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Combustion processes of organic materials also produce chlorophenols and their highly toxic dimeric products (10, 11). Polychlorophenols are among the main components of the smoke of communal waste burning plants (12). In Finland the analysis of the fly ash of such a plant showed 1 ppm chlorophenols which together with polychlorobenzenes, PCDD and PCDF caused a mass disappearance of several nesting birds on the nearby area (13).

The mutagenicity of pulp mill effluents and chlorophenols has been studied by Ander et al. and Räsänen et al. (14, 15). The latter study was conducted by the Ames test and 11 chlorophenols as well as 5 chlorocatechols all gave negative response. Nestmann and Lee (16) studied 42 compounds of a pulp mill effluent by *Saccaharomyces cerevisiae*. The two chlorophenols of the study gave negative response in yeast whereas 4,5 - dichlorocatechol and 4,5-dichloroguaiacol were positive. Ames test and yeast were used in the study of the genetic activity of bleached kraft chlorination stage effluents (17). The XAD-2 resin concentrate of the effluent induced mutations in three *Salmonella* strains and the extract had genetic activity in yeast.

In the study of Kinae et al. (18) 2,4,6-trichlorophenol and 3,4,5,6-tetrachloroguaiacol exhibited high DNA damaging potency in *Bacillus subtilis*.

In the present study we used a cell-mediated assay developed by Huberman and Sachs (19) in which Chinese hamster lung cell line, V79 is used. In this assay we studied 4 chlorophenols, 4- chlorocatechols, 3 chloroguaiacols, one chlorinated methoxyphenol and one commercial fungicide mixture containing chlorinated phenols and methoxyphenols.

MATERIALS AND METHODS

Chemicals

The chemical compounds chosen for this study are either used as wood preservatives or they appear in the effluents of wood pulp industry as a result of reactions of chlorine with natural phenolis compounds of the wood and the bleaching process.

The compounds studied are presented in Figure 1 and in addition, one commercial fungicide mixture KY-5 was included in the experiment. It contains mainly 246TCP and 2346eCP and some methoxyphenols.

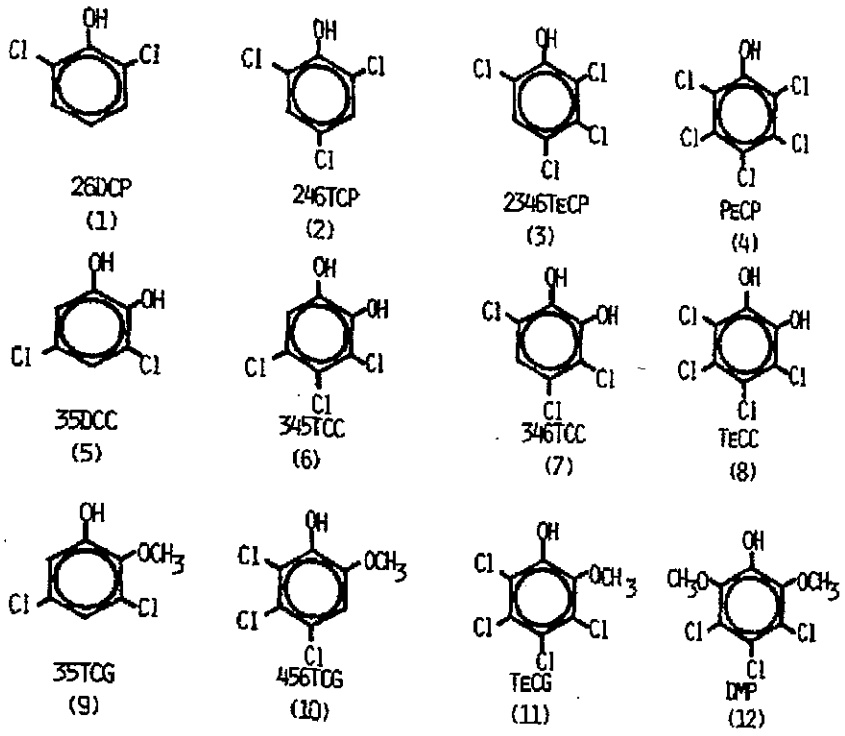

Figure I. Structures and notation of the compounds of the study.

