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MONODONTA TURBINATA (BORN); TOXICITY AND BIOACCUMULATION OF Cu AND Cu + Cr MIXTURES

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The effects of copper and chromium are studied on the cosmopolite marine prosobranch *Monodonta turbinata* (Born). The influence of Cu and of the mixture Cu + Cr was tested by the determination of the LC50 (48 h), the LT50, the respiration and bioaccumulation rates. The tested concentrations of metals caused a significant reduction of the respiration rate of *Monodonta*. Cu accumulates progressively in the tissues of the prosobranch proportionally to the Cu concentrations of the tested media. In all experiments [LC50 (48 h), LT50, oxygen consumption and bioaccumulation] when Cu and Cr act jointly, an antagonism is observed: the mixtures caused less pronounced effects than Cu and Cr acting alone. Generally *Monodonta* was found to be much more sensitive to Cu than other benthic species.

KEY WORDS: Copper, chromium, heavy metal toxicity, *Monodonta turbinata*

INTRODUCTION

Copper and chromium, essential elements for many life processes, are also potentially highly toxic to most organisms (Winner and Farrel, 1976; Trabalka and Gehrs, 1977).

Hexavalent chromium affects reproduction, feeding activity and respiration rates (Oshida and Reish, 1975; Capuzzo and Sasner, 1977; Raymond and Shields, 1964). Copper usually increases the respiration rates of marine organisms, reduces egg production, decreases the feeding rate and produces changes in the blood (O'Hara, 1971; Reeve *et al.*, 1977a; Moraitou-Apostolopoulou and Verriopoulos, 1979; Christensen *et al.*, 1972).

Marine and especially coastal ecosystems are increasingly endangered by the large amounts of these metals which enter and concentrate in the environment. The use of chromates in industrial processes and of copper in antifouling paints, algicides etc. increases the interest in the effects of these metals on aquatic organisms.

The evolution, in a biotope, of tolerant strains of some species indicates that a contaminant is exerting a very important effect on the existing population.

In sea waters multicontaminant pollution appears to be the rule rather than the exception. Thus, for a realistic approach to pollution effects it becomes necessary to estimate combined toxicity. Mixtures of heavy metals may produce unexpected effects (Braek *et al.*, 1976; Verriopoulos and Dimas, 1988).

In the present paper, we have studied the lethal effects, the oxygen consumption and the bioaccumulation rates of the mixture of copper and chromium as well as those of copper alone, on *Monodonta turbinata*.

MATERIAL AND METHODS

Monodonta turbinata (Born) is a typical prosobranch gastropod belonging to the Trochidae and living at the coastal zone. For this study we used specimens collected from the North-East part of Saronikos gulf (gulf of Athens) where it is very common.

In order to focus on animals of the same age, only organisms with a shell height of 26 ± 2 mm were used for the tests. Following shell cleansing all individuals were transferred to constant temperature rooms ($18 + 0.5°C$) for acclimation with a photoperiod of 12 hours darkness and 12 hours light. No food was attributed during the acclimation (4 days) and the experimetal period (up to 4 days).

The experiments concerned tests—in 2 to 4 replicates—for the estimation of acute toxicity of Cu and the mixture of Cu and Cr in *M. turbinata*. Specimens were exposed to metals in 200 ml glass containers using Cu and Cr in the form of $CuSO_4$ and Na_2CrO_4 diluted in artificial sea water (ultramarine synthetica sea salts) of 38% salinity. For each test solution we determined:

a) *LC50 48 h* (concentration of a toxicant lethal to 50% of the test animals after 48 hours of exposure). It was determined according to the Bliss (1938) method.

b) *LT50* (Time needed for a certain toxicant concentration to cause the death of 50% of the test animals). It was calculated by continuing a number of the LC50 experiments.

