Study on Subacute Toxicity of Carbon n-Hexane-Acetone Extract Recovered from Drinking Water

by

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Industrial, agricultural and domestic wastes are increasing recently and polluting aquatic environments in Japan. Citizens in Kyoto city are utilizing Lake Biwa as a main source of drinking water. The conventional water purification process, i.e. coagulation, sand filtration and chlorination, has been adopted by the water-works bureau of Kyoto city. However, removal of pollutants with this process is incomplete and the drinking water quality has deteriorated in proportion to the pollution which is attributed to the industrialization and increase in population of the circumference. TAKENOBU et al. (1) reported that the concentration of organic substances recovered with the carbon chloroform extract (CCE) method from the tap water of Kyoto city occasionally exceeded the maximum permissible concentration of 1962 USPHS Drinking Water Standards (2).

Several authors (3,4) reported the toxicological effects on laboratory animals of organic substances which were recovered from surface or subsurface water with the CCE or CAE (carbon alcohol extract)method. However, little information is available concerning the toxicity of drinking water. The present study was intended to investigate the sub-acute toxicity of organic substances present in the tap water of Kyoto city using a carbon n-hexane-acetone extraction method.

MATERIALS AND METHODS

Carbon n-Hexane-Acetone Extract (CHAE)

Organic substances in the tap water were recovered with the carbon adsorption method as follows. Approximately 3 kg of 12-32 mesh activated carbon (Nishio Industries Co. Ltd.) was packed in a 15X52 cm. column. 40 m^3 of the tap water was passed through this column at a velocity of 3 liters per minute without any

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pretreatment. For this study, 9.5 kg of carbon was used for the filtration of 125 m^3 of water. After the filtration, the water-treated carbon was dried at 38°C for 2 days, then it was extracted with n-hexane and acetone (46v/54v) at 64-66°C for 20 hours in a Soxhlet apparatus. A portion of the water treatment carbon was extracted with chloroform for a comparison of CHAE and CCE value. As a blank, carbon which was not treated with water was used.

To obtain an approximate recovery efficiency of CHAE and CCE, chemical oxygen demands (CODs) were measured using potassium dichromate(5).

Diets

CHAE was added to powder diet (Nihon Clea Co. Ltd.) and the concentration was adjusted to 800mg per kg diet. For control, organic substances from blank carbon were added to the basal diet.

Animals

Both sexes of 4-week-old ICR-JCL mice, purchased from Nihon Clea Co. Ltd., were individually housed in an air-conditioned room and allowed to adjust to the environment for 1 week. They were divided into two groups consisting of 24 male and female mice each. The test group was fed a diet containing 800 mg of CHAE/kg and the control group received a diet containing the substances from the CHAE-treated blank carbon. Diets and distilled water were given ad libitum. Food consumption and body weight were measured twice a week during the experiment. After 1 and 3 mo of these dietary regimens, 12 mice of each group were sacrificed by exsanguination under anesthesia. After an autopsy examination, brain, lungs, heart, liver, spleen, kidneys and gonads were excised and weighed. They were fixed in Bouin's solution and examined histologically (6 males and females of each group). Hypophysis, thymus and adrenals were also examined histologically. Hematological and blood biochemical studies were performed on all animals. Hematological examination included the determination of erythrocyte count(RBC), leukocyte count(WBC), hemoglobin concentration(Hb) and hematocrit(HCT). Total proteins, urea nitrogen(BUN), calcium, glucose, cholesterol, alkaline phosphatase activity(AP), glutamic pyruvic transaminase(GPT) and glutamic oxaloacetic transaminase activity(GOT) were quantitatively examined. The results were analyzed statistically according to the student t test.

RESULTS AND DISCUSSION

0.396 mg of organic substances were recovered from 1 liter of the tap water with the CHAE method, which is 2.3 times greater than recovered by the CCE method (Table 1). The COD values of CHAE and CCE were 1.53 and 1.90 mg/mg extract respectively, while that of the tap water was 2.86 mg/l on the average. The recoveries based on the COD values were found to be 21% for CHAE and 11% for CCE (Table 1).

TABLE 1

Organic substances recovered from the tap water with the carbon adsorption method.

Organic Extract	mg Extract/l Water	COD(mg)/mg Extract	Recovery(a) (%)
CHAE	0.396	1.53	21
CCE	0.172	1.90	11

(a): (COD of extract recovered from 1 liter of the water)x100/(COD of 1 liter of the water(2.86 mg/l on the average).

A preliminary acute toxicity study showed that the po LD50 value of CHAE was over 5 g/kg body weight, although it was difficult to determine the exact value because of its high viscosity.

