Chronic Toxicity of
2-Methyl-4-Chlorophenoxyacetic Acid
(MCPA) in Mice

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TAKAGI, S. Chronic Toxicity of 2-Methyl-4-Chlorophenoxyacetic Acid (MCPA) in Mice. Tohoku J. Exp. Med., 1990, 160 (2), 97-107 — One of the phenoxy herbicides, 2-methyl-4-chlorophenoxyacetic acid (MCPA) was soaked with pellet dissolved by corn oil and given to ICR male mice for 18 months at levels of 40, 200, 1,000 and 5,000 ppm (about 3.9, 19.0, 95.6 and 566.4 mg/kg/day, respectively). The animals were then placed on a control diet for 12 months. Treatment with 5,000 ppm of MCPA resulted in a significantly decreased survival rate of the mice. Histopathological examination of mice treated with 40 ppm MCPA revealed the occurrence of leukemia which showed neoplastic infiltration in the liver. No adverse effect of the gallbladder, except for papillary proliferation, was noted at all the dose levels of the experimental groups.

The death rate for biliary tract cancer (BTC) in Niigata Prefecture is the highest in Japan (Tominaga et al. 1979; Yamamoto et al. 1988a). In addition, it is noted that the prefectures with higher death rates are clustered in the areas where the rice production is also high. Based on these findings, the role of agricultural chemicals, the dietary pattern and geographical characteristics have been investigated.

Of these factors, the role of agricultural chemicals was tested first. Yamamoto et al. (1986) analyzed an ecological correlation between the use of chemicals (1962-1975) and standardized mortality ratios (SMRs) for deaths from BTC (1975) in Japan. They found that the use of 2-methyl-4-chlorophenoxyacetic acid ethylester (MCPA-E) was frequently correlated with SMRs. Although the presence of an ecological correlation does not always imply a causal relationship, it may be worthwhile to explain why such an ecological correlation was observed. As a part of the investigation into the biological characteristics of these compounds, chronic toxicity of MCPA was examined in mice. The aim of the present study is to find out whether or not MCPA has carcinogenicity in mice.

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MATERIALS AND METHODS

The animals used were ICR male mice, weighing approximately 29 g, and 4–5 weeks old. Each experimental group contained 30 animals. Five animals were housed in one cage. MCPA, of which more than 97% was purity, was purchased from Wako Pure Chemical Industries, Ltd. (Osaka). The animals were given pellets mixed with MCPA, 0 (only corn oil), 40, 200, 1,000 and 5,000 ppm, for 18 months and placed on an untreated control diet for 12 months. The animals of the untreated control group were housed without any treatment for 30 months. All of the animals were allowed to drink water ad libitum during the experiment.

The experimental diets were prepared in 1,000 g batches as follows: the appropriate quantity of MCPA at each dose was suspended in 100 g of corn oil, the corn oil was added to 900 g of solid feed and mixed thoroughly. The diets were stored at 4°C to prevent spoilage.

The room temperature was maintained at 23±1°C and the relative humidity at 60±5%. The mice were weighed once a week. Food and water consumption in each cage was also recorded weekly.

Autopsy was performed immediately after the death or the sacrifice when they were moribund or the experiment was completed. The liver with the gallbladder was weighed and processed for the histopathological study. Specimens were fixed in 10% formalin, embedded in paraffin, cut in 3μm section, stained with hematoxylin and eosin and examined by light microscopy.

The Cutler-Ederer's method was used for the analysis of survival rates and the statistical significance was evaluated by Student's t-test. Values significantly different from those of the control were indicated in the figures by asterisks as follows: *p <0.05, **p <0.01. Fisher's exact test for fourfold tables was used to compare the incidence rates of neoplastic changes. In addition, a scale of measurement combining the number of mice and the duration of observation was introduced. It is referred to as the person-years method in the field of epidemiology, but in this case, as the mouse-weeks method.

