The Mutagenicity of MCPA and Its Soil Metabolites, Chlorinated Phenols, Catechols and Some Widely Used Slimicides in Finland

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INTRODUCTION

Because a generally regarded assumption is that the mutagenicity and the carcinogenicity of a chemical compound correlate, the mutagenic properties measured by the first tier (FLAMM 1974) of several widely used compounds were tested in this work.

The use of MCPA (4-chloro-2-phenoxyacetic acid) has increased as a herbicide in the Nordic countries during the last few years. This probably dues to the fact that the herbicides used earlier (2,4-D and 2,4,5-T) contain dioxines the toxic effects of which are extensive. MCPA has been regarded as a relative safe compound (GURD et al. 1965, VERSCHUUREN et al. 1975, HATTULA et al. 1976). Although the sale of pure MCPA decreased more than 200 tons in Finland from 1973 to 1974 the sale of mixed products increased almost 300 tons (MARKKULA and TIITTANEN 1975). The last official figures in Finland (TIITTANEN and BLONQVIST 1976) show that the sale of MCPA increased from 1974 to 1975 400 tons.

4-chloro-o-cresol was first identified as the metabolite of MCPA (GAUNT and EVANS 1961) and it has later been shown to be the metabolite of other phenoxyacetic acid herbicides, too (BJERKE et al. 1972). Our own analyses show that the technical product of MCPA in Finland also contains approx. 4% 4-chloro-o-cresol as an impurity.

5-chloro-3-methylcatechol was first identified as the metabolite of MCPA by GAUNT and EVANS (1971). Generally, catechols are known as metabolites of chlorinated phenols (BOLLAG et al. 1968a, HORVATH and ALEXANDER 1970, HORVATH 1971) and the toxicity of catechols to cell is evident (HORVATH).

Chlorinated phenols are widely used as fungicides and slimicides around the world. Some of them are also known as metabolites of chlorophenoxyacetic acid herbicides (HELLING et al., LOOS et al. 1967, BOLLAG et al. 1968b, HORVATH 1971, IDE et al. 1972, FREAL and CHADWICK, CROSBY and WONG 1973, CLARK et al. 1975) and they are distributed on the areas where these herbicides have been used. Their use in sawmills has been realized to be an environmental hazard (LEVIN et al. 1976). Recently they have been discovered in the bleaching solution of lignin and according to primary results (KNUUTINEN, personal communication) the solution contained 2 ppm

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2,3,4,6-tetrachlorophenol and 0.2 ppm 2,4,6-trichlorophenol and several other chlorinated phenols (0.2 - 0.5 ppm).

The Fennosan compounds (trade name by Kemira Co., Finland) studied are widely used slimicides and fungicides in the wood-pulp industry. The toxicity of 3,5-D and the quinoline compounds is known but no data are available of the toxicity of Fennosan F-50. The toxicity of some phtalates which are present in the commercial product of Fennosan F-50 have been studied (BELISLE et al. 1975, LAKE et al. 1975). In this work, therefore both the mutagenicity of the active ingredient and the commercial product (mixture of different compounds) were tested separately.

The test method was the original Salmonella/mammalian microsome mutagenicity test by AMES et al. (1975) which is based on the use of Salmonella typhi-murium bacteria as test organisms for carcinogens and mutagens. Certain mutants of the bacterium cannot grow on a histidine deficient medium. If the mutants to be tested cause point mutations they revert the strains back to prototrophy and the mutagenic effect is detected as a bacterial growth.