Preliminary experiments determined the range of the lethal concentration in 48 hours (LC50 48 h). We tested the following concentrations of Cu: 2, 4, 6, 8, 10, 12 and 14 ppm for the LC50 and 2, 4, and 8 ppm for the LT50. For the Cu–Cr combination test the quantities of the two metals in each mixture have been calculated as parts of the relevant LC50 48 h in order to form mixtures characterised by equipotency. Given that the LC50 48 h of Cr was 51.9 ppm (Verriopoulos *et al.*, 1989), we calculated the effects on the survival of the following mixtures:

A) 6 ppm Cu (75% of LC50) + 13 ppm Cr (25% of LC50)

B) 4 ppm Cu (50% of LC50) + 26 ppm Cr (50% of LC50)

C) 2 ppm Cu (25% of LC50) + 39 ppm Cr (75% of LC50)

c) *Oxygen consumption.* Oxygen consumption was measured by the polarographic method (Radiometer type). Each animal was placed in a hermetically sealed 50 ml syringe and kept in a constant temperature room. Measurements were performed after 2, 24 and 48 hours of exposure. Only those animals which survived up to the end of the experimental period were taken into consideration. In order to obtain comparable results the oxygen consumption was calculated per gr of the test animals (ml 0_2/gr of animals/hour).

d) *Bioaccumulation rates.* (concentrations of Cu and Cr in the soft tissues of *Monodonta* after exposure to the pollutants). The bioaccumulation rates were estimated after exposure to the following media: 2, 4, 8 ppm Cu for a period of up to 72 hours. Measurements were performed after (a) 12, 24, 36, 48 and 72 hours of exposure to Cu and (b) 48 and 96 hours of exposure to the three concentrations of the mixture of Cu and Cr. Analysis for metals was made on pooled samples of six exposed individuals. The tissues were lyophilised and homogenised according to Bernhard's (1976) method. Approximately 0.5 gr of tissue was digested in 5 ml of nitric acid in teflon bombs for 8 hours at 150°C (UNEP, 1984). Readings for copper and chromium were taken with a Varian AA175 Atomic Absorption Spectrophotometer.

Statistical treatment of results was performed using the STATGRAPHICS package software of the National Center of Marine Research and includes: Two-way analysis of variance (ANOVA), Least Significant Difference Test (LSD) and Regression Analysis.

RESULTS

The results of the three experiments for the determination of the mortality of *Monodonta* specimens exposed to different concentrations of Cu did not differ significantly ($P = 0.999$). The regression analysis gave the equation describing the LC50 (48 h) of all the experiments: $Y = -2.01 + 6.27X$, where Y represents mortality (%) and X the concentration of copper. The statistics for this relation are $R = 0.935$, $F = 112.03$ and $P = 0.000$. This equation shows that the LC50 (48 h) for Cu is 8.08 ppm (Figure 1).

The results of mortality of two experiments using mixtures of Cu and Cr are given in Figure 2. The two experiments did not differ significally ($P = 0.965$). Our results show there is an important decrease in the toxicity of all three tested mixtures, but when compared to results for Cu acting alone (Figure 1), there is no significant difference ($F = 1.278$, $P = 0.340$).

The continuation of a number of the above experiments lead us to determine the LT50. The calculated LT50 for the three different Cu concentrations (2, 4 and 8 ppm) did not differ significantly ($F = 0.004$, $P = 0.952$). For the lower concentration (2 ppm) LT50 was 54.64 hours ($R = 0.977$, $F = 64.194$, $P = 0.004$), for 4 and 8 ppm it was 53.28 hours ($R = 0.982$, $F = 83.582$, $P = 0.027$) and 52.45 hours ($R = 0.966$, $F = 42.640$, $P = 0.007$) respectively. These regressions which are described by the equations $Y_{2\,ppm} = -25.8 + 1.38X$, $Y_{4\,ppm} = -24.2 + 1.39X$ and $Y_{8\,ppm} = -28.4 + 1.49X$ are shown in Figure 3.

