Acute and Subchronic Toxicity of 2-Methyl-4-Chlorophenoxyacetic Acid (MCPA) in Male Rat. I. Light Microscopy and Tissue Concentrations of MCPA

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2-methyl-4-chlorophenoxyacetic acid (MCPA) is one of the most generally used herbicides in the countries of Northern Europe. In Finland MCPA has been the most widely used herbicide for several years (MARKKULA and TIITTANEN, 1975).

Although MCPA has been used since 1950's little attention has been paid to its toxicological properties. GURD et al. (1965) studied the acute oral and intraperitoneal LD_{50}-values of MCPA in rats and mice. In their 7-month feeding study MCPA was added to the diet. VERSCHUUREN et al. (1975) studied the short term oral and dermal toxicity of MCPA in rats and rabbits. In the short term toxicity study MCPA was added to the diet of rats for 90 days and mortality, haematological changes, some liver enzyme activities and histopathological changes in several tissues were investigated.

The present investigation was undertaken in order to study the histopathological changes in the tissues and the tissue concentrations of MCPA. A new glass capillary column GLC-technique was developed for the assay of MCPA and some other chlorophenoxyacetic acids or their phenol metabolites.

MATERIALS AND METHODS

Treatment of animals and histopathology

In the acute experiment two months old male Sprague-Dawley rats were used. Sodium salt of MCPA (Kemisk Vaerk Køge A/S, Denmark, 99 % purity) was injected subcutaneously at the following concentrations: 0, 300, 400, 450, 500, 550, 600 and 700 mg of sodium-MCPA/kg of body weight. Each other groups contained 10 animals, but groups 500 and 700 mg/kg 20 animals. Autopsy was performed immediately after the death and several tissues (small intestine, liver, pancreas, kidney, testis, prostate, lung, heart, skeletal muscle, brain, spleen, adrenals and thyroid gland) were taken for the histopathological study or chemical analyses. Histopathological specimens were fixed in buffered 10 % formalin or Bouin's fluid and paraffin sections were stained with haematoxylin-eosin, Weigert-vanGieson or PAS-haematoxylin. Tissue samples for chemical analyses were wrapped in aluminium

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foil and kept in -20°C until analyzed.

In the subchronic experiment two months old male Sprague-Dawley rats weighing 217-35 g were allowed to drink sodium-MCPA solution ad libitum for 9 weeks. Different concentrations of sodium-MCPA in drinking water (100, 500, 1 000, 2 000 and 3 000 mg/l) were used and water consumption was studied in order to determine the daily doses. At the end of the subchronic exposure the animals were killed with ether and autopsy were carried out. Animals and organs were weighed and histopathological specimens and tissue samples for chemical analyses were taken as in the acute experiment.

Chemical analyses

For chemical analyses tissue sample was weighed and homogenized in Sorvall omnimixer with anhydrous sodium sulphate (4 g of Na_2SO_4/g of wet tissue). After drying for 48 hrs the homogenate was extracted in a shaker with the mixture of chloroform and ether (1:1 v/v) three times for 30 min. The amount of the solvent was 30 ml for 1-2 g and 50 ml for 2-5 g of wet tissue. Extraction solution was then filtered (Whatman No 40) and the combined extracts were evaporated in Büchi Evaporator. The residue was dissolved in 1-2 ml of n-hexane and one half of the sample was chromatographed by GLC. The other half was methylated with diazomethane (1-2 drops for 1 ml solution). Methylation was carried out in a test tube with a glass stopcock. Diazomethane was destroyed after 10 min with pure nitrogen and the solution was used for chromatographing. Appearance of phenols and catechols in the non-methylated fraction and MCPA in the methylated fraction were studied. In extraction the recoveries of MCPA and 4-chloro-o-cresol were >95 %.

Carlo Erba Model Fractovap 2300 gas chromatograph equipped with FID-detector and Grob-type splitless injection system was used. The column was a glass capillar of 20 m length with the inside diameter of 0.35 mm with Emulfor as a liquid phase. Samples were introduced into the column in n-hexane or diethylether and 2 µl of solution was applied at the temperature of 35°C. After the solvent peak had appeared the solution was programmed 30°C/min to 190°C.

The list of the standards used and their relative retention times (x/MCPA, when methylester of MCPA was 1) is presented below:

Compound	Relative retention time
2-chloro-p-cresol	0.65
3-chloro-o-cresol	0.74
4-chloro-m-cresol	1.04
4-chloro-o-cresol	1.05
3-chloro-o-cresol	1.09

Concentration of standard compounds in chromatographing was 10 µg/ml of n-hexane or diethylether. In the routine analyses a mixture containing equal amounts of methylester of

MCPA and different cresols was used as a reference. Only MCPA required methylation on Emulfor liquid phase. All other compounds listed above could be chromatographed without derivatization. A sensitivity was used which gave ca. 10 cm peak hight for 20 ng of MCPA.

RESULTS

Acute experiment

In the acute subcutaneous toxicity study LD_{50}-value of sodium-MCPA was 500 mg/kg. All MCPA injections caused a myotonia in 15-30 min. Also diarrhea occurred. In autopsy of the dead animals extensive haemorrhages were observed in the gastrointestinal and urinary tracts.