(1) 26DCP,2,6-dichlorophenol, (2) 246TCP,2,4,6-trichlorophenol, (3) 2346TeCP,2,3,4,6-tetrachlorophenol, (4) PeCP, pentachlorophenol, (5) 35DCC,3,5-dichlorocatechol, (6) 345TCC,3,4,5-trichlorocatechol, (7) 346TCC, 3,4,6-trichlorocatechol, (8) TeCC, tetrachlorocatechol, (9) 35DCG,3,5-dichloroguaiacol, (10) 456TCG,4,5,6-trichloro-guaiacol, (11) TeCG, tetrachloroguaiacol, (12) DMP,3,4,5-trichloro-2,6-dimethoxyphenol.

The chlorinated phenols were purchased from Fluka A.G. Switzerland and they were purified at the Department of Chemistry, University of Jyväskylä to the purity >99.95 %. Final checking was made on Perkin Elmer Sigma 3 gas chromatograph equipped with FI and EC detectors and an SE-30 quartz capillary column. No dioxins were observed at ppb level.

The chlorinated catechols and guaiacols were synthetized at the Department of Chemistry, University of Jyväskylä (20, 21).

3,4,5-trichloro-2,6-dimethoxyphenol was prepared by chlorination of 2,6-dimethoxyphenol (Fluka) with chlorine gas in CS_2 at 20°C. The structures of the synthesized compounds were verified by GLC, Jeol FX-60-NMR and Varian MAT-212 GC-MS.

KY-5 was obtained from KYMI C.O., Finland.

In the hepatocyte-mediated assay (24), primary hepatocytes of 2-3 months old male Sprague-Dawley rats were used and only one concentration of 8 compounds was examined by the method. As a positive control we used DMN, (N-nitrosodimethylamine, 98% pure Sigma).

V79 cells were seeded at 5 x 10^5 cells/25 cm^2 T-flask. After 18 hr 2 x 10^6 primary rat hepatocytes were seeded on the V79 cells in 4 ml Leibovitz L-15 medium (Gibco) containing 2 mM glutamine, 10% fetal calf serum, 100 IU penicillin/ml and streptomycin 100 µg/ml. After 3 h the medium was changed and the chlorophenols were added in 4 ml of fresh medium. The cells were dispersed 18 hr later with 0.05% trypsin and 0.02% EDTA. The experiment was conducted as in the direct method. The following compounds were studied in this method: 26DCP, 246TCP, 2346TeCP, PeCP, 346TCC, TeCC, 456TCG and the commercial fungicide, KY-5.

RESULTS

The study was started simultaneously as a direct and fibroblast-mediated assay with part of the chemicals. The results of the cell-mediated assay with fibroblasts are presented in Table I.

Table I. Induction of 6-thioguanine resistant mutants in the fibroblast-mediated assay by polychlorophenolic compounds and DMBA as a positive control.

	Concentration µg/ml	Cloning efficiency %	6-thioguanine resistant mutants per 10^6 survivors
Control		74.9	0
26DCP	30	45.5	0
"	90	55.1	2
"	150	58.5	0
2346TeCP	10	79.7	0
"	15	56.0	0
"	20	50.0	0
35DCC	1.5	44.8	0
"	3.0	60.6	2
"	10.0	52.8	2
KY-5	2.4	75.3	0
"	8	78.0	0
"	24	67.2	1
DMBA	0.01	56.6	264
"	0.03	41.8	490
"	0.10	21.4	645

Because no response was obtained in the cell-mediated assay it was discontinued and all compounds were studied in the direct method. Table II shows those compounds which caused the induction of 6-thioguanine resistant mutants the number of which was three-fold or more as compared to the control.

Table II. Induction of 6-thioguanine resistant mutants in the direct method by chlorophenolic compounds and MNNG as a positive control.

	Concentration µg/mg	Cloning efficiency %	6-thioguanine resistant mutants per 10^6 survivors
Control	0	61.7	0
246TCP	10	58.0	1
"	20	55.0	13
"	30	55.1	53
"	45	47.5	25
"	60	40.5	11
Control	0	74.3	0
2346TeCP	3.5	75.3	12
"	7	73.0	17
"	10	70.8	35
"	15	68.5	18
"	20	60.0	10
Control	0	54.5	0
246TCC	3	51.2	0
"	4	45.0	11
"	5	38.3	16
"	6	27.6	20
Control	0	51.3	0
456TCG	10	51.5	5
"	30	48.8	16
"	50	40.0	27
Control	0	78.0	0
KY-5	2.4	68.0	5
"	8	67.7	4
"	24	50.6	2
Control	0	65.5	0
MNNG	0.5	54.5	471
"	1.0	45.5	799

In the direct method positive results were obtained with 246TCP, 2346TeCP, 346TCC, 456TCG and KY-5, the commercial fungicide micture. Even the highest mutagenicity observed was roughly one tenth or less of the mutagenic power of MNNG, the positive control. The compounds which produced no mutants or less than three times as compared to the control were 26DCP, PeCP, 35DCC, 345TCC, TeCC, 35DCG, TeCG and DMP.