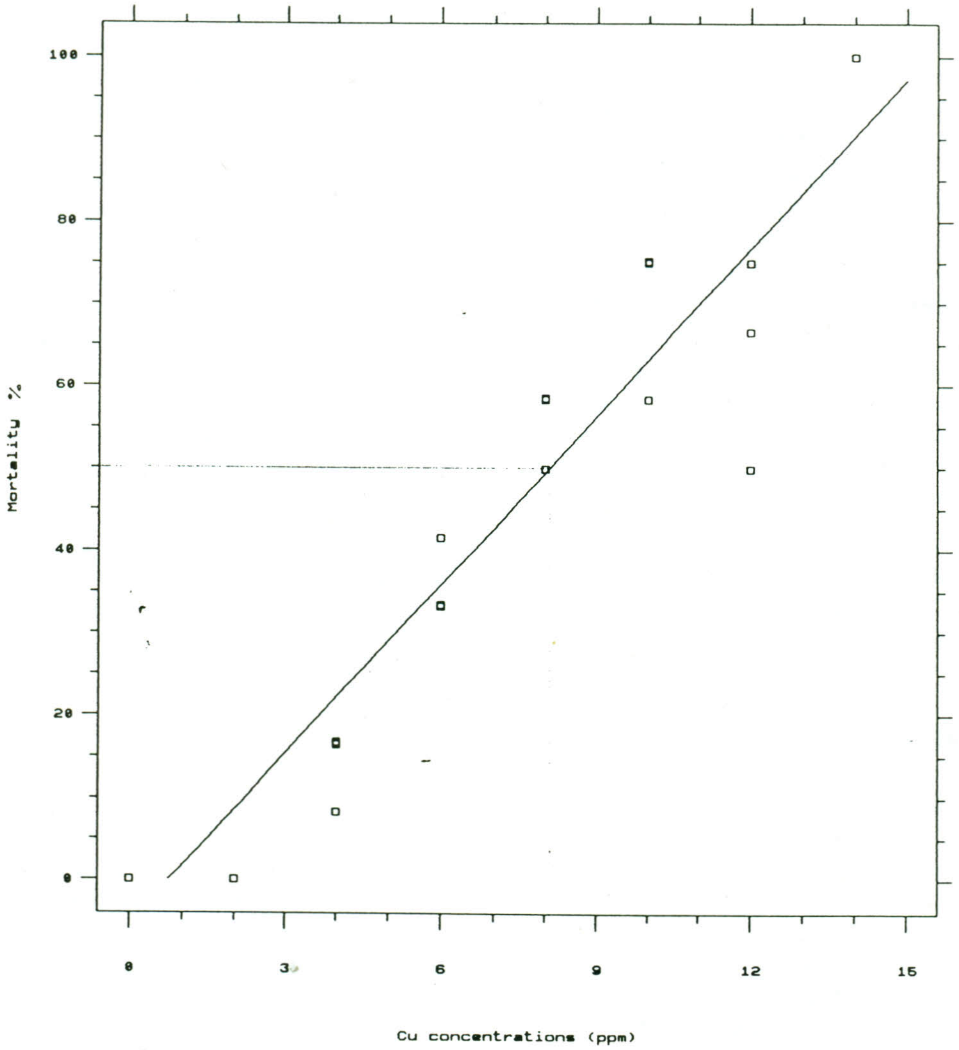

Figure 1 Mortality of *Monodonta* exposed to different concentrations of Cu. Determination of LC50 (48 h).

The three Copper-Chromium mixtures have also similar results ($P = 0.944$). The calculated LT50 for the first mixture (2 ppm Cu + 39 ppm Cr) was 96.10 hours ($R = 0.934$, $F = 41.234$, $P = 0.001$), while for the two others (4 ppm Cu + 26 ppm Cr and 6 ppm Cu + 13 ppm Cr) it was 83.58 hours ($R = 0.965$, $F = 80.772$, $P = 0.000$) and 81.06 hours ($R = 0.978$, $F = 131.719$, $P = 0.000$) respectively. These regressions, described by the equations $Y_a = -13 + 0.65X$, $Y_b = -25.6 + 0.90X$ and $Y_c = -21.4 + 0.88X$, are shown in Figure 4.

