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ACUTE TOXICITY OF FOUR HEAVY METALS TO *SPHAEROMA WALKERI* AND *CIROLANA BOVINA* (CRUSTACEA:ISOPODA)

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ABSTRACT: The median lethal concentrations (LC50) of two Isopoda species exposed to each tested metal (Cu, Co, Cd and Zn) in static tests for different exposure periods are quite variable depending on the tested metal. The LC50 values for *Sphaeroma walkeri* after 24 hours exposure to Cu and Co were estimated graphically to be 11.20 and 7.00 mg/l respectively. The correspoding values for *Cirolana bovina* exposed to Cu,Co, Cd andZn were 3.60, 11.0, 3.80 and 4.80 mg/l respectively. For 2 days the LC50 of *S.walkeri* exposed to Cd was 5.60 mg/l, but it was 10.10 mg/l for 3 days exposure to Zn. After prolonged exposure the LC50 values decreased proportionally with the exposure duration of the test. the percentages of surviving animals demonstrated a progressive decrease with increasing concentratins as a main factor from the analysis fof variance (ANOVA). The sensitivity of adult *S.walkeri* exposed to the four heavy metals for different exposure times ranked: Cd>Co>Zn>Cu. *Cirolana bovina* appeared to be more sensitive to Cu, Cd and Zn than to Co. Species in order of increasing sensitivity is *C.bovina* more than *S.walkeri.*

KEY WORDS: Isopoda, heavy metals, toxicity.

INTRODUCTION

Over the past decades a rapid increase of pollutants in Alexandria coastal waters (South-Eastern Region of Mediterranean Sea) has greatly accelerated the deposition of the heavy metals on the bottom and subsequently in aquatic organisms. These compounds "xenobiotics" are usually accumulated in organisms to levels greater than the concentration in aquatic ecosystem. Toxic xenobiotics are well known to be carcinogenic and mutagentic to animals and man (Arinc and Sen, 1992; Bolognesi *et al.,* 1992). Many studies have been carried out on the bological effects of different pollutants specially on marine organisms for ecotoxicological tests. Most of these studies dealt with phytoplankton (Unsal, 1992), zooplankton (Verriopoulos, 1992), *Artemia* (Tayel *et al.,* 1992), barnacles (El-komi and Tayel, 1992), molluscs (Pavicic *et al.,* 1992; Quiniou and Toularastel, 1992) and sea urchins (Waterman, 1937; Pagano *et al.,* 1992) to measure their response and sensitivity to variety of xenobiotics and to assess the degree of environmental contamination in the study area. Two isopod species were considered in this study because of thier abundance among the fouling assemblages in Alexandria Harbour (El-komi, 1991, 1992), it is highly significant for environmental contamination levels and constituing significant members of the marine food chain.

The present study aimed to evalute the effects of four heavy metals (Cu, Co, Cd and Zn) on the survival of two Isopoda *Sphaeroma walkeri* Stebbing and *Cirolana bovina* Barnard.

MATERIAL AND METHODS

The sampling sites were slected from Alexandria coastal waters, namely the Eastern and Western Harbours. The samples were collected among the fouling assemblages attached to submerged objects in seawater. The test animals from two isopod species namely *Sphaeroma walkeri* and *Cirolana bovina,* were isolated in large quantities in separate aquaria (ca 101) and were maintained for a week prior to the experiments to acclimatize the animals under the laboratory conditions (salinity 39‰ and temperature ranged from 20^{o} to 25^{o} C). The aquarium was aerated; the sea water was changed every two days and the animals were fed with *Ulva lactuca, Enteromorpha intestinalis, Chaetomorpha aerea* and *Cladophora perolifera* which were associated with the collected fouling assemblage.

The experiments were conducted in static (non-continuous flow) method under the following conditions:

1) the test animals were *Sphaeroma walkeri* and *Cirolana bovina.*
2) adult isopods of 4-7 mm long were used.
3) 8 - 10 individuals were placed in each container with 100 ml toxicant solution.
4) the test metals were $CuCl_2$, $Zn(NO_3)_2$, $CdCl_2.4H_2O$, and $CoCl_2$.
5) three replicates were used at each exposure concentration and for each control.
6) the concentration of the test metals ranged from 1.0 to 10.0 mg/l and
7) the times of exposures were 24 , 48, 72 and 96 hours.