MATERIALS AND METHODS

The chemicals tested

MCPA (99.9% purity, Kemisk Værk, Denmark)
5-chloro-salicylic alcohol (hydroxymethyl compound of MCPA)(+)
3-chloro-o-cresol (+)
4- " Fluka purum
5- " "
5-chloro-3-methylcatechol (+)
3,4-dichlorocatechol (+)
3,5- " (+)
3,6- " (+)
3,4,5-trichlorocatechol (+)
2,3-dichlorophenol Fluka purum
2,4- " "
2,5- " "
2,6- " "
3,4- " "
3,5- " "
2,3,5-trichlorophenol "
2,3,6- " "
2,4,5- " "
2,4,6- " "
2,3,4,6-tetrachlorophenol "
Fennosan F-50 [active ingredient 1,4-bisbromoacetoxy-2-butene (U.S. Patent n = 2.840,598, June 24, 1958)], Kemira Co., Finland.
Fennosan H-30 (8-hydroxyquinoline), Kemira Co.
Fennosan B-100 (3,5-D = 3,5-dimethyltetrahydro-1,3,5-thiadiazine-2-thione), Kemira Co.
Fennotox 45 (Copper-8-hydroxyquinolate), Kemira Co.

benzo(a)pyrene	Fluka
2-aminofluorene	Sigma
9-aminoacridine	Fluka
MNNG	(N-methyl-N'-nitro-N-nitrosoguadinine) Sigma

The compounds denoted by a (+) were synthesized in the Department of Chemistry, University of Jyväskylä and the purity was tested by UV-, IR-, NMR and mass spectrometry.

The media used were as follows: Vogel-Bonner -stock solution, minimum-glucose agar, nutrient agar, broth solution, top agar, 0.5 mM histidine-biotine solution, 0.2-M phosphate buffer and the cofactor solution as described by AMES et al. (1975).

The enzyme inducer used was Aroclor 1254 (Monsanto) dissolved in sterile corn-oil, concentration 200 mg/ml.

The bacteria used were Salmonella typhi-murium tester strains originating in strain LT2: TA98, TA100, TA1535, TA1537.

The rats were male Wistar-rats, 2-3 months old, served as a source of the liver homogenate. The rats were induced by Aroclor 1254 5 days before the sacrifice and the food was removed one day before the sacrifice.

The homogenate was made as described by AMES et al. (1975) and it was denoted by S-9. The final S-9Mix (liver homogenate + cofactor solution) contained per ml 0.1 ml S-9, 8 µmoles $MgCl_2$, 33 µmoles KCl, 5 µmoles glucose-6-phosphate, 4 µmoles NADP and 100 µmoles phosphate buffer. (pH was 7.4.)

Experimental

The test was carried out as described by AMES et al. (1975). Firstly the bacteria strains were tested by testing the histidine requirement, spontaneous mutations, chrystal-violet sensitivity, sensitivity to UV-light and to ampicillin, respectively. The mutagenesis tests were carried out as plate incorporation assays. All compounds studied were dissolved in dimethylsulphoxide (MERCK, p.a.).

The compounds were tested over a wide range of concentrations (0.5, 5, 50 and 500 µg/plate) both in the presence and absence of S-9Mix. Also the water-soluble compounds (MCPA, Fennosan F-50, Fennosan H-30, Fennosan B-100 and Fennotox 45) were dissolved in dimethylsulphoxide. The revertant colonies were calculated after incubation of 48 hours at 37°C.

Benzo(a)pyrene (10 µg/plate), 2-aminofluorene (10 µg), 9-aminoacridine (100 µg) and MNNG (5 µg) served as positive controls. Also different amounts of S-9/S-Mix were tested with some of the compound by the tester strains TA98 and TA100 because, as described by AMES et al. (1975), the amount of the liver homogenate fraction which gives the optimum mutagenic effect may vary with test compounds. In this test MCPA, 4-chloro-4-o-cresol and 5-chloro-3-methylcatechols were used.

RESULTS

The results of 5 µg of test compounds/plate and positive control mutagens are shown in Table 1. None of the test compounds caused significant increase in revertant colonies under conditions where increased back mutations occurred in the plates containing control mutagens. The number of colonies by using 0.5 and 50 µg of test compounds per plate were comparable to those observed in Table 1 but in the presence of 500 µg/plate the number of colonies decreased due to the toxic effect of the compounds. The toxicity of Fennosan F-50, its active ingredient and Fennosan H-30 was obvious and the results are based on 0.005 µg/compound/plate.