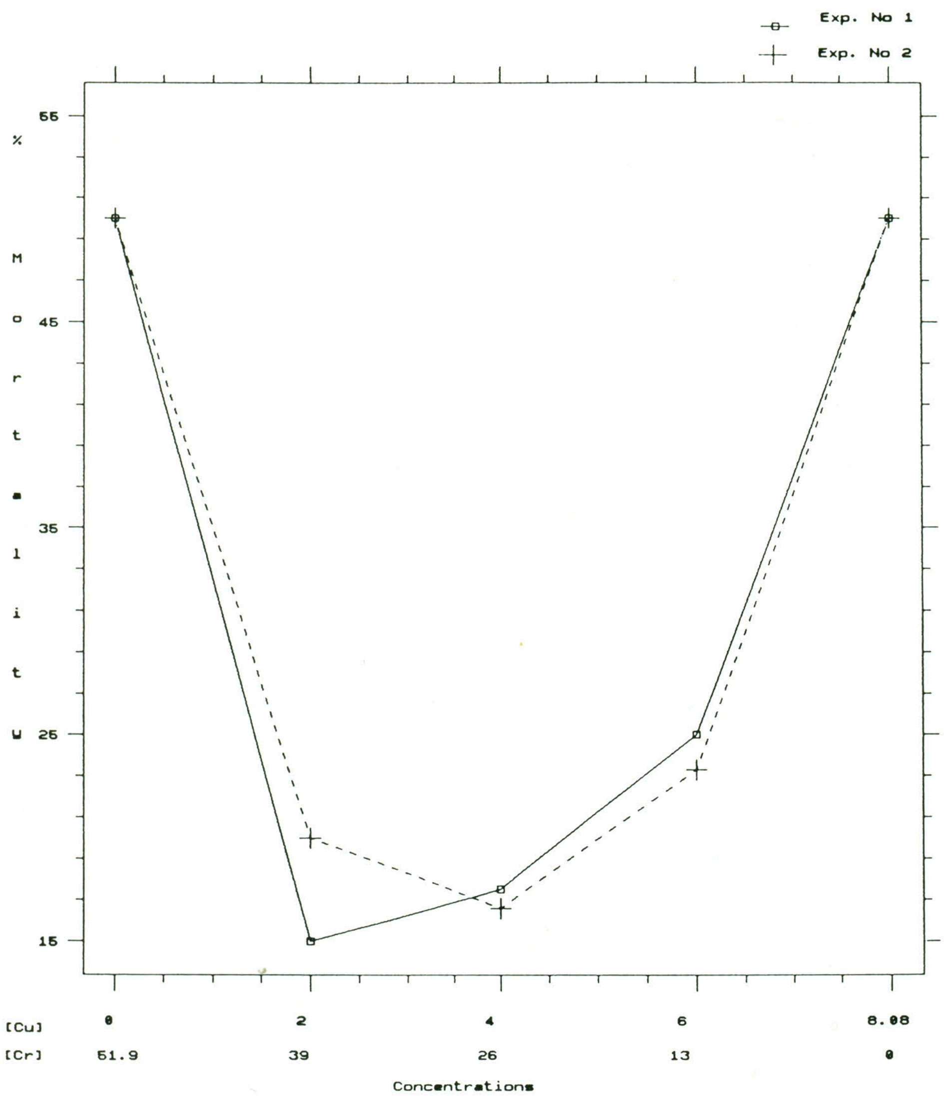

Figure 2 Mortality of *Monodonta* (LC50 (48 h)) exposed to different combinations of Cu and Cr.

Figure 5 shows the effects of Cu on the respiration rate of *Monodonta* after 0, 24 and 48 hours of exposure. The two-way ANOVA showed statistically significant differences between respiration rates of specimens exposed to various Cu concentrations (Table 1). An important decrease of the respiration rate was noticed for all Cu concentrations in comparison with controls, while the least significant difference test (LSD) also showed that the lower concentrations (2 and 4 ppm) provoked a more pronounced effect (decrease) than the higher ones (6 and 8 ppm). Furthermore, in all

