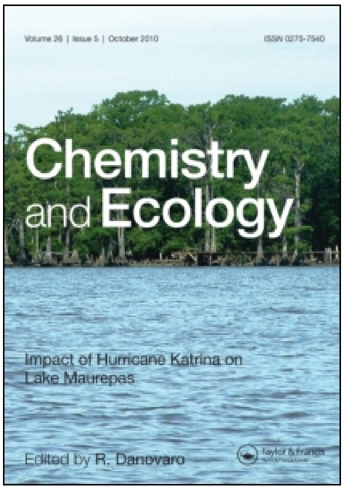

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STUDY OF JOINT ACTION OF MERCURY AND LEAD ON DISCHARGES OF THE ANTENNULE-INNER RAMUS NERVE OF THE CRAYFISH (*CAMBARUS CLARKII*).

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The joint action of mercury and lead on the discharge of antennule-inner ramus nerve of crayfish (*Cambarus clarkii*) has been studied by means of a multichannel record of electrophysiological signals. The results show:

1. When the concentration of mercury (Hg^{2+}) in water is only half of the restricted concentration (0.1 ug l^{-1}) but there is also 12.5 ug l^{-1} lead (Pb^{2+}) present, the joint inhibition of the two heavy metallic ions on the discharge is much stronger than that of the restricted concentration of mercury present. The slope of a linear regressive equation which indicates the frequency of discharges (F_n/F_1) and action time is $< -0.08129 \pm 0.00357$ ms. Further, in a certain concentration range, the higher the concentration of lead (Pb^{2+}), the inhibition is stronger.

2. When the concentration of lead (Pb^{2+}) in water is only 1/2 of the restricted level (100 ug $l^{-1}Pb^{2+}$), but when there is 0.025 ug l^{-1} Hg^{2+} present, the joint inhibition of the two heavy metal ions on the discharge is much stronger than the restricted level of lead. The slope of a linear regression indicates a frequency of discharge (F_n/F_1) and action time is $< -0.07732 \pm 0.00451$ ms. Within a certain concentration range, the value is higher with a higher concentration of mercury.

The results suggest that there are possibly enhanced effects of the toxicity of mercury and lead together. More attention might be made of this in the evaluation of water quality.

Keywords: Hg^{2+} and Pb^{2+}, joint action, crayfish (*Cambarus clarkii*), antennular nerve reaction

INTRODUCTION

There is almost no natural source of water which has not been contaminated. The sources of these polluting materials will harm water organisms first. The heavy metals and many organic poisons are present often at levels which are antagonistic to water biota (Alabaster and Lloyd, 1980; Alabaster *et al.*, 1994; Liu, 1994). The antennular surface of crayfish (*Cambarus clarkii*) contains sensory hairs which are sensitive to chemicals and mechanical waves in water (Hodgson, 1958; Vedele, 1985; Tazaki, 1975; Tautz *et al.*, 1981). The heavy metal divalent ions, such as mercury, lead, zinc and chromium can obviously inhibit antennular nervous discharge of crayfish (Zhang *et al.*, 1991 a, b, c). If there are several substances present, a joint toxic action may occur (Forstner, 1974; Zhang Qiuqi and Chen, 1993; Alabaster *et al.*, 1988, 1994). We can expect to develop a biological monitoring technique to evaluate water quality, but this is often difficult to determine since a joint action of pollutants with the physiochemical methods is seldom known (Cairns *et al.*, 1975; Alabaster *et al.*, 1988, 1994). Using electro-physiological methods, we studied the joint action of mixed mercury (Hg^{2+}) and lead (Pb^{2+}) solutions in different mixtures on the discharge of the antennular-inner ramus nerve of crayfish. The feasibility of using the characteristic change of discharge frequency was considered for monitoring water sources contaminated by heavy metals.

MATERIALS AND METHODS

Animals: Crayfish (*Cambarus clarkii*) were used. Each was 20 to 30 grams in weight, 6 to 11 cm long. Male or females were used (forty for each experiment) since there was no evident difference in statistics, $p > 0.05$. The crayfish were kept in fresh unpolluted water for 24 hours prior to the experiment.

Test solution: mercury ($HgCl_2$) and lead ($PbCl_2$) (analytical reagents) were mixed with redistilled water as follows:

1. Hg^{2+} alone at 0.1 $ug\,l^{-1}$ (restricted level);
2. Hg^{2+} alone at 0.05 $ug\,l^{-1}$;
3. Hg^{2+} 0.05 $ug\,l^{-1}$ mixture with Pb^{2+} at 12.5 $ug\,l^{-1}$, 25 $ug\,l^{-1}$, and at 50 $ug\,l^{-1}$;
4. Pb^{2+} alone at 100 $ug\,l^{-1}$ (restricted level);
5. Pb^{2+} alone at 50 $ug\,l^{-1}$;

6. Pb^{2+} at 50 ug l^{-1} mixture with Hg^{2+} at 0.0125 ug l^{-1}, at 0.025 ug l^{-1} and at 0.05 ug l^{-1}

The solution was made just prior to the experiment since the concentration of heavy metals changes with time.