After the specified time of exposure all the containers were examined to remove the dead animals and surviving isopods in the various concentrations and control were counted.

The results were analysed graphically in order to calculate the median lethal concentration value (LC50) and 95% confidence intervals for each test metal using the probit analysis methods described in Reference Methods for Marine Pollution Studies No. 43 (UNEP, 1989).The significance of the results was obtained from variance analysis (ANOVA) in test of longer duration (according to the methods described in MAP Technical Reports Series No.68, UNEP, 1992).

RESULTS AND DISCUSSION

The results (Figs. 1 and 2) reveal that the cumulative percentage mortality (CPM) of two isopod species namely *S. walkeri* and *C. bovina* is higher in media containing high concentrations of test metal. For example, the CPM after 96 hrs of *S. walkeri* in medium containning 5.6 mg/l of Cd, Co and Zn was 97%, 27% and 0% respectively. But it was 54% in 6.4 mg/l of Cu. The average CPM was 17.5% in medium containing 7.5 mg/l of tested metals, whereas the value exposed to Cd was generally high ranging from 30% to 100%. On the other hand , the results of *C. bovina* show that CPM after 96 hrs was 79%, 77%, 60% and 43% in medium containing 3.2 mg/l of Cu, Cd, Zn and Co respectively. The average was 15.7 and it was high to Cu ranging from 4% to 79% in medium containing 3.6 mg/l. Therefore, the concentration of the tested substance was about two times greater than those for *C.bovina.*

The median lethal concentration (LC50) values for *S. walkeri.* and *C. bovina* were estimated graphically to range from 3.60 to 11.20 mg/l after 24 hrs exposure to the four tested metals and are listed in Tables I and II. The effect of Cu on adults of isopod

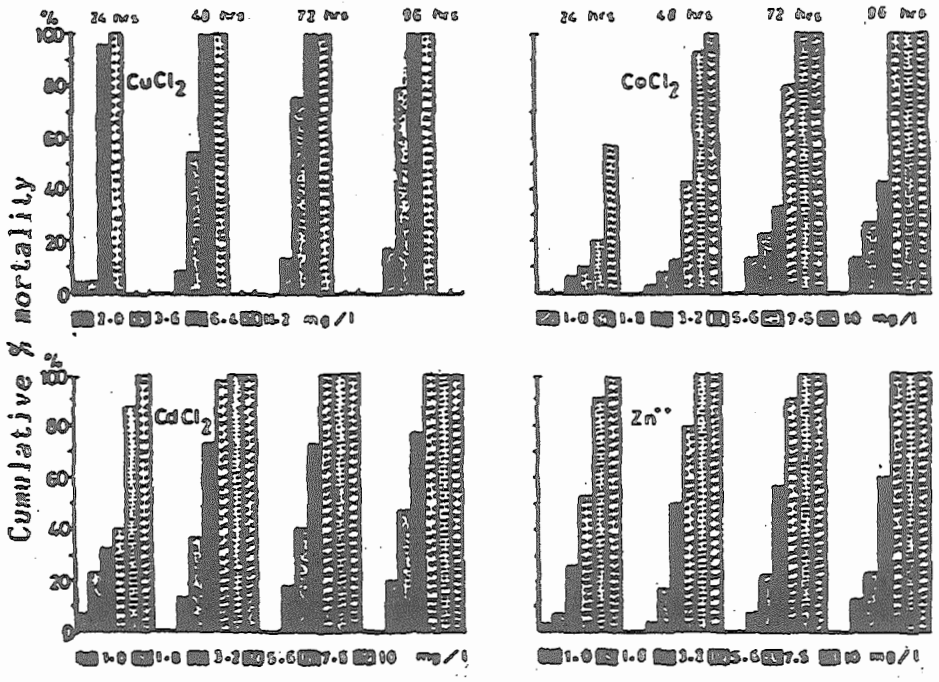

Fig. 1. The cumulative percentage mortality of *Sphaeroma walkeri* exposed to different heavy metals at various concentrations.