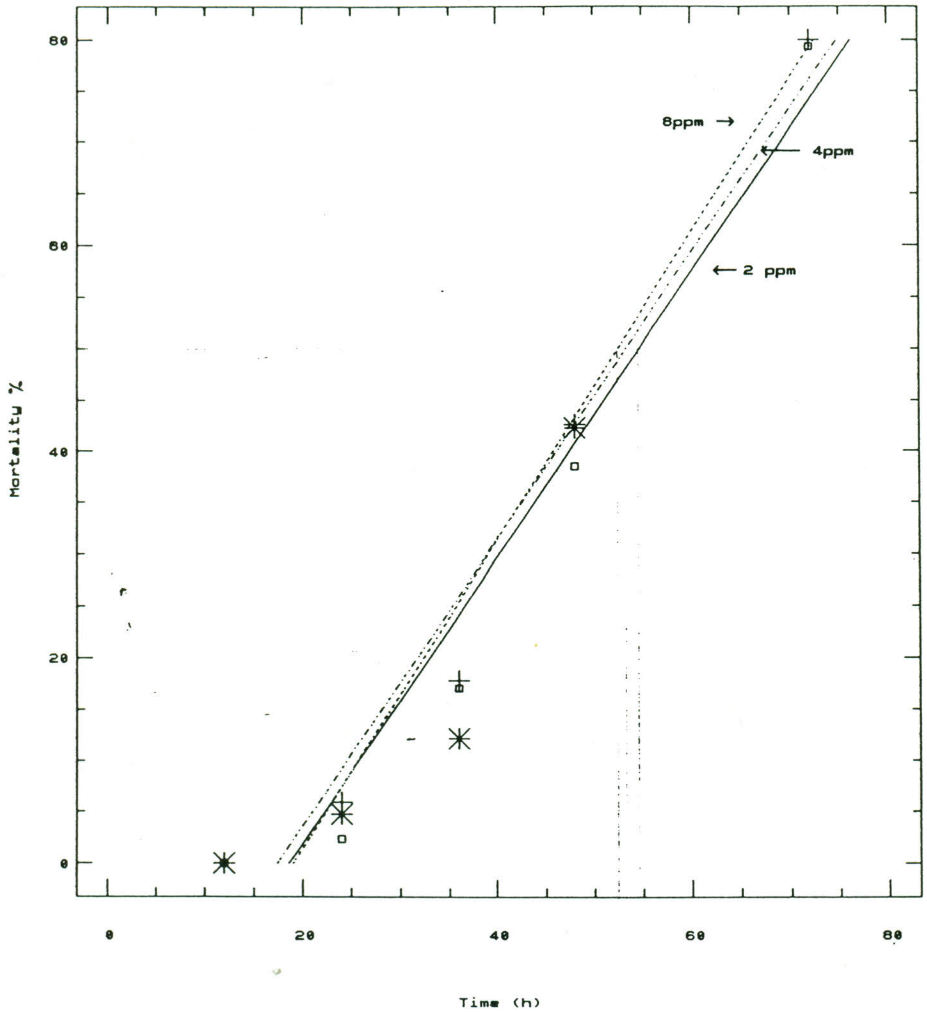

Figure 3 Determination of LT50 of *Monodonta* exposed to 2, 4 and 8 ppm of Cu.

cases (controls and tests) higher respiration rates were noticed for the 24 hour measurements (Table 1; Figure 5). An interaction between the factors "Cu concentration" and "time of exposure" was also noticed (Table 1); this means that "time" influences oxygen consumption of specimens exposed to different Cu concentrations.

The mixtures of the two metals significantly reduce the respiration rate of *Monodonta* (Figure 6; Table 1). The Least Significant Difference test showed no important difference between the impact of the three mixtures (theoretically equitoxic) (Table 1). In this case too, an interaction was noticed between the two factors: metal

