study

is motivated by the fact that both pesticides are still widely used in Indonesia (Sembel *et al.* 1991, pers. obs.).

MATERIALS AND METHODS

Diazinon [O, O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], an organophosphorous insecticide, and glyphosate [N-(phosphonomethyl)glycine], an organophosphorous herbicide (Gallo & Lawryk 1991), were used as test chemicals. Both chemicals were obtained from a pesticide drugstore.

Box mussels *S. bilocularis* L. were collected on the shore of Tongkaina, northern part of Sulawesi, Indonesia. The weight ranged from [mean ± standard error (SE)] 0.18 ± 0.03 g dry weight for diazinon and 0.17 ± 0.02 g dry weight for glyphosate experiments. Encrusting organisms were removed and mussels held in stagnant sea water. They were not given food other than that occurring naturally in the water surrounding them. The mussels were stored in the Laboratory of Marine Sciences, Faculty of Fisheries and Marine Sciences, University of Sam Ratulangi, Manado, Indonesia. All water for experiments was taken from the site where the specimens were collected. Sea water was autoclaved at 121 °C and suspended matter allowed to settle before use. Distilled water was used to dilute the water to obtain the salinity needed.

The experimental set-up and measurement of oxygen consumption were adapted from Johnson (1973) and Bayne *et al.* (1985). Depletion of dissolved oxygen was measured on groups of three mussels placed in containers with pure water (control) and water with sublethal concentrations of diazinon (0.6, 6, and 30 ppm) and glyphosate (480, 720, and 960 ppm). These concentrations were chosen because preliminary studies showed that mortality occurred above the highest concentration of each of the tested chemicals. For measurement of oxygen consumption, groups of 3 mussels with 9 replicates were used. Oxygen was measured for

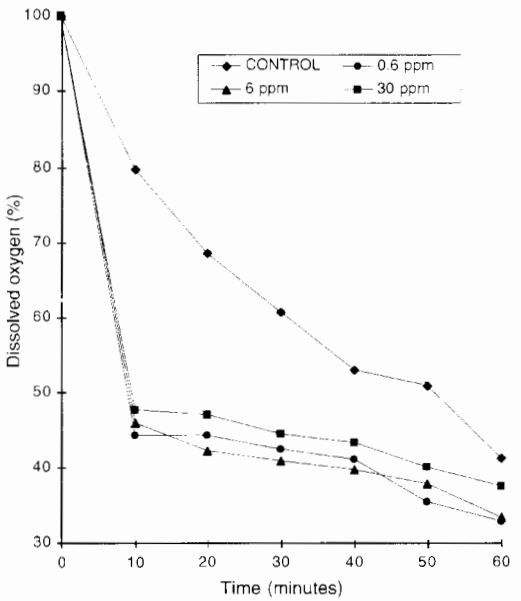

Figure 1. Relative changes of dissolved oxygen when the control is compared with containers with *S. bilocularis* exposed to diazinon for one hour. Each point is the mean of 3 measurements.

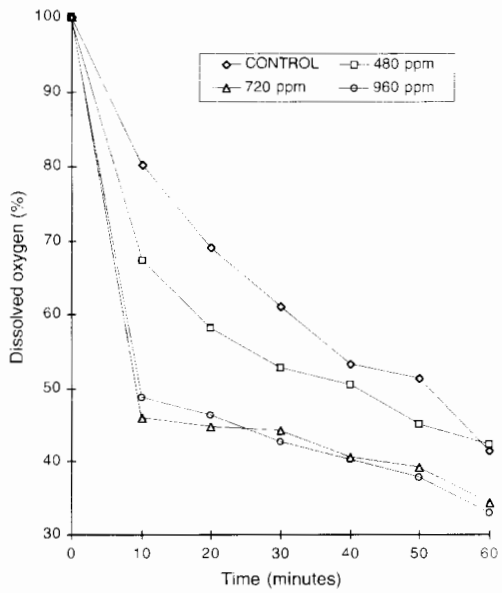

Figure 2. Relative changes of dissolved oxygen in the control compared with containers with *S. bilocularis* exposed to glyphosate for one hour. Each point is the mean of 3 measurements.