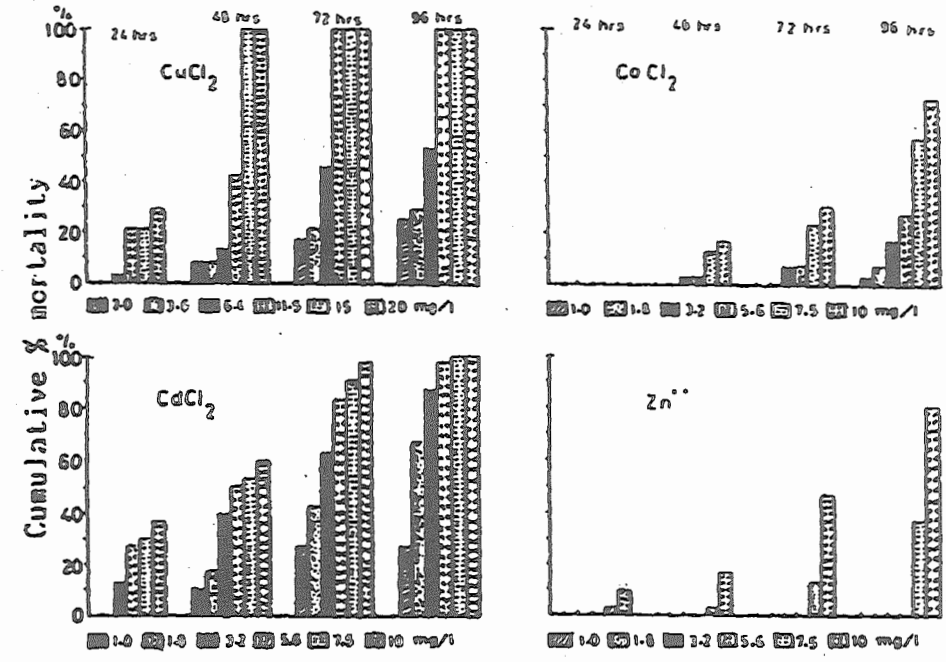

Fig. 2. The cumulative percentage mortality of *Cirolana bovina* exposed to different heavy metals at various concentrations.

TABLE I. COMARISON OF THE ESTIMATED LETHAL TOXIC EFFECT OF ISOPOD, *SPHAEROMA WALKERI* TO THE TESTED METALS

Toxicant	Duration	LC50 (hrs)	S (mg/1)	95% confidence Upper	Lower
$CdCl_2$	48	5.60	3.70	9.35	3.53
	72	2.00	2.33	3.07	1.30
	96	1.48	2.03	2.50	1.00
$CoCl_2$	24	7.00	2.56	10.51	6.64
$CuCl_2$	24	11.20	2.06	16.87	7.44
$Zn(NO_3)_2$	72	10.10*			
	96	8.00*			

* Estimated by the long-concentration versus percentage survival method. S = the slope function.

TABLE II. COMARISON OF THE ESTIMATED LETHAL TOXIC EFFECT OF ISOPOD, *CIROLANA BOVINA* TO THE TESTED METALS

Toxicant	Duration	LC50 (hrs)	S (mg/1)	95% confidence Upper	Lower
$CdCl_2$	24	3.80	2.27	5.44	2.65
	48	2.08	1.75	2.94	1.47
$CoCl_2$	24	11.00	1.93	12.54	7.80
	48	6.80	2.45	10.70	4.32
	72	2.70	1.82	3.91	1.86
$Zn(NO_3)_2$	24	4.80	2.07	7.54	3.06
	48	3.20	1.91	4.45	2.30
	72	2.70	1.84	3.94	1.85
$CuCl_2$	24	3.60*			
	48	3.50*			
	72	2.80*			

* Estimated by the long-concentration versus percentage survival method. S = the slope function.

S. walkeri produced 50% mortality, to be caused by addition of 11.20 mg/1. It was 3.60 mg/1 for *C. bovina.* Concentrations of Zn have slight effect on the adults of *S. walkeri* (10.10 mg/1) compared to *C. bovina* (4.80 mg/1). Contrarily, the LC50 values and significant levels show that the two species reacted to Cd in a similar manner, measuring 5.60 and 3.0 mg/1. The results indicated that the values of LC50 for various exposure times in general demonstrated a progressivee decrease with increasing the duration of exposures.