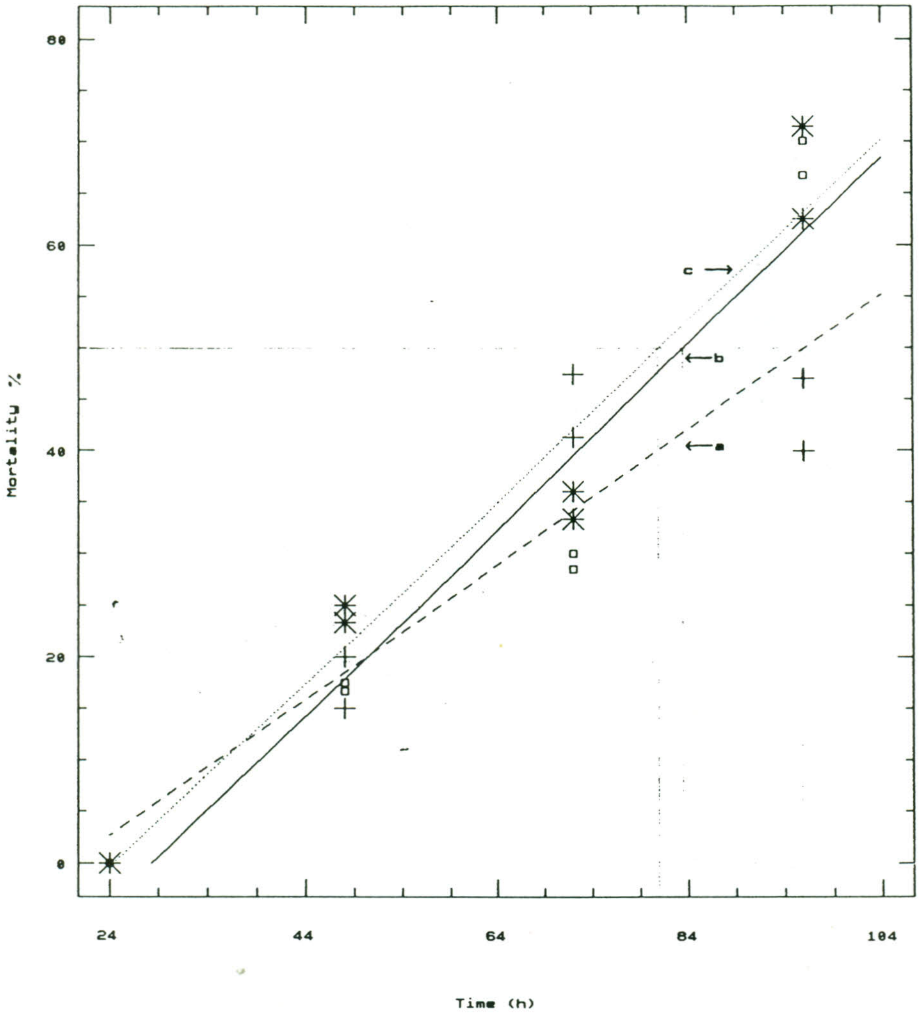

Figure 4 Determination of LT50 of *Monodonta* exposed to different combinations of Cu and Cr. (a) 2 ppm Cu + 39 ppm Cr, (b) 4 ppm Cu + 26 ppm Cr, (c) 6 ppm Cu + 13 ppm Cr).

accumulation and time of exposure. All three tested mixtures caused a reduction of the respiration rate which was significantly less than that of copper acting alone in the 8 ppm concentration.

The accumulation of Cu in the tissues of *Monodonta* after exposure to the three copper concentrations is shown in Figure 7. The concentration of Cu in *Monodonta* showed progressive increase with time throughout the experimental period of 72 hours ($F = 72.032$, $P = 0.000$). Furthermore, bioaccumulation was proportional to the Cu concentrations of the three tested solutions ($F = 29.757$, $P = 0.000$). The

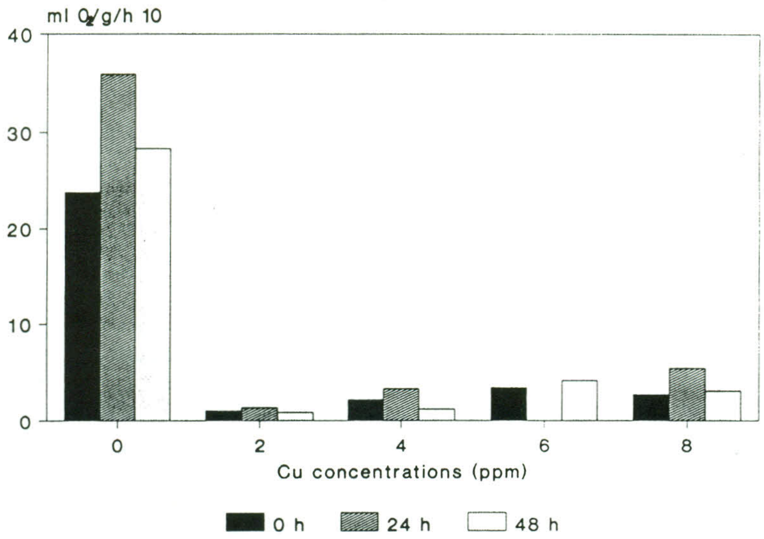

Figure 5 Oxygen consumption of *Monodonta* after exposure for 0, 24 and 48 hours to different concentrations of Cu.

maximum measured Cu concentration was 180 ppm after exposure for 72 hours to the 8 ppm solution.

Figure 8 shows the concentration of Cu in the tissues of *Monodonta* after exposure to the three mixtures of Cu and Cr. A progressive increase with time was also noticed in the concentrations of Cu in *Monodonta* tissues ($F = 2.198$, $P = 0.212$); bioaccumulation was also proportional to the Cu concentrations in the mixtures ($F = 44.016$, $P = 0.001$).