Finally, all the compounds which gave positive response in the direct method plus 26DCP, PeCP and TeCC, one concentration of each were conducted in the hepatocyte-mediated assay and DMN was used as a positive control. The results are shown in Table III and it shows either decrease or total disappearing of the mutagenicity.

Table III. Induction of 6-thioguanine resistant mutants in the hepatocyte-mediated assay by polychlorophenolic compounds and DMN as a positive control.

	Concentration	Cloning efficiency %	6-thioguanine resistant mutants per 10^6 survivors
Control	µg/ml	54.7	34
26DCP	100	49.9	12
246TCP	30	43.3	0
2346TeCP	10	61.0	0
PeCP	15	55.3	0
346TCC	5	34.1	0
TeCC	5	54.1	0
456TCG	30	36.7	6
KY-5	10	51.2	3
	mM		
DMN	0.3	49.3	102
	1.0	53.1	300
	3.0	37.6	347

CONCLUSION

The present investigation was undertaken in an attempt to find mutagenic compounds among chlorinated phenols and related compounds in a mammalian cell mutagenesis assay. The compounds studied are widely spread as environmental pollutants. 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, 3,4,6-trichlorocatechol and 4,5,6-trichloroguaiacol and one commercial fungicide mixture were found mutagenic and there was a dose related increase in the number of mutant colonies. The mutagenicity, however, decreased or disappeared in the hepatocyte-mediated assay. 2,3,4,6-tetrachlorophenol and KY-5 which gave positive response in the direct method gave negative responses in the fibroblast-mediated assay. Similar decrease or disappearance of mutagenicity of XAD-resin concentrate of chlorination stage pulp mill efflunets was observed in three strains of *Salmonella typhimurium* (17) in the presense of S9 mix, S9 mix without cofactors or heat activated S9 mix.

In our study the maximum response was roughly one tenth as compared to MNNG at 0.5 µg/ml level, which shows the compounds studied weak mutagenic. The maximum response was

a factor of 50 higher compared to the control at 30 µg/ml of 246TCP and only a factor of 5 of the commercial fungicide mixture.

In the study of pulp mill effluent by a bacterial assay (17) the mutagenic response was a factor 3.6 times higher at 1 mg pulp mill extract/ml level as compared to the background level. In the study of Kinae et al. (18) high DNA damaging potency was obtained by 246TCP and 3456TeCG at 0.5 and 0.1 mg/disk level.

It is important to know the mutagenic activity of pulp industry effluents which are complex mixtures but it is also important to know the mutagenicity of single chlorinated phenols and related compounds because they seem to appear from new sources to the environment like in the precipitation of snow and concentrate in animal tissues, too (1).

Chlorophenols did not give positive response in the *Salmonella typhimurium* and *Saccharomyces cerevisiae* assays (15, 16) although one chloroguaiacol and -catechol gave positive response in the yeast. However, both a chlorophenol and chloroguaiacol gave positive response in *Bacillus subtilis*.

It is obvious that several *in vitro* systems, including the mammalian cell assay should be used simultaneously in the study of the mutagenicity of chlorinated phenols and related compounds. The final evaluation of their mutagenic potency and risk to the environment can, however, be ascertained when more data of their dilution in waters and the concentration coefficients in the tissues of different organisms is available.

