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Subacute Toxicity of Phenoxy Herbicides, MCPA- and MCPB-Ethylesters, in Mice

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Summary. **Phenoxy herbicides, 2-methyl-4-chlorophenoxyacetic acid ethylester (MCPA-E) and 2-methyl-4-chlorophenoxybutyric acid ethylester (MCPB-E) were evaluated for subacute toxicity by gavage in male mice. Groups of 10 mice were given daily doses of MCPA-E (50, 100 and 200 mg/kg) or MCPB-E (25, 50 and 100 mg/kg) 5 days/wk for 6 weeks. After completion of the treatment, histopathological changes were noted. In addition to this 6-wk experiment, MCPA-E or MCPB-E was given for 12 weeks, and a comparison was made between the two experiments. The relative liver weight of mice in the group treated with MCPA-E for 6 weeks increased significantly. Histopathological changes were seen in the livers of mice at all dose levels of MCPA-E and MCPB-E, both in the 6 and 12-wk experiments. Hepatic cell atypia and central necrosis were the major findings in mice with hepatomegaly. As for the histology of the gallbladder, no specific change was observed in mice treated with MCPA-E and MCPB-E.**

INTRODUCTION

Epidemiological studies on the biliary tract cancer (BTC) have been undertaken in our laboratory, one reason for this being that the death rate for BTC in Niigata Prefecture is the highest in Japan.[1,2] In addition, it has been noted that prefectures with the higher death rates are clustered in the areas where rice production is also high. Based on these findings, we investigated the role of agricultural chemicals, dietary patterns, and other geographic characteristics in the drinking water or soil. In this paper, the role of agricultural chemicals is reported.

Yamamoto et al.[3] analysed an ecological correlation between the use of chemicals (1962-1975) and standardized mortality ratios (SMRs) for BTC (1975) in Japan and found that the use of MCPA-E and MCPB-E was frequently correlated with SMRs. Although the presence of an ecological correlation does not always represent the causal one, it is worthwhile to research why such a statistical association should be observed. As a part of the investigation into the biological characteristics of these compounds, subacute toxicity was examined in mice and the results were compared with those of previous reports by others.[4-6] Since the previous studies were conducted using rats in which the gallbladder was absent, we used mice with intact gallbladders in order to examine the histopathological effects of these chemicals on this organ.

MATERIALS AND METHODS

Six and 12-wk experiments were carried out using the same methods as follows:

Chemicals. MCPA-E and MCPB-E were extracted from their commercial products with dichloromethane. Their purities were analysed by making use of ECD-GC (Shimadzu 9A); those of MCPA-E and MCPB-E were 96.0% and 94.0%, respectively. Contaminants were not chemically identified for the present experiment.

Test procedures. In the present experiment, 4-5 week-old male ICR mice were used. Each test group contained 10 animals. The room temperature was maintained at 23±1℃ with the relative humidity at 60±5%. The test compound was given with the aid of a gastric catheter for either 6 or 12 weeks (5 days/week). Each concentration of MCPA-E and MCPB-E stock solutions was 60 mg/ml, dissolved in corn oil.

Working solutions were prepared daily just prior to use. Based on the previous experiments by Vainio et al.[7], the dose levels of MCPA-E and MCPB-E were set at 50, 100 and 200 mg/kg body weight. In the case of MCPB-E treatment, however, the highest dose was lowered to 100 mg/kg because of the occurrence of drowsiness, diarrhea and death in the treated mice. Finally, the three levels in MCPB-E were established as 25, 50 and 100 mg/kg. The volume of solution was about 0.1 ml per mouse. The control animals were given corn oil by gavage (corn oil control) or left alone without any treatment (untreated control). The mice were weighed twice a week, and food and water intake was recorded weekly. All animals were sacrificed 24 h after the last treatment.

Histopathology. The liver, together with the gallbladder, lung, spleen, kidney and testis were removed, weighed and fixed by 10% formalin. For light microscopic observation, these organs were treated with paraffin embedding. The sections were stained with hematoxylin and eosin, and d-PAS.

Statistical analysis. Student's t test was used to evaluate statistical significances. Values significantly different from those of the controls are indicated in the tables by asterisks, as follows; $*p<0.05$ and $**p<0.01$.

