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EVALUATION OF THE SYNERGISTIC EFFECT OF SELENIUM ON THE ACUTE TOXICITY OF MERCURY IN FISH LARVAE

by
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RESUME

Les concentrations lethales, CL 50 48 h du mercure, du sélénium et du mercure + sélénium ont été étudiées pour évaluer l'effet synergique du sélénium sur la toxicité aigue du mercure et pour déterminer la sensibilité des larves de poissons (Mugil auratus) exposé à ces polluants. En outre, l'effet du prétraitement avec le sélénium sur la toxicité du mercure a été aussi étudié. La toxicité du mercure était significative aux concentrations basses du sélénium ($\leq$750 ppb) lorsque les larves ont été exposées en même temps à ces deux éléments coexistants dans le milieu du test. Le prétraitement avec le sélénium a augmenté légèrement la résistance des larves à la toxicité du mercure. Le sélénium seul était moins toxique que le mercure.

ABSTRACT

The lethal concentrations, 48 hr LC 50 of mercury, selenium and mercury + selenium were investigated to evaluate the synergistic effect of selenium on the acute toxicity of mercury and to determine the sensitivity of fish larvae (Mugil auratus) exposed to these pollutants. Furthermore, the effect of selenium pretreatment on mercury toxicity was also studied. The toxicity of mercury was found to be significant at low selenium concentrations ($\leq$750 ppb) when the larvae were exposed simultaneously to both elements co-existing in the medium. Selenium pretreatment increased slightly the resistance of larvae against the toxicity of mercury. Selenium alone was less toxic than mercury.

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INTRODUCTION

The main sources of selenium in marine environment are the weathering of selenite containing rocks (Fowler and Benayoun,1976 a) and fossil fuel combustion (Bertin and Goldberg,1971). A small amount of selenium of industrial origin can also reach the marine environment.

Selenium in trace amounts is known to be an essential element in animal nutrition (Harr,1978) and in enzyme systems (Stadtman,1974), but it is considered to be a nutritional marine pollutant (Fowler and Benayoun,1976 b) and is toxic at elevated levels (Sato et al.,1980). The results related to the interaction of selenium with mercury and other metals have been obtained by several investigators (Koeman et al., 1973; Hill,1975; Rimerman et al.,1977; Lucu and Skreblin,1981). It is suggested by these results that selenium counteracts the toxic effect of some of these metals, such as mercury, cadmium etc. Previous studies have mostly been concentrated on this protective effect, in other words, on the antagonistic effect of selenium against the toxicity of mercury (Ganther et al.,1972); Sumino et al.,1977; Sheline and Schmidt-Nielsen, 1977) and very few studies have been carried out on the synergistic effect of selenium on the acute toxicity of mercury.

The present study is aimed towards the evaluation of the synergistic effect of selenium pretraetment on the acute toxicity of mercury in fish larvae, Mugil auratus .

MATERIALS AND METHODS

1. Synergistic Effect:

The fish larvae (27± 1.2 mm) were caught from the harbour and placed in continously aerated fiberglass aquaria containing 50 l sea water. They were acclimatized to laboratory conditions for ten days prior to the initiation of the experiments. During this acclimatization, the larvae were fed on artificial fish food (JBL GmbH, W.Germany) which does not contain mercury and selenium. Following this acclimatization, the organisms were trahsfered to two groups of glass aquaria each containing 2.5 l sea water of 35 ‰ and 20 fish larvae. The water was filtered through a 55 µm filter and treated with ultra-violet. In the first group, the larvae were exposed to different mercury concentrations (as $HgCl_2$) ; in the second group, they were simultaneously exposed to selenium (as Na_2SeO_3) and mercury co-existing at the same concentrations in the medium. The experiments were conducted at 18± 1°C for a total duration of 48 hrs and during this time the sea water was not changed and the organisms were not fed. The tests were repeated four times for each concentration used. At the end of experiments, the number of dead organisms were determined.

2. Effect of Pretreatment :

The fish larvae, acclimatized as above, were pretreated with 0.25 mgl^{-1} and 0.5 mgl^{-1} selenium (as Na_2SeO_3) for 24 hr. After pretreatment they were transfered to clean aquaria and were subjected to the same concentrations of mercury as were

Table 1. 48 hour IC_{50} calculation using the Bliss method for fish larvae, *Mugil curvicus* exposed simultaneously to selenium and mercury.