REFERENCES

1. J. Paasivirta, K. Heinola, T. Humppi, A. Karjalainen, J. Knuutinen, K. Mäntykoski, R. Paukku, T. Piilola, K. Surma-aho, J. Tarhanen, L. Welling, H. Vihonen and J. Särkkä, Chemosphere, in press.
2. J-O. Levin and C-A. Nilsson, Chemosphere, 6, 443 (1977).
3. K. Lindström and J. Nordin, J. Chromatog., 128, 13 (1976).
4. L. Lander, K. Lindström, M. Karlsson, J. Nordin and L. Sörensen, Bull. Environ. Contam. Toxicol., 18, 663 (1977).
5. N. Gjøs and G.E. Carlberg, Adv. Mass Spectrom., 813, 1468- (1980).
6. J. Paasivirta, Environmental Chemistry Comprehensive Course, Assoc. Finn. Chem. Soc., Helsinki 114 (1983).
7. J. Paasivirta, J. Särkkä, T. Leskijärvi and A. Roos, Chemosphere, 9, 441 (1980).
8. J. Knuutinen, J. Tarhanen and M. Lahtiperä, Chromatographia, 15, 9 (1982).
9. J. Knuutinen, J. Chromatog., 248, 289 (1982).

10. K. Olie, P.L. Vermeulen and O. Hutzinger, Chemosphere, 7, 419 (1977).
11. H.R. Buser and H.P. Bosshardt, Chemosphere, 7, 419 (1978).
12. T.O. Tiernan, M.L. Taylor, J.H. Garret, G.F. Van Ness, J.G. Solch, D.A. Deis and D.J. Wagel, Chemosphere, 12, 595 (1983).
13. J. Paasivirta, Kemia-Kemi, 11, 452 (1984).
14. P. Ander, K.-E. Erikson, M.-C. Kolar, K. Kringstad, U. Rannug and C. Ramel, Svensk. Papperstid., 80, 454 (1977).
15. L. Räsänen, M.L. Hattula and A.U. Arstila, Bull. Environ. Contam. Toxicol., 18, 565 (1977).
16. E.R. Nestmann and E.G.-L. Lee, Mutat. Res., 119, 273 (1983).
17. O.P. Kamra, E.R. Nestmann, G.R. Douglas, D.J. Kowbel and T.R. Harrington, Mutat. Res., 118, 269 (1983).
18. N. Kinae, T. Hashizume, T. Makita, I. Tomita, I. Kimura and H. Kanamori, Water Res., 15, 17 (1981).
19. E. Huberman and L. Sachs, Int. J. Cancer, 13, 326 (1974).
20. J. Knuutinen, J. Chromatog., 209, 446 (1981).
21. J. Knuutinen and E. Kolehmainen, Chromatographia, 15, 707 (1982).
22. R. Langenbach, H.J. Freed, D. Raven and E. Huberman. Nature, 276, 5685,
23. E. Huberman and T.J. Slaga, Cancer Res., 39, 411 (1979).
24. C.A. Jones and E. Huberman, Cancer Res., 40, 406 (1980).

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MUTAGENESIS OF MAMMALIAN CELLS IN CULTURE BY CHLOROPHENOLS, CHLOROCATECHOLS AND CHLOROGUAIACOLS

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ABSTRACT

The mutagenicity of 4 chlorinated phenols, 4 chlorinated catechols, 3 chlorinated guaiacols, one chloromethoxyphenol and one wood preservative mixture was studied in a mammalian cell assay, in which Chinese hamster cells V79 are used. Of the compounds studied 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, 3,4,6-trichlorocatechol, 4,5,6-trichloroguaiacol and the wood preservative were mutagenic in the test system used.

INTRODUCTION

The chlorophenolic compounds are widely spread as environmental pollutants the main sources of which are the fungicides, chlorine bleaching in wood pulp industry and combustion processes of organic materials (1). About 200 000 tons of chlorophenols are manufactured annually for use as pesticides (2). The wood pulp industry produces chlorophenols and related compounds which appear in the bleaching liquor as a result of chemical reaction of chlorine with the natural phenolic compounds of the wood. The first study of chlorophenolics in European pulp bleachery effluents was conducted by Lindström and Nordin (3) and later by Landner et al. (4) and Gjøs and Carlberg (5).

In Finland 1000 tons of chlorophenolic wood preservatives are used annually and the estimated amount of chlorophenols from pulp industry is of the order of 75000 tons (6). The first observations of chlorinated phenols in wildlife samples in Finland were

made in 1973 and later also guaiacols and catechols were identified in pike (7). The main route of chlorocatechols and guaiacols in the waters is wood pulp industry. Knuutinen et al. (8) identified 4 chlorocatechols in chlorination stage spent liquor and 9 guaiacols of the 15 theoretically possible ones were identified in the extraction stage spent bleach liquor (9).