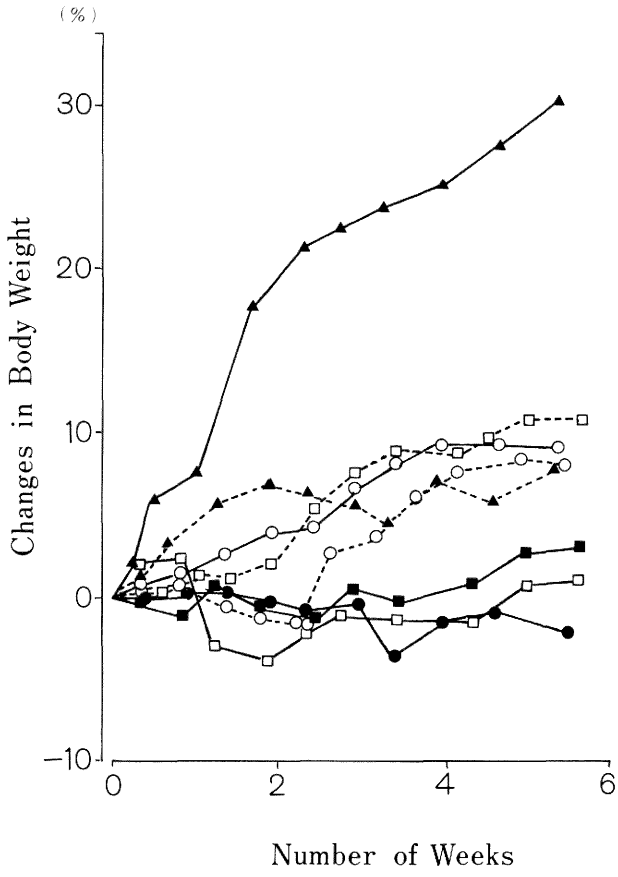

Fig. 1. Changes[1)] in body weight of mice treated with MCPA-E and MCPB-E for 6 weeks.
Notes 1): Changes in body weight are expressed as the difference from the body weight from the initial day of the treatment. 2): ▲—▲, untreated control; ▲·····▲, corn oil control; ○—○, MCPA-E 50; ●—●, MCPA-E 100; ○·····○, MCPA-E 200; □—□, MCPB-E 25; ■—■, MCPB-E 50; □·····□, MCPB-E 100

RESULTS

Six-week experiment

The body weights of mice untreated or treated with only corn oil increased by an average of 19.0% during the treatment period (Fig. 1). On the other hand, those of mice treated with MCPA-E and MCPB-E increased slightly by an average of 4.2% and 1.5%, respectively.

Regarding food and water intake in each group, the only significant finding was a reduction of food consumption by the corn oil control, as compared with the untreated control.

The high dose of MCPA-E produced a few tremors and drowsiness in the mice 3 to 4 days after the initiation of treatment. Tremors and drowsiness also occurred in all mice treated with MCPB-E. In addition to these symptoms, diarrhea was frequently observed, especially in the higher dose groups. A total of 2 mice died during the experiment.

Wet and relative organ weights are shown in Table 1. All of the wet weights of the liver in the MCPA-E groups increased significantly, as compared with those in the corn oil control. A decrease in the relative liver weight in the 100 mg/kg and 200 mg/kg MCPA-E groups was evident. However, changes in other organs were only sporadically found as shown in Table 1.

Concerning the histopathology of the organs, anisonucleosis of hepatocytes markedly increased in mice receiving MCPA-E and MCPB-E. In contrast, no change in hepatocytes was found in the mice of both control groups. Spotty necrosis of the central vein in the liver and late nodule as a reaction after the central necrosis was recognized in those animals treated with MCPA-E and MCPB-E. In the treated groups, a considerable increase in the number of Kupffer's cells was noted after d-PAS stain. Pathological changes of the gallbladder were found in the

Table 1. Wet and relative organ weights[1)] in mice treated with MCPA-E and MCPB-E for 6 weeks

