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International Council for  
the Exploration of the Sea

C.M. 1971/K:16  
Shellfish and Benthos Committee  
Ref.: E(Fish. Improve. Cttee)

The acute toxicity of heavy metals  
to the larvae of some marine animals

by

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#### SUMMARY

The toxicity of copper, mercury and zinc to the larvae of Ostrea edulis, Crangon crangon, Carcinus maenas and Homarus gammarus is examined over a period of up to 70 hours. Mercury was found to be more toxic than copper and zinc which had similar levels of toxicity. Over the experimental period, the relationship between toxicity and concentration was linear. Larvae were from 14 to 1000 times more susceptible than adults of the same species. The median lethal concentrations ( $LC_{50}$ ) of each metal to the most sensitive species of larvae, tested over a 48 hour period, exceeded the concentrations found in natural sea water by a factor of 100. For longer test periods, the  $LC_{50}$  would be considerably less and this factor would then be considerably reduced. Hence the continued addition of these metals to confined waters should give cause for concern.

#### INTRODUCTION

Corner and Sparrow (1956) showed that several species of small marine crustaceans are very susceptible to poisoning by heavy metals, and Wisely and Blick (1967) showed that certain invertebrates are even more sensitive. However, little is known about the relative sensitivity of larvae and adults of the same species. Portmann (1968) at this laboratory determined the toxicity of certain heavy metals to several marine species, and since the larvae of these species were available, an ideal opportunity to study the relative sensitivity of larvae and adults was presented.

#### METHOD

Larvae of several marine species (Ostrea edulis, Crangon crangon, Carcinus maenas and Homarus gammarus) were obtained from laboratory tanks in which stocks of adults were held for toxicity experiments. These tanks were orientated in such a way that daylight fell on one side only;

healthy active larvae were pipetted from the tank as they swam towards the light. All larvae were from 1-3 days old at the start of the experiment.

Experiments were carried out in a series of tanks made of opal "Perspex" measuring 5 x 5 x 2.5 cm deep. Toxin was added to sea water in these tanks by a micro-pipette and after stirring to ensure complete mixing, larvae were introduced to each tank - about 50 per tank for Ostrea, 10 per tank for Carcinus and Crangon, and 5 per tank for Homarus. The tanks were maintained at 15°C for up to 70 hours in a constant temperature room throughout the experiment and were not aerated, since the large surface area/depth ratio ensured sufficient aeration by diffusion. The larvae were not fed during the experiment but some food organisms were probably present in the water. Larvae in the control tanks survived the experimental period in an apparently healthy condition. The effect of the toxin was determined by examination of the larvae in the tanks at intervals throughout the experiment; any which failed to respond when touched with a "Perspex" needle were considered to be dead and were removed. The number of dead larvae found at each inspection was recorded.

## RESULTS

The distribution of the survival times of the larvae was found to be log-normal and was analysed by means of Litchfield's (1949) solution of the time-response curve. The method, briefly, is as follows:

The cumulative percentage mortality for each concentration is plotted against time on log-probability paper, and a straight line is drawn through the points by eye. The median effective time ( $ET_{50}$ ) is obtained from the 50 per cent intercept, and values for  $ET_{16}$  and  $ET_{84}$  are similarly obtained. From these, the slope of the line is calculated, and the nomographs printed in Litchfield's paper are used to calculate the confidence limits of the  $ET_{50}$ s.

Table 1 shows the median effective time (in hours) for the death of larvae, with 95 per cent confidence limits, for each concentration of metal. Using these data, survival/concentration curves are drawn for each toxin on logarithmic scales by plotting each  $ET_{50}$  against its respective concentration (Figs. 1, 2 and 3). From these figures the concentrations of toxin killing 50 per cent of larvae in 48 hours ( $LC_{50}$ ) have been obtained. The  $LC_{50}$  values for adults of the species tested were obtained from Portmann (1968). Comparison of  $LC_{50}$  values for larvae and adults is made in Table 2.

## DISCUSSION

Over the period of time of the experiments (20-70 hours) the relationship between toxicity (as measured by the  $EC_{50}$ ) and concentration was linear for all species of larvae with all metals. Of these, mercury was more toxic than copper in all cases, with zinc having a toxicity similar to copper. Crangon larvae were more sensitive than Carcinus larvae.