RESULTS

No marked changes in behavior and appearance were observed in the animals that were given lower doses of MCPA. In the 1,000 and 5,000 ppm groups the mice showed tremor, drowsiness and piloerection during the MCPA treatment and, in addition to these symptoms, diarrhea continued throughout the treatment.

The MCPA treated and untreated control groups were compared with the corn oil control group for survival rates (Fig. 1). The survival rate, as compared with the corn oil control, significantly decreased in the 5,000 ppm MCPA group at 8–16 weeks (p <0.05), 32–64 weeks (p <0.01) and 96 weeks (p <0.05). The survival rates, on the contrary, were found to be higher in the groups 40 and 200 ppm than in the corn oil control throughout the experimental period. A significant increase of the survival rate was, however, only present at 80 weeks in the 200 ppm group (p <0.05).

In all of the experimental groups, except for the 5,000 ppm group, the body weight of the mice increased for 40 weeks after the initiation of treatment. In the 5,000 ppm group the body weight decreased for the first 4 weeks and then increased for 48 weeks after the initiation (Fig. 2). The body weight increase in the
corn oil control, 40 and 200 ppm groups were accelerated in the rate during the first 40 weeks, as compared with the untreated control. During the recovery period (81–132 weeks), the body weights decreased in the untreated and corn oil controls and 5,000 ppm groups. In the 40, 200 and 1,000 ppm groups, on the other hand, the decrease started after 96 weeks.

As to the changes of food consumption, similar changes were observed in all the groups (Fig. 3). The average doses of MCPA were about 3.9, 19.0, 95.6 and 566.4 mg/kg/day in the groups of 40, 200, 1,000 and 5,000 ppm, respectively, during the treatment period. The decrease of the water consumption was observed at the first 8 weeks after the treatment in all the groups. But, little changes were seen in the successive treatment period (Fig. 4).
Histopathological findings, except for the neoplastic changes of the liver in the MCPA treated animals, were similar to those of the control groups. In the MCPA treated groups anisonucleosis of hepatocytes and Kupffer's cells in the liver of animals were scattered at spotty levels. The nuclear size of hepatic cells were measured. But the mean sizes of nuclei in the MCPA treated groups were not
Fig. 4. Average water consumption in mice treated with MCPA. Symbols are the same as those in Fig. 2.

Table 1. Incidence of neoplastic lesions in the liver and gallbladder of mice treated with MCPA

<table>
<thead>
<tr>
<th>Organ and Type</th>
<th>Group</th>
<th>MCPA (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated control</td>
<td>Corn oil control</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of animals examined</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>Neoplastic nodule</td>
<td>0</td>
<td>2(8.7)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>0</td>
<td>1(4.3)</td>
</tr>
<tr>
<td>Leukemia&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1(4.8)</td>
<td>0</td>
</tr>
<tr>
<td>Number of animals with neoplastic lesions</td>
<td>1(4.8)</td>
<td>2(8.7)</td>
</tr>
<tr>
<td>Gallbladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of animals examined</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Papillary proliferation&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2(12.5)</td>
<td>2(15.4)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Showing neoplastic infiltration
<sup>b</sup> Referring to the hyperplastic changes, such as papilloma and adenoma
<sup>c</sup> p = 9.5 × 10<sup>-3</sup> and p = 2.9 × 10<sup>-3</sup>, respectively as compared with the corn oil control by Fisher's exact test of probability.
significantly different from those in both the untreated and the corn oil controls (the data not shown). In all the groups, vein expansion and fatty change of the liver were observed.