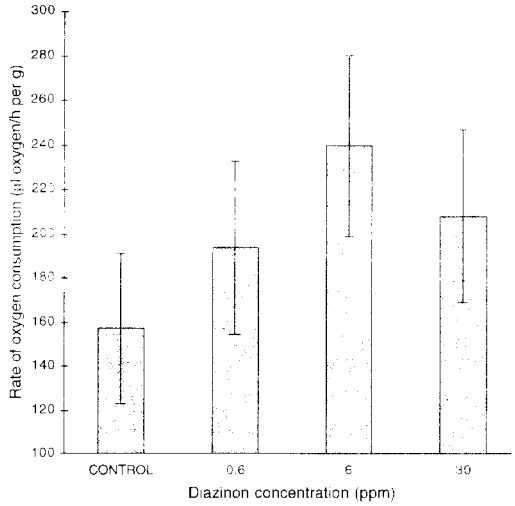

Figure 3. Oxygen consumption (ml O_2 h^{-1} g^{-1}) of the mussel *S. bilocularis* in the control compared with indicated concentrations of diazinon during one hour. Vertical lines are standard errors (S.E.).

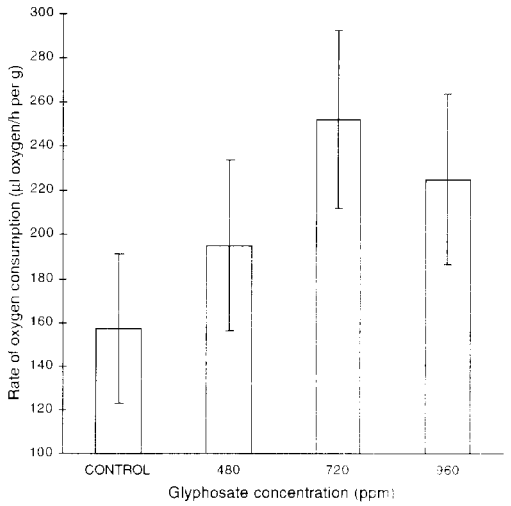

Figure 4. Oxygen consumption (ml O_2 h^{-1} g^{-1}) of the mussel *S. bilocularis* in the control compared with indicated concentrations of glyphosate during one hour. Vertical lines are standard errors (S.E.).

one hour and depletion of the dissolved oxygen was measured every 10 minutes for 2 h to the nearest 0.01 ppm with a Dissolved Oxygen Meter mounted in the upper part of the sealed container. Water was stirred by a magnetic stirring bar inside the containers for 1-3 minutes prior to readings. Temperature was stabilised by an air conditioner. Water temperature was 22.85 ± 0.44 °C, salinity 30.00 ± 0.00 ‰, and pH 7.88 ± 0.24. The three variables were measured before and after each experiment.

The oxygen consumption was measured as a rate of oxygen uptake (Johnson 1973). According to Johnson (*op. cit.*) the rate was calculated from the formula:

$$R = [(C_i - C_t) \cdot V \cdot 700] \cdot [t \cdot w]^{-1},$$

where R is the rate of oxygen (O_2) consumption (ml h^{-1} g^{-1} d.w.), C_i is the initial concentration of dissolved O_2 (ppm), C_t is final concentration of dissolved O_2 (ppm), 700 is a conversion factor for O_2 adapted from Johnson (1973) (1 ppm = 700 ml l^{-1}). V is the volume of water in the container (l), t is time (h), and w is dry weight (g).

To avoid errors due to handling, the first readings during the first hour were not used in the calculation. After the tests, the animals were dissected and soft parts were dried at 105 °C overnight to obtain the dry weight.

The rate of oxygen consumption (R) for both diazinon and glyphosate, was analysed by means of One-way ANOVA (Analysis of Variance) and Tukey-test (Sokal & Rohlf 1981; Fowler & Cohen 1990). Both statistical tests were applied to test whether the concentrations of the two pesticides had an effect on the oxygen consumption.

RESULTS

In preceding pilot experiments, mortality occurred when animals were exposed to concentrations of diazinon above 30 ppm, and above 960 ppm for glyphosate.

Figs. 1 & 2 show the relative depletion of dissolved oxygen in experiments with sublethal concentrations of diazinon and glyphosate using groups of three mussels with 3 replicates. The values are expressed as a percentage of the control. Both diazinon and glyphosate influence the ability of the

mussels to take up the oxygen. However, there is no significant difference ($p>0.05$) between the control and the treatments.

