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Mutagenicity of Heavy Metals

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Since Ames and his colleagues (Ames et al. 1973) introduced the bacterial system to detect mutagens and carcinogens, a large number of environmental pollutants such as organic compounds (McCann et al. 1975), dyes (McCann et al. 1975) as well as pesticides (Waters et al. 1981) were found to be mutagenic or carcinogenic by using this Salmonella/mammalian-microsome mutagenicity tests (Ames et al. 1975).

Certain heavy metals are required, as trace elements for normal cellular functions. However, heavy metals are toxic to cells once their levels exceed their low physiological values. The toxicity of heavy metals on microorganisms (Gadd and Griffith 1978), on plants (Ichikura et al. 1970), and on animals (Eisler and Hennekey 1977) has been well-documented. Eichhorn (1975) described the binding of heavy metals to the phosphate, deoxyribose and heterocyclic base residues of DNA. These interactions may induce the alteration of the primary as well as secondary structures of the DNA and result in mutation(s).

Though the rec assay with Bacillus subtilis and the reversion assay with Escherichia coli were used to assess the mutagenicity of some heavy metals (Green et al. 1976, Nishioka, 1975), the present communication reports the results in determining the mutagenicity and carcinogenicity of ten heavy metals commonly found in polluted areas by using the Salmonella/mammalian-microsome mutagenicity test.

MATERIALS AND METHODS

Heavy metal stock solutions (10 parts per thousand, ppt)

were prepared by dissolving chloride salts of different heavy metals in distilled water containing 0.1 mM citric acid and sterilized by autoclaving.

Nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G-6-P), L-histidine, D-biotin and 2-aminofluorene were purchased from Sigma Chemical Co. USA. Bacterial media (except nutrient broth no.2 from Oxoid Co. USA) were purchased from Difco Co. USA. Other chemicals used in this study were of reagent grade.

Salmonella typhimurium TA98, TA102, TA1535, and TA1537 tester strains were kindly provided by Dr. Bruce Ames (Department of Biochemistry, University of California at Berkeley). The liquid culture, storage and preparing the bacterial cultures for mutagenicity tests were carried out according to Maron and Ames (1983).

The toxicity of heavy metals on the growth of the tester strains were determined by growing the bacterial cells in VBE medium (Vogel and Bonner 1956) supplemented with 0.4% glucose, 0.25 mM L-histidine and 3 μM D-biotin in the presence of different concentrations (in parts per million, ppm) of heavy metals. The growth of the bacteria cells was monitored by a Klett-Summerson photoelectric colorimeter with a red filter (wavelength of 640-700 nm). The 50% toxic doses (the concentrations of heavy metals that exhibit a 50% inhibition in growth) and 90% toxic doses (the concentrations of heavy metals that exhibit a 90% inhibition in growth) to the tester strains were obtained from these growth inhibition experiments.

The plate-incorporation method was used for the mutagenicity test (Maron and Ames 1983). The concentrations of heavy metals used in the mutagenicity tests were those between the 50% toxic dose and 90% toxic dose determined in the growth inhibition experiments. S9 preparation and the mutagenicity tests in the presence of S9 were performed as described (Lin et al. 1986). The metabolic activity of S9-mix was determined by assessing the activation of the mutagenicity of 2-aminofluorene to the tester strains (Maron and Ames 1983).

RESULTS AND DISCUSSION

Figure 1 shows the toxicity of different concentrations of cadmium (0 to 120 ppm) to the tester strain TA98. The 50% toxic dose of cadmium to strain TA98 was 20 ppm, and growth of strain TA98 was about 90% inhibited by 120 ppm cadmium (this was 90% toxic dose and the high concentration of cadmium used in the mutagenicity

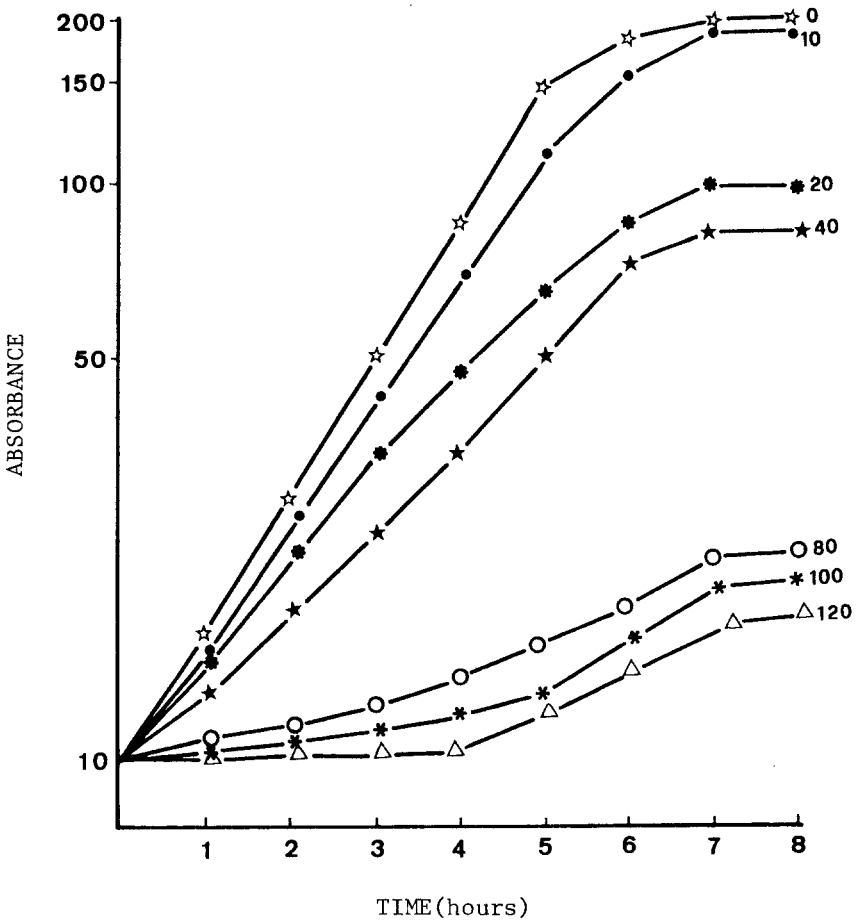

Figure 1. Growth of strain TA98 in the presence of different concentrations (in ppm) of cadmium