From the analysis of variance (ANOVA) it was shown that the concentration is the main factor affecting on the survival of animals (Tables III and IV). These results

TABLE III. COMPARISON OF DISPERSION ANALYSIS FOR THE RESULTS OF BIOASSAY ON THE *SPHAEROMA WALKERI* EXPOSED TO THE TESTED METALS

Toxicant	Source of variation	df	SS	MS	F actual	F from Table 0.01	F from Table 0.05
$CuCl_2$	Subgroups	27	796.9	29.51	-	-	-
	Between time(A)	3	196.5	65.50	198.48	2.78	4.17
	Between conc.(B)	6	495.2	82.53	250.09	2.30	3.15
	Time x conc.(AxB)	18	105.2	5.84	17.70	1.81	2.29
	Error (within group)	56	18.7	0.33	-	-	-
$CoCl_2$	Subgroups	27	261.3	9.68	-	-	-
	Between time(A)	3	80.9	26.97	56.19	2.78	4.17
	Between conc.(B)	6	101.2	16.87	35.15	2.30	3.15
	Time x conc.(AxB)	18	79.2	4.4	9.17	1.81	2.29
	Error(within group)	56	26.7	0.48	-	-	-
$CdCl_2$	Subgroups	27	1017	37.67	-	-	-
	Between time(A)	3	360.4	120.13	120.3	2.78	4.17
	Between conc.(B)	6	574.25	95.71	95.71	2.30	3.15
	Time x conc.(AxB)	18	82.35	4.58	4.38	1.81	2.29
	Error(within group)	56	56.0	1.00	-	-	-
$Zn(NO_3)_2$	Subgroups	27	267.7	9.91	-	-	-
	Between time(A)	3	28.9	9.63	14.16	2.78	4.17
	Between conc.(B)	6	153.1	25.52	37.53	2.30	3.15
	Time x conc.(AxB)	18	85.7	4.76	7.0	1.81	2.29
	Error(within group)	56	38	0.68	-	-	-

TABLE IV. COMPARISON OF DISPERSION ANALYSIS FOR THE RESULTS OF BIOASSAY ON THE *CIROLANA BOVINA* EXPOSED TO THE TESTED METALS

Toxicant	Source of variation	df	SS	MS	F actual	F from Table 0.01	F from Table 0.05
$CuCl_2$	Subgroups	15	537	35.80	-	-	-
	Between time(A)	3	29.2	9.77	6.34	2.91	4.47
	Between conc.(B)	3	460.9	153.63	99.76	2.91	4.47
	Time x conc.(AxB)	9	46.9	5.21	3.38	2.19	3.03
	Error (within group)	32	49.3	1.54	-	-	-
$CoCl_2$	Subgroups	27	1302.1	48.23	-	-	-
	Between time(A)	3	216.6	72.20	92.56	2.78	4.17
	Between conc.(B)	6	934.5	155.75	199.68	2.30	3.15
	Time x conc.(AxB)	18	151	8.39	10.76	1.81	2.29
	Error(within group)	56	42.7	0.76	-	-	-
$CdCl_2$	Subgroups	23	1004.6	43.68	-	-	-
	Between time(A)	3	80.5	26.83	9.25	2.81	4.24
	Between conc.(B)	5	871.6	174.32	60.11	2.42	3.44
	Time x conc.(AxB)	15	52.5	3.50	1.21	1.84	2.36
	Error(within group)	48	139.3	2.90	-	-	-
$Zn(NO_3)_2$	Subgroups	23	1055.4	45.89	-	-	-
	Between time(A)	3	39	13	11.50	2.81	4.24
	Between conc.(B)	5	989	197.8	175.04	2.42	3.44
	Time x conc.(AxB)	15	27.4	1.83	1.62	1.84	2.36
	Error(within group)	48	54.3	1.13	-	-	-

TABLE V. COMPARISON OF VALUES OF LEAST SIGNIFICANT RANGE "LSR2 OF *SPHAEROMA WALKERI*

Toxicant	MS within group	Q 0.05	Arrange mean of no. of survival in ascending order	LSR
			Conc. 2 3.6 6.4 11 15 20	
$CuCl_2$	0.33	4.2	24 21 20.5 17 8.3 7.3 4.3	0.13
			Conc. 1.0 1.8 3.2 5.6 7.5 10	
$CoCl_2$	0.48	4.2	30 29.8 29.5 28 27.3 23 21	0.05
			Conc. 1.0 1.8 3.2 5.6 7.5 10	
$CdCl_2$	1.00	4.2	30 25.3 20.5 14.8 10.8 9.5 8	0.35
			Conc. 1.0 1.8 3.2 5.6 7.5 10	
$Zn(NO_3)_2$	0.68	4.2	30 30 30 30 30 25.8 18.5	0.24