In Figure 1 are shown the results of the testing of the amount of S-9 on the mutagenesis of TA100. The results show that there were no difference in the number of colonies when concentrations 0.05, 0.1, 0.2 or 0.4 ml S-9/S-Mix were used with the tester strain TA100.

DISCUSSION

Because of the extensive use of 2,4-D and 2,4,5-T also their toxicity, mutagenicity and carcinogenicity have been widely studied. MCPA was taken in use at the time when little attention was paid to the possible toxicity of the compound. The studies carried out on MCPA (GURD et al. 1965, VERSCHUUREN et al. 1975, HATTULA et al. 1976) show the relatively low toxicity of the compound. The degradation products of MCPA in soil, however, have not been studied practically at all. The results of the present study show that MCPA and its metabolites, chlorinated phenols and catechols studied do not show a positive result. This result must be regarded very important because of the increasing use of MCPA and the wide distribution of chlorinated phenols as environmental contaminants. However, we want to point out that according to the tier system of mutagen testing (FLAMM 1974) the test system used belongs to the first tier and a negative result in the test used does not completely exclude the possible risk of the mutagenicity and carcinogenicity of the compounds studied. As long as the use of the MCPA is increasing and new possible sources of chlorinated phenols and catechols are found it is important to ascertain the results observed by using the mutagenicity tests of second tier.

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TABLE 1

Reversion of the bacterial tester strains with the test compounds and positive control mutagens (number of colonies calculated) at the level of 5 µg of the compound. [(+) = S-9Mix added, (-) = without S-9Mix.]

Compounds tested	µg/plate	TA98		TA100		TA1535		TA1537	
		+	-	+	-	+	-	+	-
MCPA	5	53/55[X]	48/36	94/66	182/176	18/18	23/41	14/10	11/9
5-chlorosalicylic alcohol	"	15/38	38/42	200/148	120/116	12/20	42/25	8/8	5/6
3-chloro-o-cresol	"	58/49	61/65	164/178	186/228	15/18	18/18	9/13	8/9
4- "	"	47/55	31/36	86/66	175/66	15/18	31/41	12/10	13/9
5- "	"	44/49	34/65	126/178	190/228	10/18	23/18	18/13	9/9
5-chloro-3-methylcatechol	"	46/55	39/36	86/66	124/176	15/18	23/41	13/10	16/19
3,4-dichlorocatechol	"	144/63	110/60	314/332	272/196	23/18	28/61	20/33	19/21
3,5- "	"	55/49	64/65	238/206	200/239	17/27	10/34	2/9	4/6
3,6- "	"	56/49	45/65	136/178	154/228	18/18	18/18	12/13	19/19
3,4,5-trichlorocatechol	"	59/49	34/65	321/206	262/239	21/27	52/34	12/9	8/6
2,3-dichlorophenol	"	42/49	47/65	92/67	182/115	7/16	18/46	19/8	7/14
2,4- "	"	48/52	48/36	140/168	160/142	21/26	59/30	5/8	11/6
2,5- "	"	32/49	54/65	72/67	117/115	14/16	31/46	9/8	7/14
2,6- "	"	31/43	34/65	88/67	82/115	13/16	58/46	3/8	7/14
3,4- "	"	56/49	58/65	118/67	84/115	25/16	44/46	8/8	6/14
3,5- "	"	84/63	55/60	296/332	352/196	15/18	58/61	12/33	16/21
2,3,5-trichlorophenol	"	64/52	62/36	144/168	188/142	24/26	20/30	12/18	15/16
2,3,6- "	"	67/63	53/63	368/332	308/196	16/18	59/61	9/6	19/21
2,4,5- "	"	12/52	30/36	96/168	192/142	22/26	37/30	16/8	10/6
2,4,6- "	"	72/52	32/36	112/168	140/142	36/26	44/30	13/8	12/6
2,3,4,6-tetrachlorophenol	"	41/49	39/65	402/239	222/239	18/27	33/34	7/9	10/6
Fennotox 45	"	42/49	43/60	188/148	184/116	13/34	22/40	13/8	6/6
Fennosan B-100	"	57/49	45/60	156/48	116/116	0/34	40/40	2/8	8/6
Fennosan B-50 active ingredient	0.005	36/38	26/42	112/148	114/116	21/20	7/25	8/6	3/6
Fennosan F-50	"	33/38	52/42	144/148	200/116	14/20	9/25	5/6	0/6
Fennosan H-30	"	36/38	25/42	280/148	202/116	12/20	15/25	3/6	2/6
2-aminofluorine	10	3 480/63							
benzo(a)pyrene	10			1 244/332					
MNNG	5						13 600/46		
9-aminoacridine	100								8 200/9