From the comparison of Figures 7 and 8 it becomes clear that *Monodonta* accumulates higher concentrations of Cu when exposed (for the same period) to Cu than when it is exposed to the same concentration of Cu mixed with Cr.

Table 1 Results of two-way ANOVA of respiration rates consumption (c = concentration, t = time)

Factor		*F*	*P*	*Groups (L.S.D.)*
a) Cu				
Main effect	c	266.413	0.000	{[2], [4]} – {[4], [8], [6]} – {[0]}
	t	11.690	0.000	{(0), (48)} – {(24)}
Interaction		4.092	0.001	
Resid. = 613.7				
b) Cu + Cr				
Main effect	c	1000.000	0.000	{[6], [2], [4]} – {[0]}
	t	18.332	0.000	{(48), (96)} – {(0)}
Interaction		74.829	0.000	
Resid. = 196.2				

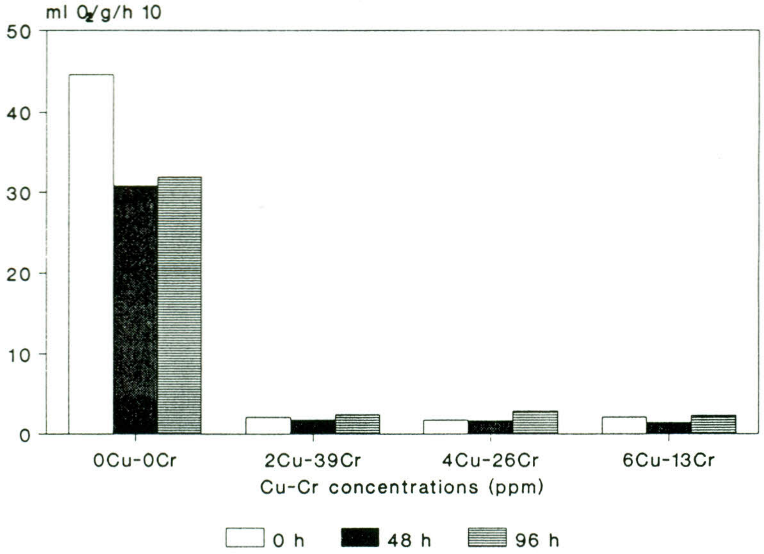

Figure 6 Oxygen consumption of *Monodonta* after exposure for 0, 48 and 96 hours at different combinations of Cu and Cr.

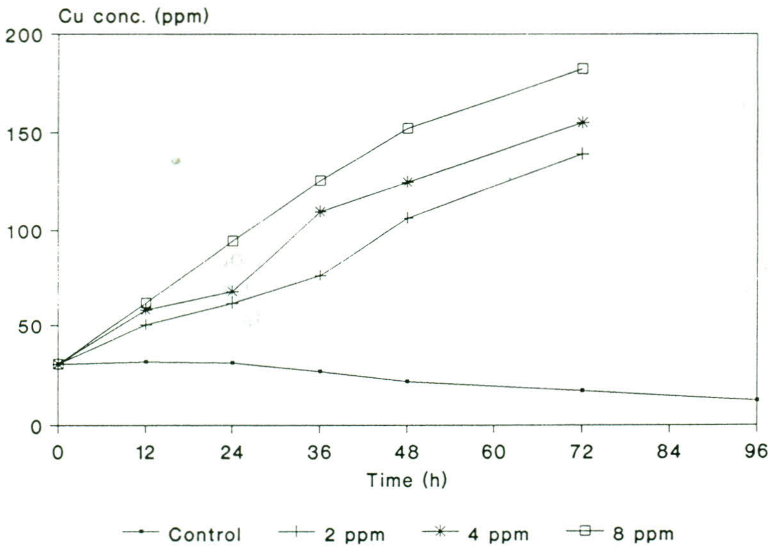

Figure 7 Accumulation of Cu in the soft tissues of *Monodonta* for different exposure times and toxic concentrations of Cu.