The type and numbers of neoplastic changes are summarized in Table 1. The neoplastic types observed in the liver were neoplastic nodule (Fig. 5), hepatocel-

Fig. 5. Neoplastic nodule in the liver of a mouse treated with 40 ppm MCPA. ×25

Fig. 6. Liver of a mouse treated with 40 ppm MCPA showing infiltration of leukemic cells. ×25
lular carcinoma, hemangioma and leukemia (Fig. 6). Of these neoplastic lesions, the occurrence of leukemia was the most frequent in the MCPA treated groups; 1 (4.8%), 0 (0.0%), 5 (29.4%), 3 (12.5%), 1 (4.2%) and 1 (5.6%) in the untreated and the corn oil controls, 40, 200, 1,000 and 5,000 ppm MCPA groups, respectively. The incidence rate of 29.4% (5/17) in the 40 ppm group was only found to be higher than that of the corn oil control group at the probability level of \( p = 9.5 \times 10^{-3} \) by Fisher's exact test. The number of animals with neoplastic lesions were 1 (4.8%), 2 (8.7%), 9 (52.9%), 7 (29.2%), 2 (8.3%) and 2 (11.1%) in the untreated

![Fig. 7. Hyperplastic changes in the wall of gallbladder from a mouse treated with 5,000 ppm MCPA. ×50](image)

<table>
<thead>
<tr>
<th>Groups (ppm)</th>
<th>Mouse-weeks at risk</th>
<th>All lesions</th>
<th>Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed Expected(^a)</td>
<td>Probability(^b)</td>
<td>Observed Expected(^a)</td>
</tr>
<tr>
<td>40</td>
<td>1610</td>
<td>9(0.559) 1.8</td>
<td>1.1 \times 10^{-4}</td>
</tr>
<tr>
<td>200</td>
<td>2079</td>
<td>7(0.337) 2.3</td>
<td>9.4 \times 10^{-3}</td>
</tr>
<tr>
<td>1000</td>
<td>1861</td>
<td>2(0.107) 2.0</td>
<td>6.8 \times 10^{-1}</td>
</tr>
<tr>
<td>5000</td>
<td>1125</td>
<td>2(0.178) 1.2</td>
<td>3.4 \times 10^{-1}</td>
</tr>
</tbody>
</table>

\(^a\)expected number was calculated by using the incidence of the corn oil control (2/1822) in the case of all lesions. But, in the case of leukemia, the incidence of untreated control (1/1758) was employed, since no prevalence of leukemia in the corn oil control made the calculation of Poisson's probability meaningless.

\(^b\)probability in Poisson's distribution.
and corn oil controls, 40, 200, 1,000 and 5,000 ppm groups, respectively. The incidence rate in the 40 ppm group was statistically higher than the corn oil control ($p = 2.9 \times 10^{-3}$ by Fisher's exact test).

Table 2 demonstrates the numbers of observed and expected animals with neoplastic changes. Poisson's probabilities of observed vs. expected numbers of all neoplastic lesions showed the significant differences in the groups of 40 and 200 ppm from that of the corn oil control. In contrast, there were no differences in 1,000 and 5,000 ppm groups. In the case of leukemia, the expected number was calculated by using the incidence rate of the untreated control, since no prevalence of leukemia in the corn oil control made the calculation of Poisson's probability meaningless. As shown in the last column of Table 2, a statistically significant increase was only evident in the 40 ppm group.

Concerning the pathological findings of the gallbladder, papillary proliferations of the epithelium were frequently observed in the MCPA treated groups. Here, the papillary proliferation stands for the hyperplastic changes, such as papilloma, adenoma, and metaplastic ones (Fig. 7). The statistical analysis revealed no difference in the occurrence of papillary proliferations between the corn oil control and the MCPA groups.

**DISCUSSION**

MCPA is one of the phenoxy herbicides and is extensively used as an agricultural chemical. In contrast to the extensive accumulation of carcinogenicity studies of chemically similar compounds; 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T), little has been investigated as to the carcinogenicity of MCPA.

Regarding the carcinogenicity of MCPA, there is one case of patient who suffered from acute leukemia after exposure to MCPA (Timonen and Palva 1980). They suggested that MCPA was probably a causative agent of leukemia. Recently, Coggon et al. (1986) reported one case of soft tissue sarcoma and three cases of nasal cancer among 4078 workers whose jobs entailed exposure to MCPA. But, they did not give a conclusive answer as to the carcinogenicity of MCPA.