In the histopathological study the most obvious changes were observed in the liver and spleen. In the liver sinusoids were hyperemic and in the parenchymal cells necroses consisting one or few cells were found. The necrotic foci contained polymorphonuclear leucocytes, degenerated parenchymal cells and nuclear debris. Rather often these necrotic foci were adjacent to portal areas. These changes were observed especially at the dose of 700 mg/kg. Pathological changes of the spleen were found mostly in the white pulp. In experimental group the periarterial lymphoid tissue was much less than in untreated control animals, and it contained only a few germinal centers. Foci of necrotic cells, polymorphonuclear leucocytes and macrophages filled with phagocytized material were found in the periarterial lymphoid tissue. This phagocytized material probably originated from the necrotic lymphoid cells of the white pulp. In other organs no histopathologic changes could be observed.

The concentrations of MCPA in some tissues of the dead animals are presented in Table I. The concentrations in tissues varied between 120 and 480 µg of MCPA/g of wet tissue. At all doses the highest concentration was observed in the kidney. Because the resolution ability of a glass capillary column is great the number of peaks in our chromatograms was also voluminous. No additional peaks, compared with controls, could, however, be observed in the acute experiment.

TABLE I

Concentration of MCPA in some tissues of dead male rats. Mean$\pm$SD are given. Number of animals is shown in parentheses.

Tissue	Concentration of MCPA (µg/g of wet tissue)		
Dose	500 mg/kg	600 mg/kg	700 mg/kg
Liver	136.5$\pm$37.5(15)	193.0$\pm$34.7(5)	403.9$\pm$71.1(15)
Kidney	235.5$\pm$43.2(15)	361.4$\pm$63.2(5)	482.0$\pm$86.8(15)
Muscle	118.8$\pm$20.9(15)	204.0$\pm$34.7(5)	276.0$\pm$96.5(15)
Spleen	205.9$\pm$89.7(15)	220.0$\pm$37.4(5)	238.1$\pm$88.0(15)

Subchronic experiment

Development of body weight, average daily water consumption and dose of sodium-MCPA during the subchronic exposure are seen in Table II.

TABLE II

Development of body weight of male rats, average daily water consumption and dose of sodium-MCPA in the subchronic experiment. Number of animals is given in parentheses.

Sodium MCPA in water (mg/l)	Water consumption (ml/kg/day)	Dose of sodium-MCPA (mg/kg/day)	Increase of body weight during exposure (%)
0	127	0	$76.1 \pm 23.7(6)$
100	130	13	$72.5 \pm 16.2(5)$
500	122	61	$81.5 \pm 54.9(6)$
1 000	112	112	$50.0 \pm 20.9(5)$
2 000	95	190	$28.5 \pm 15.9(6)$
3 000	82	246	$18.4 \pm 14.5(5)$

Water consumption of animals was decreased at the concentrations above 1 000 mg/l. Development of body weight was also delayed (1 000 mg/l $P \leq 0.05$; 2 000 and 3 000 mg/l $P < 0.001$) and relative weights of the liver (16-30 % at all concentrations), brain (11-26 % at the concentrations above 1 000 mg/l), kidney (22 % at the concentrations of 2 000 and 3 000 mg/l) and adrenals (27-45 % at the concentrations above 1 000 mg/l) were significantly increased. Autopsy did not reveal any gross pathological changes in the body.

In the subchronic experiment similar histopathological changes were seen as in the acute experiment. The amount of the white pulp in the spleen was slightly decreased and the changes resembled those of the acute experiment. In the liver focal degeneration of parenchymal cells was occasionally observed. In the testes loss of maturing spermatids and slight degenerative alterations in the semiferous epithelium were seen at the concentrations above 1 000 mg/l. In the other organs no histopathological changes compared with the controls could be observed.

The concentrations of MCPA in some tissues after the subchronic exposure are presented in Table III. The highest concentrations were observed in the liver and the lowest ones in the skeletal muscle. At the concentration of 2 000 mg/l tissue concentrations were approximately two times higher than at the concentration of 500 mg/l. A chromatogram which shows the residues in rat liver after the subchronic experiment is presented in Figure I. In the subchronic experiment

only one peak in addition to MCPA could be observed and its relative retention time was 1.05 which is equal with the retention time of 4-chloro-o-cresol. The concentration of the compound which was present only in the livers of the two greatest doses analyzed was approximately 46.9-30.8 µg/g of wet tissue.

TABLE III

Concentration of MCPA in some tissues of male rats after the 9-weeks subchronic exposure. Mean±SD are given. Number of animals is shown in parentheses.