Organ weight	Group	No. of mice	Organ				
			Liver	Lung	Spleen	Kidney	Testis
Wet weight (g)							
	Untreated control	10	1.42±0.077	0.21±0.017	0.15±0.014	0.55±0.071**	0.28±0.029
	Corn oil control	9	1.40±0.313	0.26±0.040	0.19±0.222	0.43±0.046	0.26±0.028
	MCPA-E 50 mg/kg	10	1.70±0.244*	0.24±0.036	0.12±0.022	0.39±0.070	0.26±0.025
	100	9	1.73±0.259*	0.32±0.260	0.16±0.056	0.41±0.065	0.24±0.020
	200	10	1.76±0.244*	0.34±0.132	0.16±0.043	0.39±0.043	0.24±0.022
	MCPB-E 25 mg/kg	10	1.23±0.232	0.22±0.023*	0.12±0.066	0.49±0.089	0.23±0.039
	50	10	1.19±0.128	0.23±0.043	0.12±0.043	0.42±0.066	0.23±0.029
	100	9	1.19±0.151	0.31±0.219	0.13±0.090	0.47±0.066	0.23±0.024
Relative weight (g%)							
	Untreated control	10	5.29±1.048	0.58±0.083**	0.42±0.101	1.51±0.268**	0.78±0.095
	Corn oil control	9	4.51±0.554	0.84±0.153	0.62±0.701	1.38±0.090	0.83±0.103
	MCPA-E 50 mg/kg	10	4.89±0.457	0.68±0.114*	0.36±0.082	1.42±0.204	0.75±0.069*
	100	9	6.01±0.529**	1.11±0.871	0.55±0.196	1.46±0.157	0.85±0.093
	200	10	5.59±0.459**	1.12±0.558	0.52±0.135	1.49±0.102*	0.77±0.078
	MCPB-E 25 mg/kg	10	4.49±0.906	0.82±0.163	0.47±0.308	1.43±0.191	0.85±0.135
	50	10	4.10±0.321	0.81±0.155	0.41±0.152	1.40±0.169	0.79±0.080
	100	9	4.21±0.363	1.13±0.781	0.46±0.315	1.42±0.087	0.83±0.092

Notes: 1) Values are expressed as Mean ±S.D.
*$p<0.05$, **$p<0.01$ as compared with the values of the corn oil control