Comparison of the relative sensitivity of adults and larvae after 48 hours' exposure showed larvae in all cases to be considerably more sensitive, the toxicity ranging from 14 times greater (for zinc and Carcinus) to over 1000 times greater (for mercury and Ostrea). Thus where the toxicity of industrial wastes is being assessed by means of a determination of the 48 hour  $LC_{50}$  for adult species, some caution must be shown when using the results to predict their effect on larval stages.

The accepted representative values (ARVs) for the concentrations of mercury, copper and zinc in sea water (Goldberg 1963) are shown in Table 1. All these values are approximately 1/100 of the values of the 48 hour  $LC_{50}$ s of the most sensitive species of larvae to each metal (Crangon and Homarus to copper, 48 hour  $LC_{50}$  c. 0.33 ppm; Ostrea to mercury, 48 hour  $LC_{50}$  c. 0.0033 ppm; Carcinus to zinc, 48 hour  $LC_{50}$  1.0 ppm).

The recent work reported by Wilson and Connor (1971) shows a curvilinear relationship between toxicity and concentration for adult Crangon with mercury, which only became apparent after 100 hours' exposure. Although this suggests a threshold of tolerance, the 1000 hour  $LC_{50}$  for adults (the approximate larval life of Crangon is 1000 hours) is less than 1/100 of that for 48 hours. If a similar relationship exists with the larvae of this and other species, i.e. the  $LC_{50}$  for 1000 hours' exposure is less than 1/100 of that for 48 hours' exposure, then the 1000 hour  $LC_{50}$ s of larvae would be very similar to the concentrations found in the sea.

Although more work is required in this field to make accurate assessments of the long-term effects of heavy metals on marine life, it can be concluded that the continued addition of these metals to restricted waters which do not provide adequate dilution must give cause for concern.

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Table 1 The median effective time (ET<sub>50</sub>) for the death of larvae of each species with each metal, showing 95 per cent confidence limits. Concentration of metal in natural sea water (accepted representative value) in ppm: copper, 0.003; mercury, 0.00003; zinc, 0.01

Species	Copper as CuSO <sub>4</sub>			Mercury as HgCl <sub>2</sub>			Zinc as ZnSO <sub>4</sub>		
	Conc. (ppm)	ET <sub>50</sub> (hours)	95 per cent confidence limits	Conc. (ppm)	ET <sub>50</sub> (hours)	95 per cent confidence limits	Conc. (ppm)	ET <sub>50</sub> (hours)	95 per cent confidence limits
<u>Carcinus naenas</u>	0.1	35	33.2 - 36.9	0.0033	70	44.3 - 110.6	1.0	47	27.3 - 80.8
	0.33	6.6	5.4 - 8.1	0.01	47	32.4 - 68.2	3.3	8	7.7 - 8.3
	1.0	6.6	5.4 - 8.1	0.033	22.5	20.0 - 25.2	10	1.1	1.0 - 1.2
	3.3	5	4.0 - 6.3	0.033	20.5	17.7 - 23.8	33	0.22	0.19 - 0.25
	10	1.9	1.7 - 2.1	0.033	30	28.8 - 31.2	100	0.22	0.19 - 0.25
				0.1	13.5	12.4 - 14.6			
				0.1	4.3	3.4 - 5.4			
				0.33	10	8.7 - 11.5			
				1.0	2.7	2.6 - 2.8			
				3.3	0.55	0.5 - 0.61			
			10	0.22	0.19 - 0.25				
<u>Crangon crangon</u>	0.33	54	42.9 - 68	0.033	19	17.0 - 21.3			
	1.0	29	23.0 - 36.5	0.033	21	20.4 - 21.6			
	1.0	7	6.3 - 7.8	0.1	16.5	15.6 - 17.5			
	3.3	2.8	2.6 - 3.0	0.1	12	10.7 - 13.4			
	10	0.83	0.76 - 0.90	0.33	5.1	4.6 - 5.6			
	33	0.22	0.19 - 0.25	1.0	2.3	2.1 - 2.5			
				3.3	1.5	1.44 - 1.56			
<u>Homarus gammarus</u>	0.33	24.0	23.4 - 24.7	0.1	22.5	20.3 - 25			
	1.0	4.3	3.3 - 5.6	0.1	22.5	19.7 - 25.7			
	1.0	4.0	3.1 - 5.2	0.33	4.0	3.3 - 4.9			
<u>Ostrea edulis</u>				0.0033	4.2	3.9 - 4.5			

