HEAVY METAL CONCENTRATIONS IN MARINE ORGANISMS FROM THE MEDITERRANEAN SEA (VALENCIA-CASTELLON, SPAIN)

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Total concentrations of Hg, Cd, Cr and Pb have been determined in marine organisms from Vinaroz, Castellón, Burriana, Sagunto, Valencia, Cullera and Gandía.

This work is a part of the Mediterranean Pollution Monitoring Programme, MEDPOL, which has been carried out during the months of July, October and November of 1985.

The samples were stored in a freezer at -22°C up to the moment of preparation and analyses. The organisms were classified, weighed, and their lenght measured; and the different tissues were separated, lyophilised and homogenised for use in the analyses. The material used was at all times made of Pyrex and polyethylene, and was washed in HNO_3 and rinsed with twice distilled water.

The digestion of the different tissues was carried out in teflon-lined high-pressure decomposition vessels, with 3-5 ml of conc HNO_3 (65%) per 0.3-0.5 g of lyopilised sample, at 135°C for one hour. The solutions were cooled and diluted with twice distilled water to 15 ml.

The AAS determination of total Hg content was carried out by the Cold Vapour Technique after reduction to Hg° with $SnCl_2$. Cadmium, chromium and lead were analysed by graphite furnace AAS with deuterium or Zeeman background correction, and the standard additions method was used.

The precision is about 4% for Hg, and 15% by graphite furnace. The accuracy was determined by means of samples for intercalibration. The values of accuracy were similar to the precision ones.

There were no significant variations according to the time of the year, and results obtained were generally of the same order in the different stations studied.

In Table 1 are shown the average values (in ng/g fresh weight) for each organism and tissue analysed.

Highest Hg concentrations were found in crustaceans and in fishes; levels of Cd were higher in crustaceans and in Tunnus thynus. The highest values of Cr and Pb were obtained in moluscs and also in crustaceans (specially for Cr).

Respect to the tissues analysed, the order of heavy metal concentrations was: muscle < digestive < liver , which shows a degree of metal accumulation, that in certain cases was very important.

No significative differences were observed with respect to the sex of animals.

Table 1.- Average values (in ng/g F.W.) of heavy metals in marine organisms

ORGANISM	N	I	TISSUE	Hg	Cd	Cr	Pb
Mytilus galloprovincialis	18	54	b	31.5	68.7	363	997
Venus gallina	7	68	b	21.5	65.1	267	119
Donax vittatus	7	126	b	19.4	3.9	353	132
Macropipus depurator (M)	13	10	b	334	115	660	84.1
Macropipus depurator (F)	13	11	b	329	116	467	92.4
Aristeus antennatus	1	6	m	652	51.2	103	107
			d	959	746	4941	5687
			l	1067	2088	446	222
			g	374	290	139	74.5
Palaemon serratus (M)	1	8	m	71.0	81.0	379	6.8
Palaemon serratus (F)	2	13	m	284	42.5	226	55.7
Sardina pilchardus	17	8	m	179	45.9	156	62.7
Mullus barbatus (M)	4	6	m	231	16.6	210	37.2
	4	6	d	323	62.5	279	461
	4	6	l	317	86.6	813	365
Mullus barbatus (F)	2	3	m	273	17.9	181	116
	2	3	d	342	63.1	342	1604
	2	3	l	421	109	431	3905
Mullus surmuletus (M)	14	3	m	116	8.4	42.5	99.6
	7	3	d	157	59.0	341	475
	7	3	l	212	207	274	1191
Mullus surmuletus (F)	6	2	m	153	18.8	109	41.8
	6	2	d	262	70.0	518	1095
	6	2	l	223	110	336	953
Tunnus thynus (F)	5	1	m	468	281	152	127
	4	1	d	363	235	363	499
	4	1	l	560	357	295	719
	4	1	g	238	20.5	314	114