Concentration (ppb).	Number of dead	% of death	x	Y	Y'	y	$\frac{Z^2}{Q}$	W	Products				
			Log. Conc. (ppb)	Empirical Probit	Expected Probit	Working (Corrected) Probit	Weighting Coeff.	Weights	wx	wy	wxy	wx^2	wy^2
250	2	10	2.39794	3.718	3.71800	3.71864	0.33589	6.7178	16.10888	24.98108	59.90313	38.62813	92.89564
500	4	20	2.69897	4.158	4.16978	4.15908	0.50260	10.0520	27.13005	41.80707	112.83603	73.22318	173.87896
625	6	30	2.79588	4.476	4.37829	4.47730	0.55788	11.1576	31.19531	49.95592	139.67077	87.21835	223.66765
750	12	60	2.87506	5.253	4.71648	5.27140	0.61609	12.3218	35.42593	64.95314	186.74425	101.85172	342.39396
875	14	70	2.94201	5.524	4.98870	5.50132	0.63662	12.7324	37.45882	70.04501	206.07297	111.20416	385.34000
1000	16	80	3.00	5.842	5.24041	5.76444	0.62742	12.5484	37.64520	72.33453	217.00350	112.93560	416.96788
1125	17	85	3.05115	6.036	5.86468	6.02797	0.47144	9.4288	28.76871	56.83652	173.41690	87.77771	342.60886
							Sums	74.9588	213.73290	380.913240	1095.64750	611.83685	1977.7595

$\bar{x}$ = 2.85134

$\bar{y}$ = 5.08163

b = 3.94981

log LC_{50} = 2.83067

LC_{50} = 677.12 ppb S.D.= 1.07

V (log LC_{50}) = 0.0009664

χ^2 = [illegible]

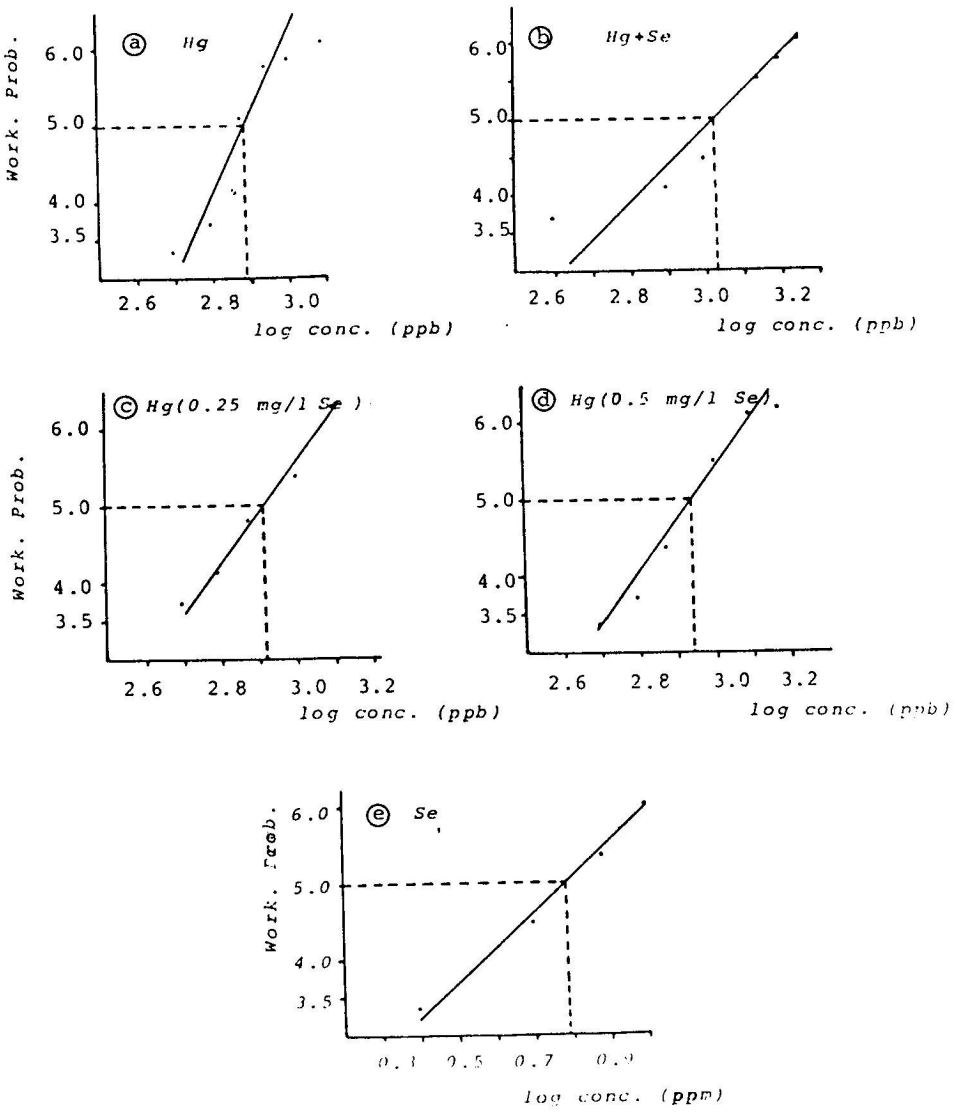

FIGURE 1. Linear regression between the percentage of mortality (working probit) and the logaritms of the concentrations of (a) mercury alone, (b) mercury+selenium, (c) mercury pretreated with 0,25 mgl^{-1}Se, (d) mercury pretreated with 0,5 mgl^{-1}Se, and (e) selenium alone.

used for mercury alone. The test conditions were similar to those described for the study of the synergistic effect. For all concentrations, the tests were repeated four times. Mercury concentration in the sea water obtained from the collection area and used during these experiments was extremly low (0.0005 $\mu g l^{-1}$) compared to the concentrations tested.