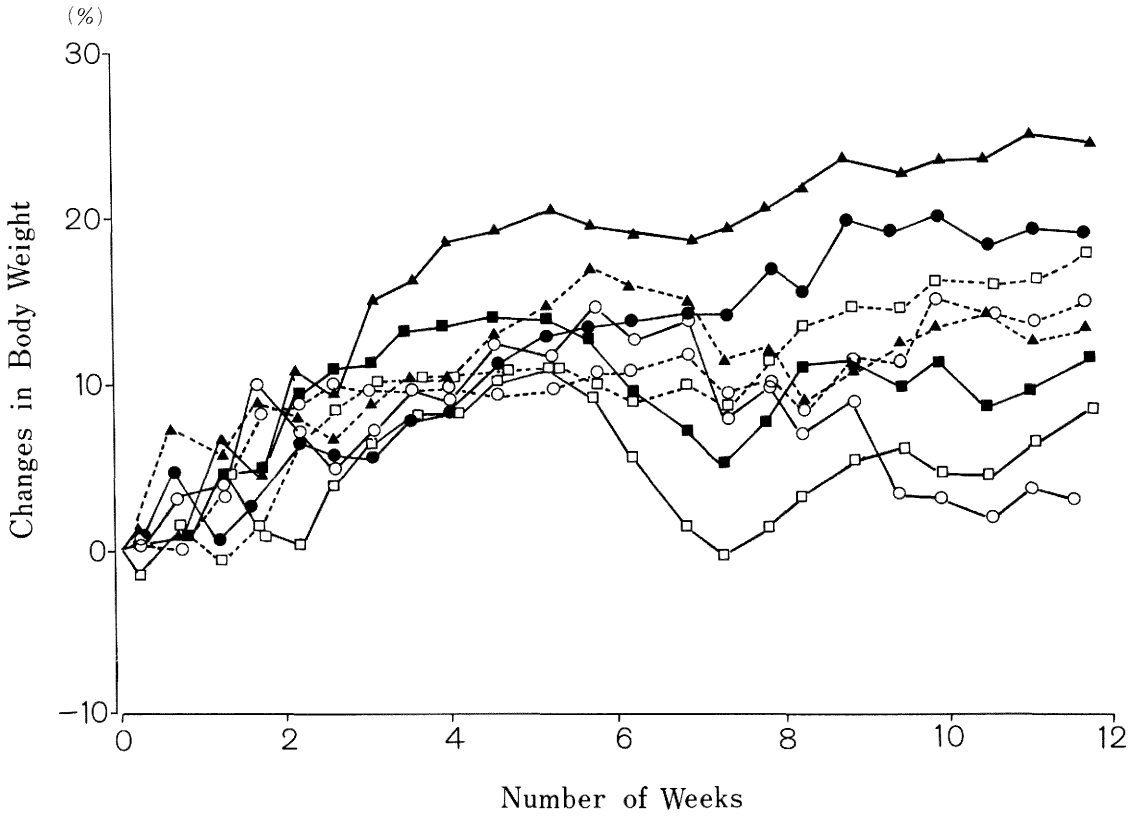

Fig. 2. Changes[1)] in body weight of mice treated with MCPA-E and MCPB-E for 12 weeks.
Notes 1): Changes in body weight are expressed as the difference from the body weight from the initial day of the treatment. 2): Symbols are the same as those in Fig. 1.

Table 2. Wet and relative organ weights[1] in mice treated with MCPA-E and MCPB-E for 12 weeks

Organ weight	Group	No. of mice	Organ				
			Liver	Lung	Spleen	Kidney	Testis
Wet weight (g)							
	Untreated control	8	1.84±0.321	0.21±0.032*	0.11±0.017	0.48±0.074	0.26±0.043
	Corn oil control	8	1.86±0.164	0.43±0.208	0.20±0.092	0.53±0.057	0.26±0.030
	MCPA-E 50 mg/kg	8	1.47±0.132**	0.25±0.038	0.18±0.071	0.46±0.055*	0.25±0.021
	100	9	1.54±0.329*	0.25±0.038	0.30±0.454	0.42±0.088**	0.26±0.037
	200	10	1.25±0.202**	0.22±0.036*	0.13±0.051	0.41±0.084**	0.25±0.029
	MCPB-E 25 mg/kg	6	1.47±0.142**	0.35±0.245	0.16±0.071	0.44±0.067	0.23±0.022
	50	9	1.88±0.293	0.30±0.205	0.26±0.192	0.51±0.106	0.31±0.126
	100	9	1.80±0.213	0.29±0.149	0.21±0.197	0.47±0.068	0.25±0.029
Relative weight (g%)							
	Untreated control	8	5.44±0.583	0.62±0.091*	0.34±0.078*	1.42±0.188	0.77±0.169
	Corn oil control	8	5.39±0.416	1.26±0.665	0.56±0.245	1.52±0.104	0.75±0.054
	MCPA-E 50 mg/kg	8	4.74±0.357	0.82±0.134	0.58±0.236	1.49±0.184	0.81±0.088
	100	9	4.61±0.740	0.77±0.161	0.82±1.059	1.29±0.329	0.81±0.235
	200	10	3.89±0.850**	0.67±0.138*	0.40±0.175	1.29±0.328	0.78±0.144
	MCPB-E 25 mg/kg	6	4.94±0.459	1.19±0.836	0.54±0.248	1.49±0.213	0.77±0.067
	50	9	5.39±0.421	0.87±0.620	0.74±0.503	1.44±0.179	0.91±0.424
	100	9	5.41±0.422	0.88±0.458	0.62±0.503	1.42±0.162	0.76±0.094

Notes: 1) Values are expressed as Mean ±S.D.
*$p<0.05$, **$p<0.01$ as compared with the values of the corn oil control

epithelium, that is, the papillary formation increased slightly in the treated groups in comparison with the corn oil and the untreated control animals.