[X]Revertant colonies in test plates/revertant colonies in control plates

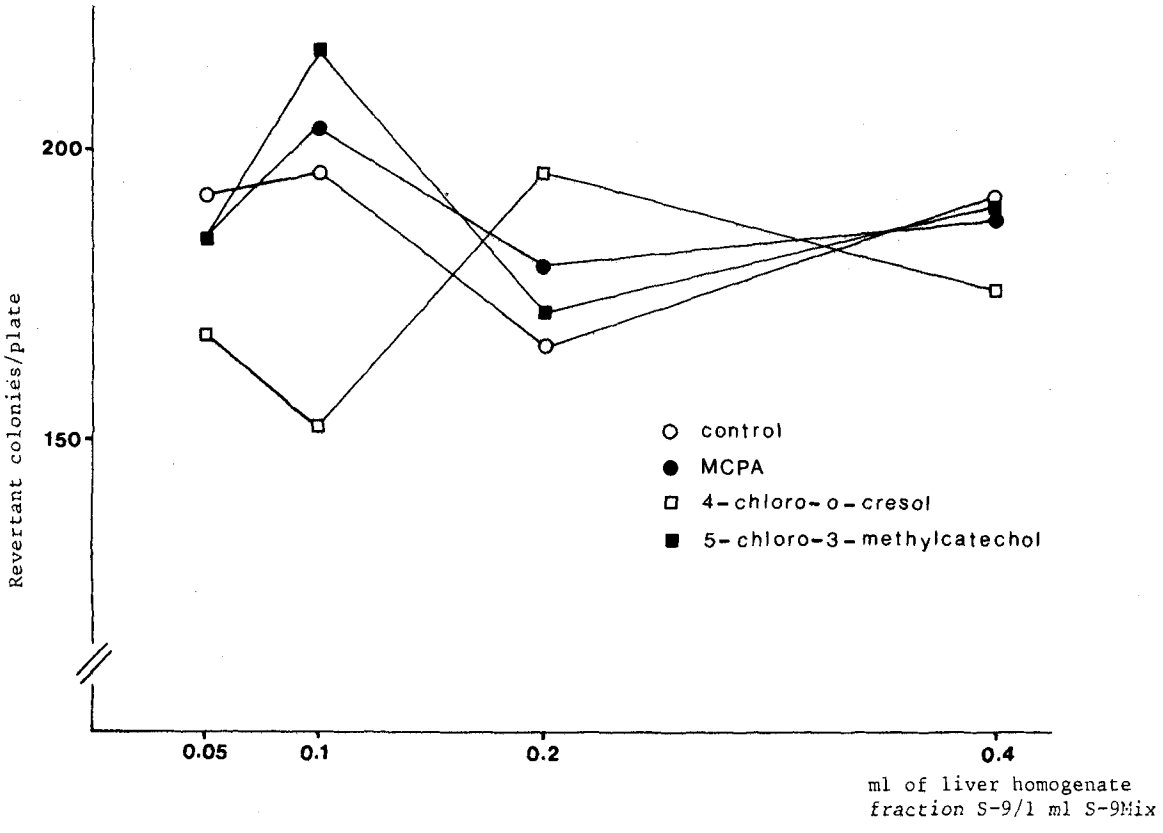

FIGURE 1. Effect of the amount of S-9 on the mutagenesis of the tester strain TA100. MCPA, 4-chloro-o-cresol and 5-chloro-3-methylcatechol were applied 5 µg/plate.

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