In regard to the chronic toxicity study by using animals, Gurd et al. (1965) published the data of a seven-month administration of MCPA to rats and reported no histopathological abnormalities. It is particularly mentioned that the experimental data were inadequate for the evaluation of carcinogenicity of MCPA to animals (IARC 1983). In addition, the use of rats primarily lacking the gallbladder made the detection of BTC impossible.

The treatment of mice with MCPA on a daily basis at the level of 5,000 ppm induced symptoms of tremor, drowsiness, diarrhea and piloerection which were similar to the findings of acute studies by Vainio et al. (1983). The histopathological findings in the liver of animals were also in agreement with the previous report in which anisonucleosis and necrosis were observed in the parenchymal cells
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(Hattula et al. 1977). Besides these observations, the high incidence of neoplastic changes in the liver of mice in the 40 ppm group is also a significant finding in the present experiment.

As to the carcinogenicity study by using ICR Crj; CD-1 mice, the spectrum of spontaneous tumors was reported by Maita et al. (1988). According to their studies, the incidence of hepatocellular carcinoma, hemangioma of the liver and leukemia in the male mice were 9.1%, 1.5% and 9.9%, respectively. Comparing with their data, the result observed in the present control group indicated the lower frequency of spontaneous tumors. Discrepancies may be explained as the difference in the genetic background, housing condition, handling procedures, diet etc., as they have already stated. In addition to these factors, the diagnostic procedure may also influence the fluctuation of the spontaneous development of tumors. As a matter of fact, leukemia was diagnosed only by the examination of the liver, since the main concern was limited to the investigation of the hepatobiliary tumors. Since the discrepancy of the factors mentioned above was already evident, the evaluation of MCPA carcinogenicity was undertaken only by making use of the present control data.

The incidence of leukemia in the 40 ppm group was statistically higher than that in the corn oil control (Table 1). This finding was confirmed by the mouse-weeks analysis, in which the number of mice and the duration of observation are concurrently considered (Table 2). However, in both methods the occurrence was found to be not dose-related. So far it is difficult to explain why such a relationship was not present.

In spite of the unclear dose-response relationship, it is of interest to consider whether or not leukemia, as observed in the 40 ppm group, is specific in relation to MCPA treatment. Concerning the mutagenicity of MCPA, Seiler (1978) reviewed that MCPA was not mutagenic to several Salmonella typhimurium strains (Rasanen et al. 1977) and did not induce chromosomal aberrations or sister chromatid exchanges in human peripheral lymphocytes in vitro (Fahrig 1974; Linnainmaa 1984). Recently, Yamamoto et al. (1988b) reported that MCPA was not mutagenic in the rec assay. The possibility has been raised that MCPA may be a potent agent amplifying the carcinogenic action. There are reports that MCPA is a suspected carcinogen (Vainio et al. 1983), or has a co-mutagenic activity (Yamamoto et al. 1988b). Based on these previous reports, it is likely to consider the promoting role of MCPA on the spontaneous occurrence of leukemia.

Concerning the ultimate aim of testing the hypothesis that MCPA may induce or promote the production of BTC, the present experimental data did not support the hypothesis. Before rejecting the hypothesis, there are several problems which remained unsolved: the number of animals used, the sex difference in susceptibility to MCPA, species specificity to MCPA and the impurity of MCPA containing. Besides these basic problems, it is of interest to see the fate of papillary proliferations of the wall of the gallbladder in mice treated with MCPA.
The pathological meaning of the small cluster of papillary proliferation of MCPA groups will require further study.

In conclusion, although the present study could not increase the occurrence of BTC in mice, the present results suggest the possibility that MCPA may be a potent agent of leukemia to nonhumans. Further study should be directed to the reproducibility of the present finding.

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References