TABLE VI. COMPARISON OF VALUES OF LEAST SIGNIFICANT RANGE "LSR" OF Cirolana bovina

Toxicant	MS within group	Q 0.05	Arrange mean of no. of survival in ascending order	LSR
			Conc. 2 3.6 6.4	
$CuCl_2$	1.54	3.5	23.8 21.3 11 0.3	0.45
			Conc. 1.0 1.8 3.2 5.6 7.5 10	
$CoCl_2$	10.76	4.2	30 27.8 25.8 22.8 12.5 6.5 3.3	0.27
			Conc. 1.0 1.8 3.2 5.6 7.5	
$CdCl_2$	2.9	4.0	30 25.8 19 10.8 4.8 1.0	0.97
			Conc. 1.0 1.8 3.2 5.6 7.5	
$Zn(NO_3)_2$	1.13	4.0	30 28 24.8 15.5 5.8 0.8	0.38

obtained for *S. walkeri* shows that all test concentrations gave significant different results compared to those of the control. According to Gotsis-Skretas and Christaki, (1992) and El-komi and Tayel, (1992) the concentration of pollutants was the main factor affecting on the growth of phytoplankton and on survival of barnacles. This difference was due to the stimulating effect of metals and their lethal effects at various concentrations. The acute lethal concentrations of the four tested metals differ more significantly between concentration than between the duration of exposures, for both species because the calculated value of F between concentrations is higher than the tabular value for 0.01. However, effect of concentration increases with time of exposure to a limit point as was reported by Gotsis-Skretas and Christaki, (1992). This was more pronounced in the culture containing Cu, where the reduction of metal concentration in the medium is due to consumption by the test organisms and by precipitation. The test of variance analysis does not, however, tell which mean is significantly different. The Least Significant Range "LSR" test would give this result.

As shown from Tables V and VI all the differences between means of number of survivors in various concentrations are greater than the calculated significant levels LSR, therefore, all these values are significantly different.

$$LSR = Q\,0.05 \underset{df}{} \times MS / n$$

Where Q. is a factor from tables called Studentized Range Table; df. degree of freedom; n. number of observations within group; MS. mean of squares.

In the present experiments, the sensitivity to the four heavy metals seemed more pronounced in the order : *Cirolana* > *Sphaeroma.* The toxicity effects of metallic salts for different exposure durations was high in the following order : Cd > Co > Zn > Cu for *Sphaeroma.* Where as *Cirolana* appeared to be more sensitive to Cu, Cd and Zn than Co.

It is difficult to make direct comparisons with the previous studies dealing with the biological effects of different pollutants because of differences in the organisms tested and the experimental conditions. Waterman, (1937) found that the toxicity of metallic salts on the development of sea urchin was indicated by following order, $HgCl_2 > CuCl_2 > ZnCl_2$, $ZnSO_4$, $Zn(C_2H_3O_2)_2 > AlCl_6$, $CdCl_2$, $NiCl_2 > FeCl_2$. The effect of heavy metals have also been reported by Kobayashi (1984) upon the fertilization and development of sea urchin eggs, which the effect of metals decreased in the following order : Hg , Cu , Zn , Ni , Cd , Pb , Cr , Mn and Co. Verriopoulos (1992) reported that the effects of sublethal concentrations of Zn , Cr , and Cu on the mortality of marine copepod *Tisbe holothuriae* and *Acartia clausi,* fed with contaminated food, increased proportionally with the exposure concentration. Further studies must be performed in comparing the effects of toxic xenobiotics on developmental stages, growth, longevity, breeding and moulting frequencies of marine isopods.

REFERENCES

Arinc, E., Sen, A. 1992. Induction of liver 7-ethoxyresourfin o-deethylase in gilthead seabream by benzo(A) pyrene and its potnial use in biochemical monitoring of environmental pollutants. *Proceedings of the FAO/UNEP/IOC Workshops on the Biological of Pollutants on Marine Organisms (Malta,10-14 September,1991), MAP Technical Reportes Series No. 69,* UNEP, Athens. Pp. 73-89.