RESULTS AND DİSCUSSION

Table 1 gives the numerical values of the 48 hr LC_{50} , calculated by Bliss method (1938). Bliss' method is particularly suitable for tests using small number of test animals and has been applied by some other investigators (Stora,1972; Castritski et al.,1980). According to this method the 48 hr LC_{50} values were computed as follows:

$Y = \bar{y} + b(X-\bar{x})$ where Y = Working(corrected)probit

X = log c (c, conc.in ppb or ppm)

a) Mercury alone: (Fig. 1,a)

Y= 5.22340 + 8.21338 (X-2.91598)

for Y=5; X= 2.88878, thus the LC_{50} = 774.07 $\pm$ 1.04 ppb

b) Mercury + Selenium: (Fig.1,b)

Y= 5.08163 + 3.94981 (X-2.85134)

for Y=5; X= 2.83067, thus the LC_{50} = 677.12 $\pm$ 1.07 ppb

c) Mercury (pretreated with 0.25 $mg l^{-1}$ Se): (Fig.1,c)

Y= 4.92116 + 6.24339 (X-2.90400)

for Y=5; X= 2.91663, thus the LC_{50} = 825.33 $\pm$ 1.05 ppb

d) Mercury (pretreated with 0.5 $mg l^{-1}$ Se): (Fig.1,d)

Y= 5.16157 + 7.07619 (X-2.96518)

for Y=5; X= 2.94235, thus the LC_{50} = 875.69 $\pm$ 1.04 ppb

e) Selenium alone: (Fig.1,e)

Y= 4.97461 + 4.53497 (X-0.78427)

for Y=5; X= 0.78987, thus the LC_{50} = 6.16 $\pm$ 1.08 ppm

We can observe from the results that the 48 hr LC_{50} value for mercury and selenium co-existing at the same concentration in the medium is lower than that obtained for mercury alone. This leads us to suggest that, selenium might have a synergistic effect on the acute toxicity of mercury.

The results of two-way analyses of variance showed that the difference in the percentage of mortality between the organisms exposed to mercury alone and those exposed simultaneously to mercury and selenium was significant ($P<0.01$) at low levels of selenium ($\leq$750 ppb), but it was insignificant at high levels. In other words, selenium occuring at low concentrations in the medium increased the toxicity of mercury, when organisms were exposed simultaneously to both elements.

Our results were different from those obtained by Glickstein (1978), who reported that in combination, high concentrations of selenium ($\geq$5000 ugl^{-1}) enhansed the toxicity of mercury in Crassostrea gigas embryos and Cancer magister larvae,whereas moderate levels (100 to 1000 ugl^{-1} Se) tended to decrease that toxicity. He also suggested that some form of antagonistic toxicity was occuring.

In the present study, high toxicity of mercury co-existed with selenium in the medium should be due to the synergistic effect of selenium. Burk et al.,(1974) observed a 15-fold increase in plasma mercury in rats when selenium and mercury were administered simultaneously. It seems probable that mercury could have accumulated in the plasma or in a critical site of fish larvae in the presence of selenium and therefore resulted in high toxicity.

Although different LC_{50} values were obtained for the group pretreated with selenium before exposure to $HgCl_2$ and for those not pretreated, these differences were not statistically significant. Our findings are not comparable to those of Bowers et al.,(1980). They reported that selenium pretreatment following liver formation in Japanese ricefish, Oryzias lapides, was protective against mercuric chloride. However, Sheline and Schmidt-Nielsen (1977) found that selenium pretreatment had no effect on the overall body retention of mercury in killifish, Fundulus heteroclitus. Several explanations have been given for the mecanism by which selenium protects against the toxicity of mercury. Burk et al., (1974) postulated that selenium was attached to the -SH groups on the protein and that mercury was attached to the selenium. This protein may play a role in preventing acute inorganic mercury toxicity by preventing a large part of the mercury dose from reaching target tissues. Sumino et al.,(1977) showed that selenium, in the form of selenite, can release methylmercury from protein linkages in various tissues and thereby influence its tissue redistribution. In the present experiment, the selenium pretreatment lasted for 24 hr. This period might have been too short for the attachment of selenium to proteins and to prevent the mercury from attching to these binding sites.

Selenium alone was not as toxic as mercury. Its 48 hr LC_{50} value was 6.16 $\pm$ 1.08 ppm. Sato et al.,(1980) found that the TL_m value for 48hr was 50 ppm in fish, Cyprinus carpio, showing that the toxic concentration of selenium was higher, which is at ppm level, than that found for mercury.

CONCLUSION

The 48 hr LC_{50} values show that mercury is more toxic than selenium for fish larvae. Some form of synergistic effect of selenium on the acute toxicity of mercury was observed when the organisms were exposed simultaneously to both toxicants. This synergistic effect is significant at low selenium concentrations. The LC_{50} values obtained for the larvae pretreated with different selenium concentrations are slightly higher than those obtained for organisms that were not pretreated. Selenium alone is less toxic than mercury.

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