METHODS OF RECORDING AND STATISTICS

The 0# insect needles (tip diameter is 100–200 um) were used as a record electrode. Four inner ramuses were cut off from the bases of four crayfish antennules and four electrodes were inserted in the ramuses. The open section is covered by vaseline to prevent drying. The ramus and the reference electrode were put into the same test solution in a shielded thermostat ($22 \pm 2°C$). The discharges of the antennular nerves were conducted by four preamplifiers and monitored by the oscilloscope and then input to the computer for drawing a sequence density histodiagram. The average frequencies, toxicosis latency and discharge durations were measured. All the data were considered with χ^2 statistics and t test. In each case, the regressive equation and regression line were evaluated and drawn out.

RESULTS

The discharge of the antennular nerve in the test solutions of mercury 0.1 ug l^{-1}, 0.05 ug l^{-1}, and mercury 0.05 ug l^{-1} with different concentrations of lead were considered. The ramuses were put into mercury levels alone, and with mercury and lead mixed solutions. The discharge was recorded to make the sequence density histogram. The sampling time is 1000 ms, and the duration of each graph is of 255×1000 ms. The average discharge frequency of each graph was counted as F. Seven of the average frequency of each graph were measured in each graph after the frequency was decreased by comparison with the beginning measurement. The F_n/F_1 (n presents the sequence of graph number 1,2···7) and the regressive equation relating the drift in value F_n/F_1 and action time were evaluated. Results are shown in Table I and Figure 1. The values of "t" were <0.5 in the

TABLE I Tests of mercury and lead additions alone or in mixtures to *Cambarus clarkii* ramus discharges, mercury and lead additions

	Concentration	*Y*	*r*
B	0.10 ug l^{-1} Hg^{2+}	1.05800-0.08129X	−0.99522
C	0.05 ug l^{-1} Hg^{2+}	1.07029-0.07100X	−0.99782
D	0.05 ug l^{-1} Hg^{2+} + 12.5 ugl^{-1} Pb^{2+}	1.09014-0.09496X	−0.99879
E	0.05 ug l^{-1} Hg^{2+} + 25 ug l^{-1} Pb^{2+}	1.06586 − 0.09918X	−0.99256
F	0.05 ug l^{-1} Hg^{2+} + 50 ug l^{-1} Pb^{2+}	1.07014 − 0.12807X	−0.96751

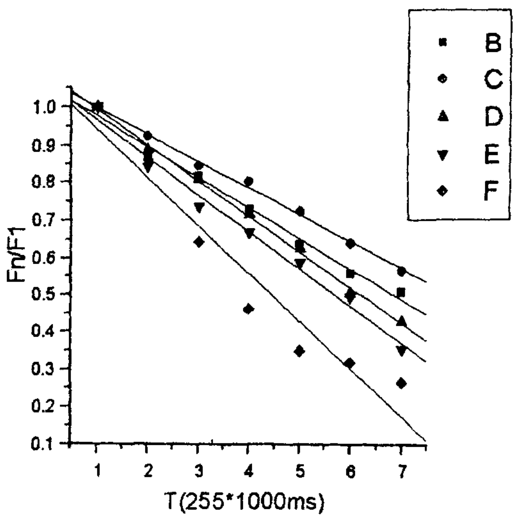

FIGURE 1 The relationship between the frequency of the discharge and action time of the antennular nerve of crayfish (*Cambarus clarkii*) in various concentrations of mercury, and in mercury and lead mixed solutions. B: 0.1 ug l^{-1} Hg^{2+}; C: 0.05 ug l^{-1} Hg^{2+}; D: 0.05 ug l^{-1} Hg^{2+} + 12.5 ug l^{-1} Pb^{2+}; E: 0.05 ug l^{-1} Hg^{2+} + 25 ug l^{-1} Pb^{2+}; F: 0.05 ug l^{-1} Hg^{2+} − 50 ug l^{-1} Pb^{2+}.

combinations of 0.05 ug l^{-1} Hg^{2+} with 12.5 ug l^{-1} Pb^{2+} 25 ug l^{-1} Pb^{2+} and 50 ug l^{-1} Pb^{2+} (N = 8).

2. The discharge of the antennular nerve in solution of lead 100 ug l^{-1} and 50 ug l^{-1}, and 50 ug l^{-1} with different concentrations of mercury, 0.125 ug l^{-1}, 0.025 ug l^{-1} and 0.05 ug l^{-1}. All the methods are the same as mentioned above. The Table II and Figure 2 show the results.