Table 2 Comparison of 48 hour LC<sub>50</sub>s (ppm) of larvae and adults

Metal	<u>Carcinus maenas</u>			<u>Crangon crangon</u>			<u>Hemarus</u> <u>gammarus</u>	<u>Ostrea edulis</u>		
	Adult	Larvae	<u>Adult</u> <u>Larvae</u>	Adult	Larvae	<u>Adult</u> <u>Larvae</u>	Larvae	Adult	Larvae	<u>Adult</u> <u>Larvae</u>
Mercury	1.2	0.014	85.7	5.7	0.01	570	0.033-0.1	4.2	0.001-0.0033	4200-1272
Copper	109	0.6	181.7	29.5	0.33	89.4	0.1 -0.33			
Zinc	14.5	1.0	14.5							

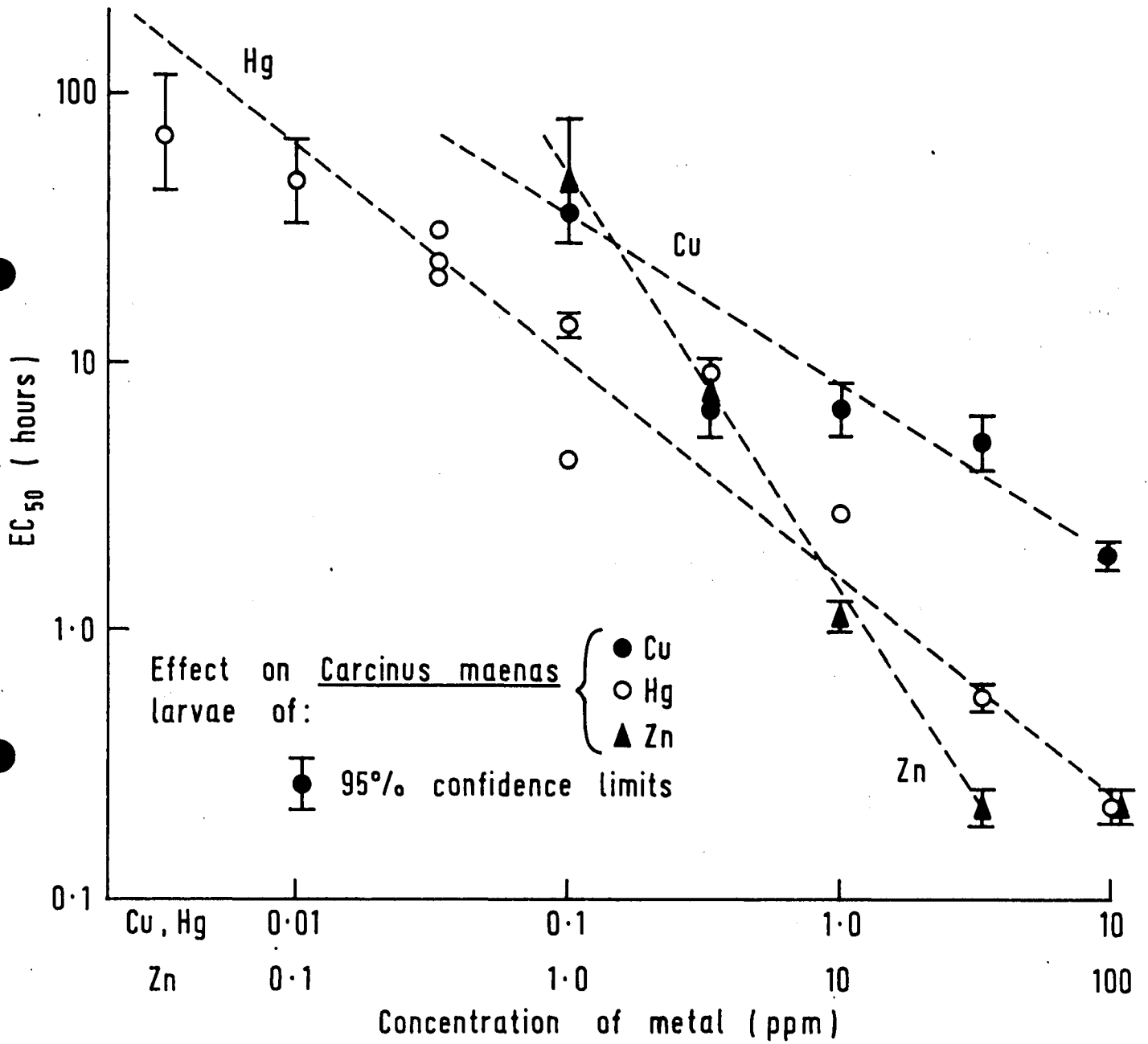


Figure 1

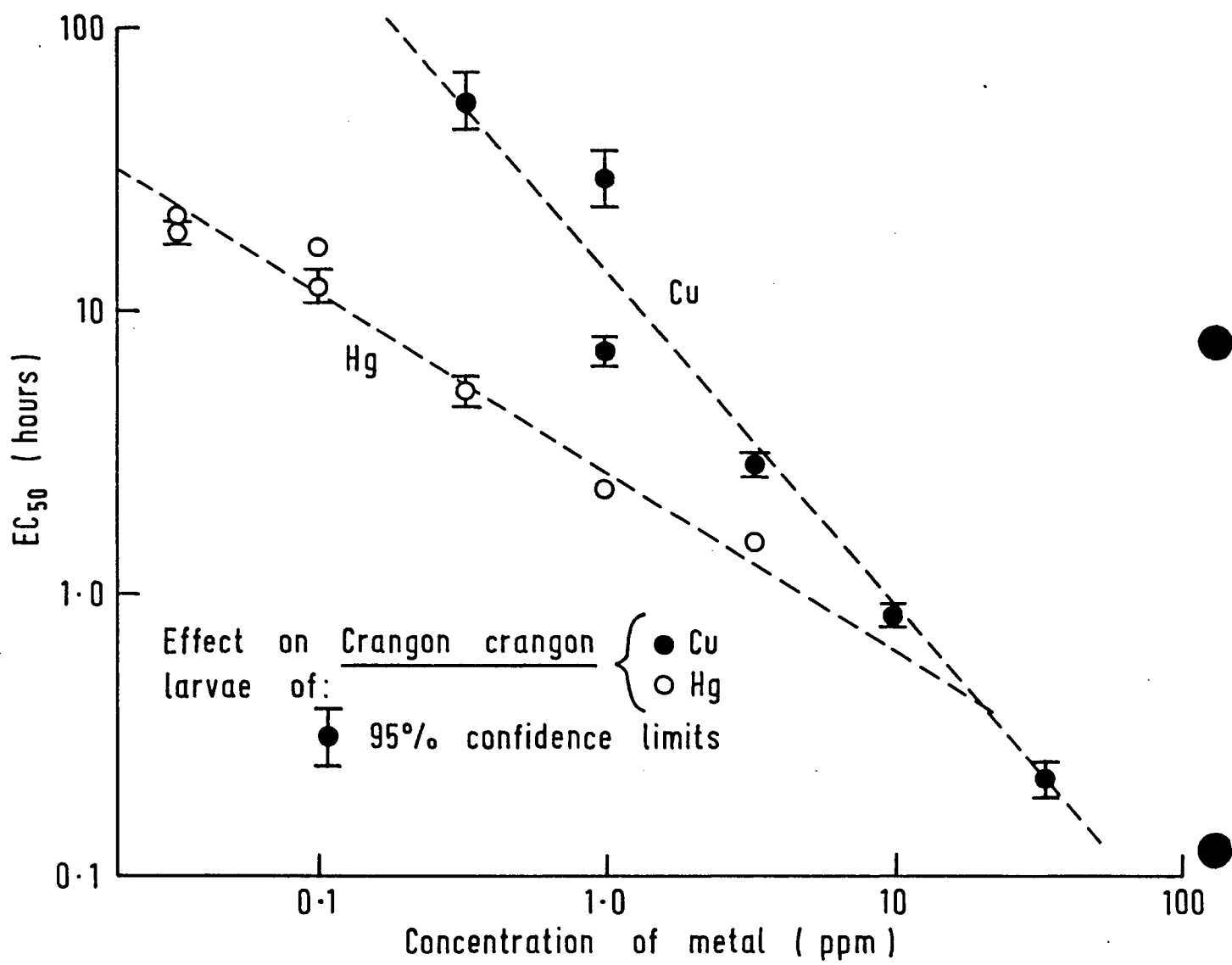


Figure 2



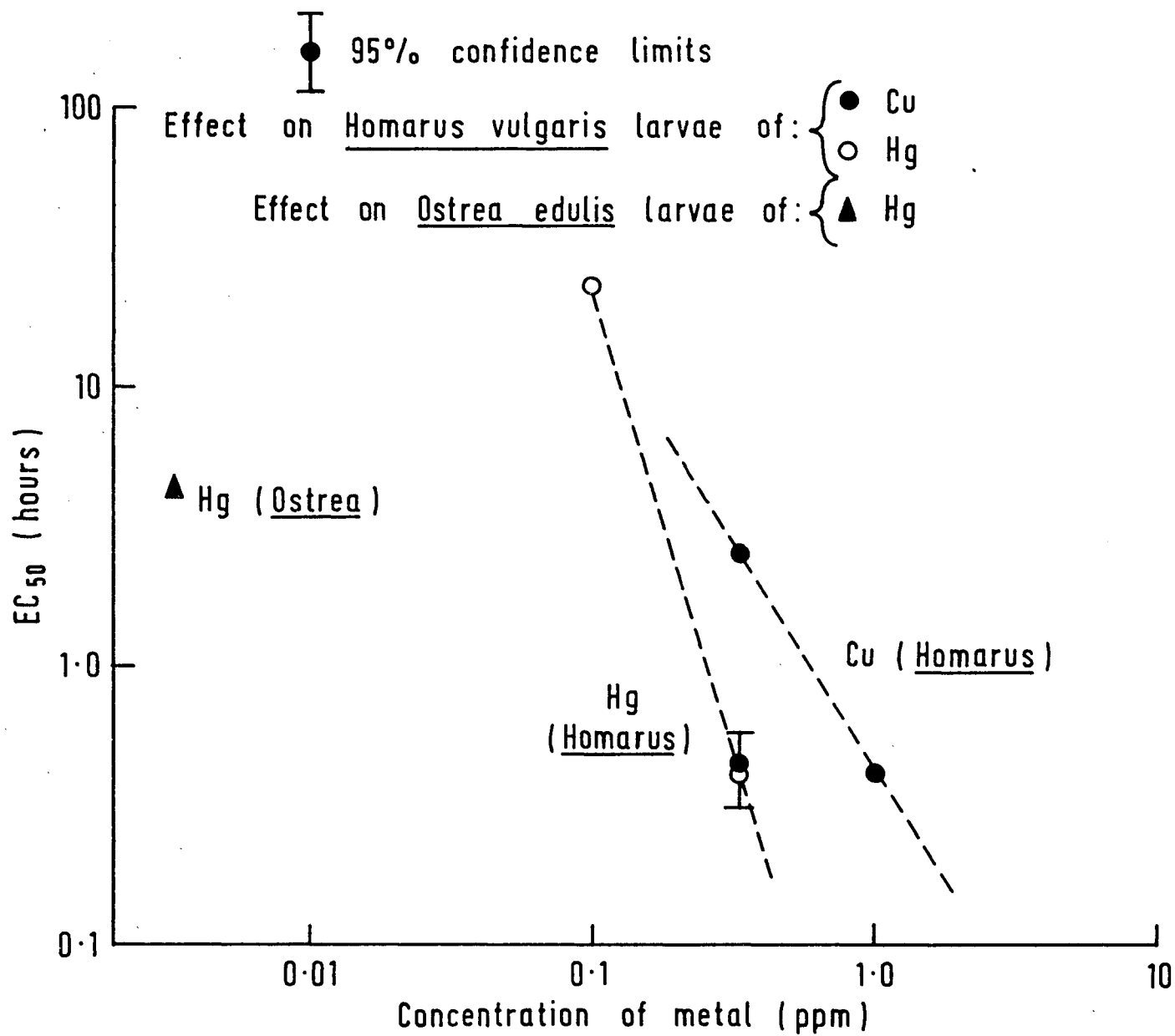


Figure 3