tests). Similar experiments were carried out to study the inhibition of growth by different heavy metals on the tester strain TA98 (data not shown) and Table 1 summarizes the 50% and 90% toxic doses of theheavy metals to strain TA98. Growth inhibition experiments were also carried out with strains TA102, TA1535 and TA1537 in order to determine the 50% and 90% toxic doses of the heavy metals to these tester strains. These tester strains showed similar growth inhibition respones to the various concentrations of the tested heavy metals as those shown by strain TA98 (data not shown). Thus, same range of concentrations of heavy metals (between 50% to 90% toxic doses) was used for all four tester strains in the mutagenicity tests.

The ten heavy metals used in the present study could be divided into three classes based on their toxicity to

Table 1. 50% and 90% toxic doses of heavy metals to growth of the tester strain TA98

Heavy metal	50% toxic dose	90% toxic dose
Cadmium	20 ppm	120 ppm
Cobalt	40 ppm	120 ppm
Chromium	0.2 ppm	0.25 ppm
Copper	160 ppm	200 ppm
Iron	160 ppm	200 ppm
Mercury	0.2 ppm	0.25 ppm
Manganese	20 ppm	120 ppm
Nickel	120 ppm	200 ppm
Lead	20 ppm	160 ppm
Zinc	120 ppm	160 ppm

the tester strains (Table 1). Class 1, including chromium and mercury was found to be very toxic to the tester strains even at relatively low concentration (0.2 ppm). Class 2, including cadmium, cobalt, manganese and lead were toxic to the tester strain at higher concentrations (20 to 40 ppm). The last one, class 3,inculding copper, iron, nickel and zinc were relatively non-toxic to the tester strains (their 50% toxic doses ranged from 120 to 160 ppm).

Plate-incorporation method was used for mutagenicity tests. To obtain a linear dose-respone curve of the mutagenciity tests, different concentraions of heavy metals between the 50% and 90% toxic doses obtained from the growth inhibition experiments were used for the mutagenicity tests. Numbers of His^+ revertants appeared on the control plates (only the cells of the tester strain and no heavy metal) and the experimental plates (containing different concentrations of heavy metals) were counted after incubated at 37°C for two days. The number of His^+ revertants of the experimental plates was substracted from that of the control plates; and the resulted number was used to plot the dose-response curve. Only the number from the linear portion of the

Table 2. Mutagenicity of heavy metals to *Salmonella typhimurium*

Heavy metal	Addition of S9-mix[a]	Mutagenicity, no. of revertants/ug[b] TA98	TA102	TA1535	TA1537
Cadmium	-	0	1.2	0	0
	+	0	0	0	0
Cobalt	-	10.6	0	0	2.8
	+	0	0	0	0
Chromium	-	4.6	0	10.8	0
	+	0	0	0	0
Copper	-	0	0	0	0
	+	0	0	0	0
Iron	-	0	0	0	0
	+	0	0	0	0
Mercury	-	0	0	0	0.8
	+	0	0	0	0
Manganese	-	0	0	0	2.8
	+	0	0	0	0
Nickel	-	0	0	0	0
	+	0	0	0	0
Lead	-	0	1.2	0	0
	+	0	0	0	0
Zinc	-	0	0	0	0
	+	0	0	0	0

[a] S9-mix: S9 plus cofactors; -, without S9-mix; +, with S9-mix.

[b] The number represents His^+ revertants taken from the linear portion of the dose-response curves. Control values (spontaneous revertants) were substracted. Average spontaneous revertants in TA98, TA102, TA1535, and TA1537 were 40, 160, 42, and 60, respectively.

dose-respone curve was used for the comparison of mutagenicity and carcinogenicity of tested heavy metals. The mutagenicities of ten heavy metals to four tester strains of *Salmonell typhimurium* were assayed with or without S9-mix and the results are tabulated in Table 2. In the absence of S9-mix, cadmium (to strain TA102), cobalt (to strains TA98 and TA1537), chromium (to strains TA98 and TA1535), mercury (to strain TA1537), manganese (to strain TA1537), and lead (to strain TA102) were found to be mutagenic. These six heavy metals belong to classes 1 and 2 toxic heavy metals as determined by growth inhibition experiments. It seems there was corelation between the toxicity and mutagenicity of heavy metals. This could be explained by that more mutagenic the heavy metal was, more lethal

mutation(s) might be induced by the heavy metal and caused harmful effect(s) on the growth of the bacterial cells.

In the presence of S9-mix, all ten heavy metals used in the present study were found to be non-mutagenic to all four tester strains (Table 2). The presence of S9-mix nullified the mutagenicity of the heavy metals might due to the presence of metalloproteins in the liver homogenate and these proteins were able to bind cadmium, copper, mercury as well as zinc (Fassett 1980). The binding of the heavy metals to these proteins would reduce the binding of the heavy metals to the residues of DNA. This might explain that the reduced mutagenicity by heavy metals in the presence of S9. However, further study is needed to prove this hypothesis.

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