Table 2 shows the body weight, food consumption and organ weights of mice after 3 mo of the dietary regimens. No significant differences were observed between control and test groups.

The results of hematological and blood biochemical examinations are summarized in Table 3. A broad range of values for WBC andsporadic elevations of AP, GPT and GOT were found. However, no significant differences were observed between control and test groups.

The gross and histological examinations revealed no abnormalities attributable to CHAE.

In this study, the average daily food consumption of mice was approximately 170 g/kg body weight and the daily intake of CHAE calculated from the food consumption was approximately 136 mg/kg body weight.

TABLE 2

Body weight, food consumption and organ weight of mice treated with CHAE for 3 mo.

	male		female	
	control	test	control	test
Initial Body Weight(g)	25.3 ± 0.9 (a)	25.4 ± 0.9	23.0 ± 0.5	22.5 ± 1.0
Final Body Weight(g)	38.6 ± 2.5	39.8 ± 2.3	31.7 ± 3.4	33.2 ± 2.8
Total Food Intake(g)	605 ± 78	600 ± 80	535 ± 39	534 ± 30
Total CHAE Intake(mg)	0	480 ± 64	0	427 ± 24
Brain(mg)	474 ± 19	443 ± 35	476 ± 25	478 ± 32
Lungs(mg)	224 ± 40	219 ± 25	209 ± 24	203 ± 23
Heart(mg)	200 ± 20	203 ± 31	145 ± 11	147 ± 12
Liver(mg)	2260 ±250	2370 ±230	1670 ±260	1640 ±170
Spleen(mg)	113 ± 29	103 ± 18	144 ± 33	143 ± 39
Kidneys(mg) left	401 ± 41	417 ± 50	244 ± 17	250 ± 30
Kidneys(mg) right	390 ± 48	403 ± 39	232 ± 20	237 ± 25
Gonads(mg) left	143 ± 15	129 ± 19	12 ± 5	11 ± 4
Gonads(mg) right	131 ± 13	112 ± 20	9 ± 4	10 ± 3

(a): Mean±SD of 12 mice. There were no significant differences in all values at 5 % level.

TABLE 3

Hematology and blood biochemical values of mice treated with CHAE for 3 mo.

Parameter	Units	male		female	
		control	test	control	test
RBC	$10^6/mm^3$	80.1[(a)] ± 6.2	80.0 ± 7.4	77.2 ± 8.5	73.3 ± 8.1
WBC	$10^2/mm^3$	93 ±39	109 ±40	81 ±45	75 ±26
Hb	g/dl	16.3 ± 1.5	17.2 ± 2.7	16.6 ± 0.9	16.7 ± 0.9
HCT	%	44.7 ± 5.9	44.8 ± 3.0	47.2 ± 5.8	48.9 ± 5.6
BUN	mg/dl	32.0 ± 5.0	28.1 ± 5.3	20.1 ± 3.4	18.3 ± 4.3
Calcium	mg/dl	8.71 ± 0.50	8.86 ± 0.60	9.15 ± 0.49	9.08 ± 0.57
Glucose	mg/ml	2.54 ± 0.43	2.68 ± 0.46	2.20 ± 0.45	2.29 ± 0.45
Cholest.	mg/ml	1.57 ± 0.30	1.45 ± 0.28	1.03 ± 0.24	1.07 ± 0.19
Proteins	g/dl	7.6 ± 0.4	7.2 ± 0.2	7.1 ± 0.3	7.3 ± 0.4
AP	B.L.	0.8 ± 0.2	0.9 ± 0.1	1.2 ± 0.3	1.3 ± 0.3
GPT	R.F.	20 ± 4	27 ± 9	14 ± 9	12 ± 4
GOT	R.F.	75 ±29	77 ±25	122 ±49	128 ±48

(a): Mean±SD of 12 mice. There were no significant differences in all parameters at 5 % level.

The CHAE was recovered from 340 liters of the tap water. This volume of water is some 2000 times greater than the average human intake (30 ml/day/kg body weight). Although the COD values indicated 21% recovery as an index, since organics were not adsorbed onto activated carbon completely and adsorbed organics were not extracted with the same efficiency, it should be noted that all trace organics in the water were not recovered with the same efficiency utilizing this adsorption method. However, it is presumed that the factor of 2000 employed in this study covers adequately the incompleteness of recovery.

From the fact that CHAE revealed no detectable effects on mice, coupled with the low po LD50 value of CHAE, it is presumed that the toxicity of organic substances in the tap water is very low and that the amounts taken in by citzens, based on realistic estimates of daily intake, may cause no adverse effects on health.

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