Twelve-week experiment

As shown in Fig. 2, the body weights of mice treated with MCPA-E and MCPB-E increased by an average of 12.0% during the treatment period, the increase being less than those of both controls (18.9% as average). In particular, the average body weight of the MCPA-E 50 mg/kg group was suppressed during the last 6 weeks. Significant changes in food and water consumption were not observed in each group. As for behavior and mortality, the mice showed symptoms common to those in the 6-wk experiment. Especially in the MCPB-E groups, tremors, drowsiness and diarrhea continued throughout the treatment. A total of 9 mice died during the experiment.

The average weights of the wet liver and kidney of mice treated with MCPA-E were found to be lower than those in the corn oil control. However, the relative weight of these organs was not statistically significant (Table 2). In the MCPB-E groups significant changes in organ weights were not found except for the wet liver weight of the 25 mg/kg group.

Histopathological findings were quite similar to those of 6-wk experiment. Anisonucleosis of hepatocytes and a numerical increase of Kupffer's cells were observed. As to the gallbladder, no epithelial dysplasia occurred. However, papillary growth of the epithelium was observed occasionally. No significant change was detected in the lung, spleen, kidney or testis.

DISCUSSION

MCPA (2-methyl-4-chlorophenoxyacetic acid) and MCPB (2-methyl-4-chlorophenoxybutyric acid) are the derivatives of chlorinated phenols, and have been used as herbicides mostly in paddy fields in Japan. It is reported that about 150 tons of MCPA and 300 tons of MCPB were produced in 1986, with an ethyl ester type of MCPA and MCPB being commonly used. Notwithstanding the extensive use of these chemicals, the levels of environmental contamination and the route of exposure to humans through water and food have not yet been clearly demonstrated.

Epidemiological surveys have suggested a possible carcinogenic action of MCPA.[8,9] According to previous reports, it is likely to be a causative agent of

leukemia in humans. Hardell et al.[10] also suggested that exposure to MCPA might be related to the occurrence of malignant lymphoma.

Treatment with MCPA-E or MCPB-E induced the lessening of any body weight increase in such mice as compared with the controls. The pattern of body weight changes was almost identical in the 6-wk and 12-wk experiments. When comparison was made between the MCPA-E and MCPB-E groups, the pattern of body weight change was not dose-related. The analysis of organ weights showed characteristic findings in which the wet and relative liver weights in mice treated with MCPA-E increased significantly in the 6-wk experiment. In contrast to this, the organ weights in the three dose groups and the relative weight in the 200 mg/kg group decreased significantly in the 12-wk experiment. The difference between the two experiments should be explained from a histopathological viewpoint of the liver.

Pathological changes in the livers of animals treated with MCPA-E and MCPB-E were confirmed in the present study. Hepatic cell atypia and central necrosis were seen in the mice treated with MCPA-E both in the 6- and 12-wk experiments. Gurd et al.[4] first reported the LD_{50} values of MCPA in rats and mice. In their 7-month feeding study, in which MCPA was added to the diet, it was concluded that MCPA itself caused little or no morphological changes in the organs which they examined. In contrast, Verschuuren et al.[5] found changes in the liver, kidney, spleen and thymus of rabbits in a subacute (3 weeks) dermal toxicity study. Hattula et al.[6] studied the acute and subchronic toxicity of MCPA in rats, and similar histopathological changes were found in the liver and the spleen. The results of the present study are consistent with those of others. Concerning the difference between the wet and relative liver weights between the 6- and 12-wk experiments, one attempt to explain it might be from the point of view of histopathology. It is inferred that the increase of wet and relative liver weights of mice in the 6-wk experiment may be caused by transient inflammation as shown by the infiltration of lymphocytes. A decrease in such lesions was evident at the end of 12-week treatment.

For the elucidation of the histopathological findings of MCPB-E, the comparison could not be made because of the lack of published data. In the present study, the livers of mice treated with MCPB-E was strongly affected as compared with that of mice treated with MCPA-E.

The papillary formation of the epithelium in the gallbladder increased slightly in MCPA-E and MCPB-E treated mice. A definitive interpretation of the present findings, however, is still forthcoming. A chronic toxicity study may be essential for the evaluation of the outcome of this pathological change. In addition to the solution of this problem, a chronic toxicity study also urges us to test the hypothesis that MCPA or its impurities are related to the occurrence of BTC.

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