Tissue	Concentration of MCPA (µg of wet tissue)		
Dose	500 mg/l	1 000 mg/l	2 000 mg/l
Liver	32.0±9.1(5)	43.0±4.7(5)	56.3±4.8(5)
Kidney	16.5±1.9(5)	25.5±2.8(5)	30.5±2.6(5)
Muscle	14.0±2.6(5)	16.0±1.4(5)	29.0±3.5(5)
Spleen			37.8 (2)
Testis	15.0±2.1(5)	26.0±1.4(5)	49.5±7.4(5)

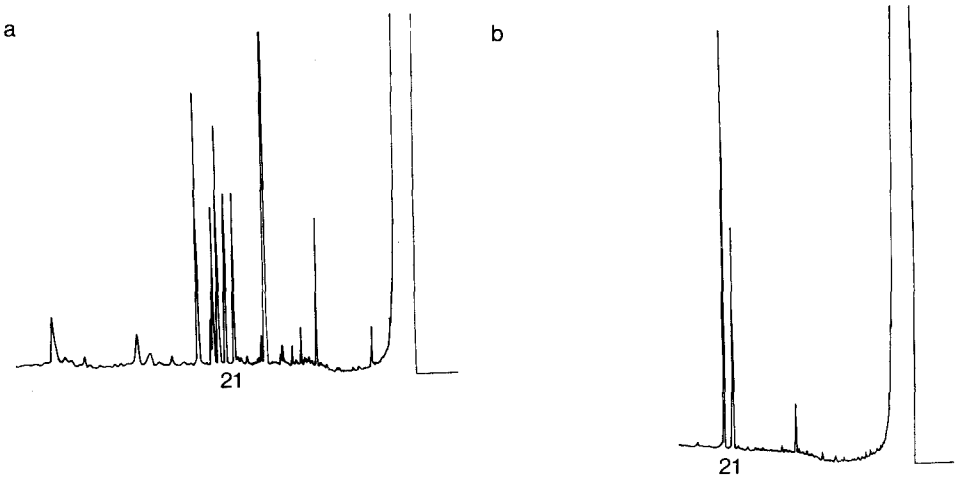

FIGURE 1. a. Chromatogram of the rat liver extract in subchronic experiment.

b. Reference chromatogram of MCPA (1) and 4-chloro-o-cresol (2).

DISCUSSION

Because of the difficulties encountered in the analysis of MCPA and other one chlorine containing related compounds a sensitive GLC method was developed in the present work. Method can be used also in the analysis of residues in wildlife, soil and water samples. The high resolution ability of this technique makes it possible to analyze residues even at picogram level.

Metabolism of 2,4-D and 2,4,5-T in the mammalian body is minor and phenols are one type of these metabolites (KHANNA and FANG, 1966; GRUNOW and BÖHME, 1974). In the subchronic experiment chromatograms of liver extracts contained a peak corresponding R_f-value of 4-chloro-o-cresol. Because no high-resolution mass-spectrometer was available the identity is based on R_f-value. The level of MCPA in the tissues of subchronic animals was relative low compared to the level in the acute experiment. Rapid elimination of MCPA principally in the urine has been reported previously (BACHE et al., 1964; ELO, 1976).

In our acute experiment LD_{50}-value of sodium-MCPA was 500 mg/kg which is in accord with the result by GURD et al. (1965), who reported LD_{50}-value 400 mg/kg for intraperitoneal injections. In the acute experiment sodium-MCPA caused myotonia, diarrhea and haemorrhages in the gastrointestinal and urinary tracts, which effects are also typical for 2,4-D and 2,4,5-T (DALGAARD-MIKKELSEN and POULSEN, 1962).

In the previous studies by GURD et al. it was concluded that MCPA itself caused little or no morphological changes in the heart, lung, kidney, liver, spleen, adrenal, stomach, ileum, pancreas, testis or ovary thyroid, brain and femoral bone marrow after feeding rats with various doses for seven months. The only significant changes were seen in the livers of the rats fed with 2 500 ppm Mecoprop 2-(2-methyl-4-chlorophenoxy)-propionic acid) . In the subacute (3 weeks) dermal toxicity study (VERSCHUUREN et al. 1975) at high concentrations histopathological changes were found in the liver, kidney, spleen and thymus of the rabbit. On the other hand no histopathological changes were found in the short term (90 days) feeding studies with rats.

In the present study essentially similar results were seen both after an acute treatment and after a short term oral administration. In the livers the degenerative changes were fairly small and confined to slight degree of parenchymal cell degeneration and possibly moderate unspecific hyperaemia. In the spleens the changes were much more striking especially after an acute administration in which cases there were almost complete disappearance of white pulp and marked lymphocyte depletion. This finding was similar to the depletion observed in the spleen and thymus of the rabbits by VERSCHUUREN et al. (1975). They concluded that this may well have been the reason for severe enteritis due to dysbacteria since it was seen only in animals fed with MCPA. On the other

hand it is difficult to explain the discrepancy of our findings and the results by VERSCHUUREN's group who did not find any histopathological changes in rats. Of course one reason may have been the different mode of administration of MCPA to the rat which in our study occurred by means of adding the MCPA into the water and in the other study by adding the compound in the food. Furthermore in the study by VERSCHUUREN et al. the rats body weight decreased as well as the food consumption which possibly also affected the levels of MCPA in the tissues.

It is obvious on the basis of this study as well as previous studies that in regard to general toxicity MCPA cannot be regarded highly toxic to rats.

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