N= number of samples; I= mean number of individuals for sample

b= whole body; m= muscle; d=digestive except liver; l=liver; g=gonads

EFFECTS OF MERCURY ON CHLORIDE FLUXES AND TRANSBRANCHIAL POTENTIALS IN PERFUSED GILLS OF CARCINUS

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In recent years a considerable effort has been directed into the development and improvement of toxicological test methods and increase understanding of mechanisms of the interaction of toxic substances in the marine organisms. Although extensive work has been done on the toxicity of mercury and its compounds, very little is known about their physiological effects on particular processes. Therefore, we studied the effect of two mercurial compounds -$HgCl_2$ and CH_3HgCl on chloride fluxes and TBP (transbranchial potentials) of the isolated perfused gills of the crustacean Carcinus mediterraneus.

The gills were perfused with diluted sea water (DSW; 460 mOsmol/l, 239 mM Cl^-) in solution identical to the external bathing solution. TBP values were measured by Keithley Instr. 601 Electrometer with Ag-AgCl Ingold electrodes. Chloride fluxes were traced by radioactive ^{36}Cl. Detailed methodological description was addressed by Lucu and Siebers (in press). Methyl mercury was dissolved in aceton and mercuric chloride in distilled water. In previous experiments aceton was added to the control solution (2 µl/50 ml) and no effect on TBP and chloride fluxes was observed. Under the control condition the TBP values were stable for several hours in the range from -3 to -4 mV (negative polarity referring to the basolateral side).

After addition of 5 µg Hg^{2+} (CH_3HgCl form) on the basolateral membrane side, the TBP values were increased from -3.5 mV to a value close to zero. However, 10 times higher Hg^{2+} concentration of $HgCl_2$ effected similar changes of the TBP values as in the case of gills treated with the organic mercury compound (Table 1.). The TBP has been described as an active potential generated by unequal distribution of ions such as Na^+ and Cl^- as a consequence of the active transport processes (Siebers et al., 1985). The effect of Cu^{2+} on positively charged potential (polarity in reference to the perfusion side) of the similar magnitude and reversed polarity compared with our results, has been described in the gills of sea water acclimated flounders (Stagg and Shuttleworth,1982).

Both mercurial compounds inhibited chloride influxes and the values were 57 to 64% of the control (Table 1.).

TREATMENT	CHLORIDE INFLUXES ($J_{Cl_{a\rightarrow b}}$; µM g^{-1} h^{-1})	TBP (mV)
Control	245 ∓ 64	-3.6 ∓ 0.6
$HgCl_2$ added; 50 µg Hg^{2+}/l	139 ∓ 43	-0.8 ∓ 1.1
CH_3HgCl added; 5 µg Hg^{2+}/l	158 ∓ 48	+0.3 ∓ 0.9

TABLE 1. Effect of mercury perfused from the basolateral side of the isolated Carcinus mediterraneus gill preparation on chloride fluxes ($J_{a\rightarrow b}$= flux from apical (a) to the basolateral (b) side, and transbranchial potentials. The perfusion solution was diluted sea water (460 mOsmol/l) identical to the external bathing solution. The values are given as the means of five observations.

At the physiological pH, membrane permeability of methyl mercury is higher than that of inorganic $HgCl_2$ (Gutknecht,1981), and that could be explanation for the more severe effects of the methyl mercury which we have demonstrated at a concentration of one magnitude lower than in the case of $HgCl_2$. Chlorides were also inhibited on the basolateral perfusion side by specific inhibitors such as furosemide and ouabain (Lucu and Siebers, in preparation). We assume that Na/K coupled Cl^- absorption is secondary active transport, whereby energy for Cl^- transport is apparently provided by counter ion. Therefore, Na/K exchange, sensitive to ouabain and in our case damaged by mercurial compounds, and consequently the changes in K fluxes, may affect the hypothetical KCl symport located on the basolateral membrane side.

Further investigations using this technique could provide us with knowledge about mechanisms of mercury interaction with ionic regulatory processes in the marine organisms.

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