Bolognesi, C., Parrini, M., Rogieri, P., Ercolini, C., Pellegrina, C. 1992. Carcinogenic and mutagenic pollutants: impact on marine organisms.*Proceedings of the FAO/UNEP/IOC Workshops on the Biological of Pollutants on Marine Organisms (Malta, 10-14 September, 1991), MAP Technical Reportes Series No. 69,* UNEP Athens. Pp.113-121.

El-Komi, M. M. 1991. Incidence and ecology of fouling organisms in the Eastern Harbour of Alexandria, Egypt. *Marine Pollution Bulletin,23:*289-298.

El-Komi, M. M. 1992. Field and laboratory studies on the ecology of marine fouling in Alexandria Harbour, Egypt. *Bulletin of the Institute of Oceangraphy and Fisheries 18:*115-140.

El-Komi, M. M., Tayel, F. T. 1992. Influence of organophosphorus herbicides on barnacles (Crustacea:Cirripedia). *Proceedings of the Second International Confrence on Environmental Protection Is. A Must, 24-27 February 1992, Egypt, in Co-operation with USPD.* Pp. 173-181.

Gotsis-Skretas, O., Christaki, U. (1992). Physiological responses of two marine phytoplanktonic species to copper and mercury. *Proceedings of the FAO/UNEP/IOC Workshops on the Biological of Pollutants on Marine Organisms (Malta, 10-14 September, 1991) , MAP Technical Reports Series No. 69, UNEP Athens.* Pp. 151-164.

Kobayashi, N. (1984). Marine ecotoxicology testing with echinoderm. In: G.Persoone, E. Jaspers and C. Claus, eds., *Ecotoxicological testing for the Marine environment,* 1:341-405. State University of Ghent and Inst. Mar. Sci. Res.,Bredene, Belgium.

Lurie, Ju, U. (1975). Handbook of analytical chemistry. *English Translation Mir Publisher.* Pp. 488.

Pagano, G., Esposito, A., Guida, M., Melluso, G., Trieff, N. M. (1992). Sublethal toxicity testing in sea urchin fertilization and embryogensis: a study of polluted water and sediment from two rivers in Campania, Italy. *Proceedings of the FAO/UNEP/IOC Workshops on the Biological of Pollutants on Marine Organisms (Malta, 10-14 September, 1991), MAPTechnical Reportes Series No. 69* UNEP, Athens. Pp. 209-215.

Pavicic, J., Raspor, B., Branica, M. (1992). Metal binding proteins of *Mytilus galloprovincialis,* similar to metallothioneins, as a potential indicator of metal pollution. *Ibid. :* 217-234.

Quiniou, F., Toularastel, F. (1992). Biological effects of contaminated water tested by marine bivalve embryobioassay. *Ibid. :* 245-254.

Tayel, F.T., El-Komi, M. M., El-Shenawy, M. A. (1992). Acute toxicity of six organophosphorus herbicides tested by nauplii of brine shrimp, *Artemia* sp.. *Bulletin High Institute of Public Health, 22(1)* :173-183.

UNEP, 1989. Test of the acute lethal toxicity of pollutants to marine fish and invertebrates. *Reference methods for marine pollution studies 43* : 27p.

UNEP/FAO/IOC (1992). Evaluation of the Trianing Workshops on the Statistical Treatment and Interpretation of Marine Community Data. *MAP Technical Reports 68* , UNEP, Athens: 221 p.

Unsal, M. (1992). Effects of herbicides on the growth of marine phytoplankton. *Proceedings of the FAO/UNEP/IOC Workshops on the Biological of Pollutants on Marine Organisms (Malta, 10-14 September, 1991), MAP Technical Reports Series 69* UNEP, Athens. Pp. 255-264.

Verriopoulos, G. (1992). Effects of sublethal concentrations of zinc, chromium and copper on the marine copepods *Tisbe holothuria* and *Acartia clausi. Ibid.* Pp. 265-275.

Waterman, A. J. (1937). Effects of salts of heavy metals on development of the sea urchin, *Arbacia punctulata. Biological Bulletin* 73:401-420.

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