TABLE II Lead alone and with lead and mercury additions in mixtures to *Cambarus clarkii* ramus discharges.

	Concentration		
B	100 ug l^{-1} Pb^{2+}	1.06343–0.07732X	−0.99162
C	50 ug l^{-1} Pb^{2+}	1.06671–0.06518X	−0.99093
D	50 ug l^{-1} Pb^{2+} + 0.0125 ug l^{-1} Hg^{2+}	1.05314–0.08186X	−0.99177
E	50 ugl^{-1} Pb^{2+} + 0.025 ug l^{-1} Hg^{2+}	1.07557–0.10275X	−0.99690
F	50 ug $l^{-1}$$Pb^{2+}$ + 0.05 ug l^{-1} Hg^{2+}	1.17014 – 0.12807X	−0.96751

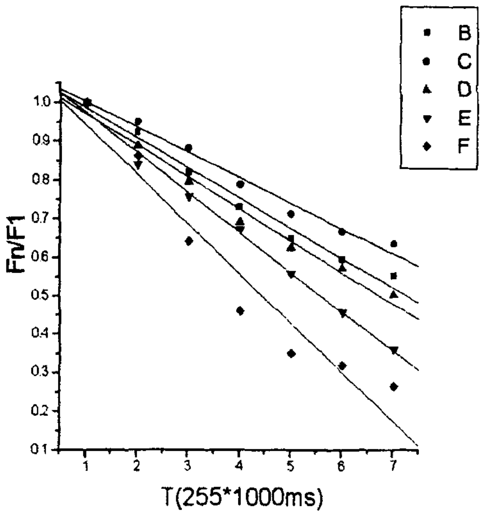

FIGURE 2 The relationship between the frequency of discharges and the action time of the antennular nerve of crayfish (*Cambarus clarkii*) in various concentration of lead (Pb^{2+}) and mercury (Hg^{2+}) mixed solution. B: 100 ugl^{-1} Pb^{2+}; C: 50 ug l^{-1} Pb^{2+}; D: 50 ug l^{-1} Pb^{2+} + 0.0125 ug l^{-1} Hg^{2+}; E: 50 ug l^{-1} Pb^{2+} + 0.025 ug l^{-1} Hg^{2+}; F: 50 ug l^{-1} Pb^{2+} + 0.05 ug l^{-1} Hg^{2+}.

These tests showed the relationship of a pollutant alone or in mixtures are different, with t tests above the $p > 0.05$ limit at levels above $t > 0.5$ are shown with Pb^{2+} at half the limiting concentration with 0.459 at 0.025 ug l^{-1} Hg^{2+} and at 0.463 at this concentration of Pb^{2+} and 0.05 ug l^{-1} Hg^{2+}.

DISCUSSION

The characteristic of antennular nerve discharge recorded with a polychannel electro-physiological method is mostly similar to our previous studies (Zhang *et al.*, 1991, a,b,c) and matching Hodgson's work (Hodgson, 1958). The results showed that in the solution which had restricted heavy metal concentration for aquarium water (Hg^{2+} 0.1 ug l^{-1}, Pb^{2+} 100 ug l^{-1} in our country) the b slope of a regresion χ^2 equation relating to the average discharge frequency and action time for crayfish antennule is -0.08129 ± 0.00357 ms and 0.07732 ± 0.00451 ms. This is similar to what we measured before (Zhang *et al.*, 1991, b, c). It illustrates that our method is repeatable and it is feasible to use the b value as an index for estimating the quality of water contaminated by complexed heavy metal ions using such a polychannel electro-physiological equipment.

The Figure 1 and Figure 2 illustrate that when the concentration of mercury (Hg^{2+}) or lead (Pb^{2+}) was only half the restricted concentration for aquarium water but accompanied with Pb^{2+} 12.5 ug l^{-1} or Hg^{2+} 0.05 ug l^{-1} respectively, the two heavy metal ions could exert a joint action and inhibit the ramus nerve discharge. The difference is remarkable ($p < 0.05$) compared with the solution of Hg^{2+} 0.05 ug l^{-1} or Pb^{2+} 50 ug l^{-1}, and the toxicity was strengthened as the concentration of the other heavy metal increased. We should consider that the joint action increased the toxicity.

Although many researchers have studied the joint action of poisonous substances, those relating to mercury and lead have been barely reported. Mercuy (Hg^{2+}) and lead (Pb^{2+}) can obviously inhibit enzyme activity (Zhang Qiuqi and Chen, 1993). Is this the reason why these metals inhibit the antennular nerve discharge? Is there the same poisonous action a simple addition effect or a coordination (Alabaster *et al.*, 1994)? All these issues should be studied in advance in comparing the toxicity of different chemicals together in the field or the laboratory.

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