Figs. 3 & 4 show the rates of oxygen consumption during 2 h in tests at sublethal concentrations of diazinon and glyphosate. In concentrations of 0.6, 6, and 30 ppm diazinon, the rates were 193.46 ± 38.84, 239.77 ± 40.36, and 208.05 ± 38.57 ml O_2 h^{-1} g^{-1} respectively. In concentrations of 480, 720, and 960 ppm glyphosate, it was 195.26 ± 43.06, 252.28 ± 36.06, 225.43 ± 22.40 ml O_2 h^{-1} g^{-1} respectively. In the control it was 157.27 ± 34.10 ml O_2 h^{-1} g^{-1}. Concentrations of 6 and 30 ppm diazinon, 720 and 960 ppm glyphosate were required to show an effect on the oxygen consumption. The effect was statistically significant ($p<0.05$).

DISCUSSION

The oxygen uptake of bivalves depends on the flow of water across the gills (Jørgensen 1990). Water is drawn into the mantle cavity through the inhalant aperture; it passes between the gill filaments into the suprabrachial cavity and is ejected through the exhalant aperture (Redpath & Davenport 1988). The water current through the mantle cavity is generated by the lateral cilia of the gills (Silvester & Sleigh 1984). The flow through the mantle cavity is laminar and then oxygen accumulated in the water is taken up by diffusion process through the epithelium lining of the mantle cavity (Famme & Kofoed 1980; Jørgensen *et al.* 1986), as well as through the tissues of the body; transport via the blood circulation being slight (Booth & Mangum 1979) or significant (Famme 1981). In the latter case the gills are of marginal importance in the overall oxygen consumption (Famme & Kofoed 1980).

The presence of a contaminant can affect the oxygen consumption in two ways. First, in a mechanical way by reducing the gape of valves and/or by acting directly on the ciliary pump (Jørgensen 1990). Second, in a biochemical way related to the effect on enzymes. Both effects can occur separate or together.

The concentrations of diazinon and glyphosate are important for the effect on mussel respiration. In diazinon, the consumption of oxygen increased and reached the highest level at a concentration of 6 ppm. It decreased when the concentration was increased (30 ppm). This was significant ($p<0.05$) compared to the control (Fig. 3). In glyphosate, the consumption increased and reached the highest level at a concentration of 720 ppm. It decreased at the concentration of 960 ppm (Fig. 4). This was significant ($p<0.05$) compared to the control. In both pesticides, the effects can be explained biochemically.

Rodriguez & Monserrat (1991) have shown the effects of parathion (insecticide) on the oxygen consumption of the marine crab *Chasmagnathus granulata*. The effect was caused by acetylcholine (Ach) inhibition. Ach is widely distributed throughout the nervous system of marine animals, including mussels. It is acting as a neurotransmitter in sensory nerve fibres and in certain neuromuscular junctions, such as those innervated by the stomatogastric ganglion.

Rodriguez & Monserrat (1991) showed the effect of herbicide (2,4 D) on oxygen consumption in the marine crab *C. granulata*. This compound is a typical uncoupler of the respiratory chain-oxydative phosphorylation.

Apparently no previous information exists on the effects of pesticides on the oxygen consumption of bivalves. In relation to other contaminants, Brown & Newell (1972) found that both zinc and copper inhibited ciliary activity. Davenport & Manley (1978) showed that *Mytilus edulis* responded with valve closure at a concentration of 0.021 ppm copper sulphate ($CuSO_4$) when concentrations were gradually raised. Stainken (1978) showed that there were significant differences in respiratory rates in clams exposed to low concentrations of oil. He suggested that the lowest concentrations of oil caused

a doubling of the respiratory rates and greater oil concentrations caused a depression in rate. The respiratory rates of the clams exposed to low oil concentrations decreased as the hydrocarbon content of the water and clam tissues decreased, but remained significantly different from the controls.

ACKNOWLEDGEMENTS

We are much indebted to the Tropical Marine Mollusc Programme (TMMP) sponsored by DANIDA for the opportunity to present this paper at the Eighth Conference/Workshop of TMMP in Hua Hin, Thailand. We wish to thank Dr I.F.M. Rumengan, the head of Marine Sciences Laboratory, for experimental facilities, Dr L.J.L. Lumingas for discussions, Mr B. Pratasik for reading the first manuscript and our colleague Mr J. Sumampouw for data collection.

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ISSN 0858-3633