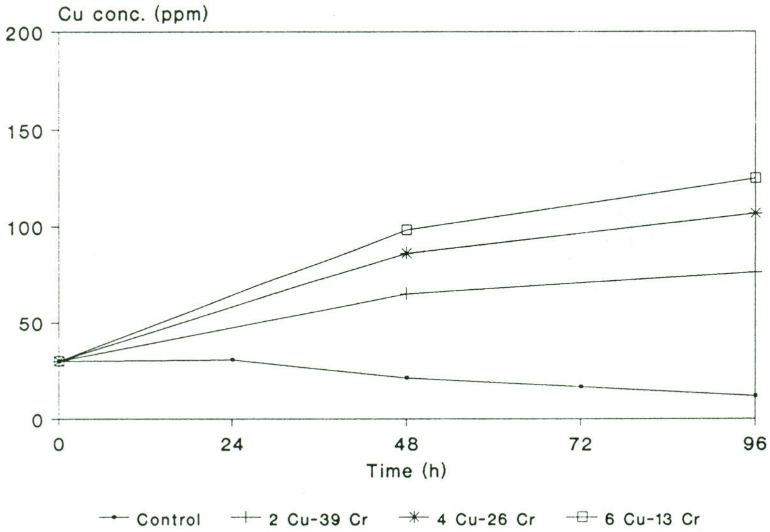

Figure 8 Accumulation of Cu in the soft tissues of *Monodonta* for different exposure times and toxic mixtures of Cu and Cr.

DISCUSSION

Monodonta proved much more resistant to Cu, as far as survival is concerned, than the planktonic or epibenthic crustaceans living in the same area (Moraitou-Apostolopoulou and Verriopoulos, 1982) but much more sensitive than other benthic animals such as *Carcinus maenas* (Portmann, 1972).

Respiration represents an important physiological index of an organism since respiratory rates reflect the metabolism and the functional well-being of an animal. Tests of respiration have been widely used for the estimation of the impact of pollutants. The test has been considered as an important index (Struhsaker *et al.*, 1974; Kapoor and Griffiths, 1976;). Respiratory rates are valuable in assessing the effects of pollutants because they are relatively easy to monitor.

In the present experiments significant effects of Cu on the respiration rate have been observed even at the lowest test concentrations (2 ppm). This seems important because respiratory changes have been reported at high pollutant concentrations (Reeve *et al.*, 1977b). It is also important to notice that the measurements realised after two hours of exposure were not considerably different from those of 24 and 48 hours.

All combined toxicity results are well in accordance with the infra-addition type (a form of antagonism) of interraction proposed by Warren (1971).

When the two metals were acting jointly on *Monodonta*, an infra-addition mode of interraction (type of antagonism) was observed both in acute and sublethal toxicity tests. In all cases, the mixtures of metals caused less pronounced effects on survival and respiration than the theoretically equipotent solutions of copper or chromium.

Concerning the interaction between the two metals it must be noted that the toxicity of Cr is influenced by pH levels and that copper is known to cause changes in the pH (Van der Potte *et al.*, 1981).

The antagonistic type of interaction of the two metals expressed both in survival and respiratory tests, must be closely related to the results of bioaccumulation tests. During these tests it was shown that *Monodonta* accumulated more Cu when exposed only to copper than when Cu is acting jointly with Cr. This could be attributed to a competition between the two metals. The problem of toxic action of pollutants acting jointly seems complicated. The mode of action depends not only on the pollutants but also on different factors such as the chemical form of the pollutants, the organism tested (Braek *et al.*, 1976) and even the effect considered, e.g. mixtures of pollutants may be additive in producing acute toxicity but interact in unpredictable ways in producing chronic effects (Verriopoulos *et al.*, 1987).

In the range of our experimental conditions (concentrations of Cu from 2 to 8 ppm and up to 72 hours of exposure) *Monodonta* has not shown a regulation capacity for copper.

Tolerance and regulation of heavy metals appears to depend strictly on the type of metal and the organism tested. For most decapod crustaceans and fishes essential metals are regulated whereas non essential ones seem to be less well regulated (Pentreath, 1976). Regulation of all metals is generally poor in plants, bivalve mollusks and gastropods (Bryan, 1979).

In conclusion *Monodonta turbinata* was found to be more resistant to Cu than planktonic or epibenthic crustaceans but much more sensitive than other benthic animals. Cu affects its respiration rate. When Cr acts jointly with Cu to *Monodonta* an infra-addition mode of interaction was observed; the mixture causing less pronounced effects than the theoretically equipotent solutions of Cu and Cr acting alone.

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