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In the study of Kinae et al. (18) 2,4,6-trichlorophenol and 3,4,5,6-tetrachloroguaiacol exhibited high DNA damaging potency in *Bacillus subtilis*.

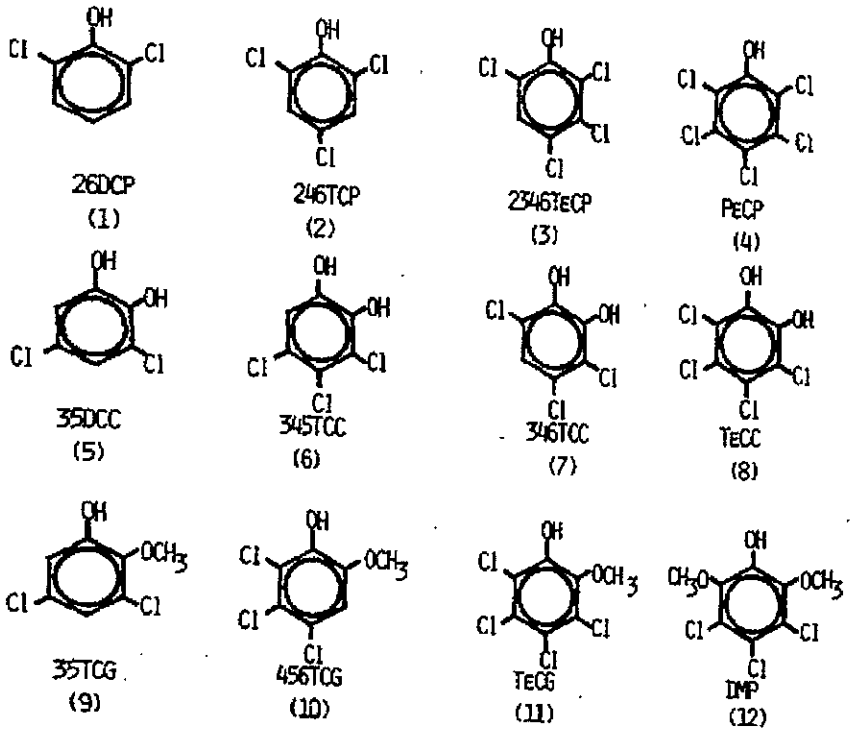
In the present study we used a cell-mediated assay developed by Huberman and Sachs (19) in which Chinese hamster lung cell line, V79 is used. In this assay we studied 4 chlorophenols, 4- chlorocatechols, 3 chloroguaiacols, one chlorinated methoxyphenol and one commercial fungicide mixture containing chlorinated phenols and methoxyphenols.

MATERIALS AND METHODS

Chemicals.

The chemical compounds chosen for this study are either used as wood preservatives or they appear in the effluents of wood pulp industry as a result of reactions of chlorine with natural phenolis compounds of the wood and the bleaching process.

The compounds studied are presented in Figure 1 and in addition, one commercial fungicide mixture KY-5 was included in the experiment. It contains mainly 246TCP and 2346eCP and some methoxyphenols.



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KY-5 was obtained from KYMI C.O., Finland.

MUTAGENESIS ASSAY

A cell mediated mutagenesis assay (19) was used as a direct method without metabolizing cells for all the compounds and part of the material was studied in a cell-mediated assay with irradiated fibroblasts and hepatocytes (22, 23, 24).

The Chinese hamster cells V79 derived from clone V79-4 were kindly supplied by Dr. E. Huberman, Oak Ridge National Laboratory, Tennessee, USA.

The cells were grown in Dulbecco's modified Eagle medium (Gibco Grand Island, N.Y.) supplemented with 10% fetal calf serum (Gibco G.I., N.Y.) and 2 mM glutamine (K.C. Biological, Kansas, USA), penicillin 100 IU per ml, streptomycin 100 µg and fungizone 250 ng per ml (Gibco, Grand Island, N.Y.) and incubated at 37 °C. The cultures were incubated in a humified incubator supplied with a constant amount of 10% CO_2 in air.

In the direct method 10^6 V79 cells in 10 ml medium were seeded in a tissue culture dish (ø 10 cm) and the test compound was added 24 hr after the V79 cells in 1 ml of medium. The maximum amount of acetone which was used as a solvent of the test compounds was 50 µl and it was added to controls, too. The cultures were incubated for 2 days and the cells were dissociated with trypsin-EDTA (0.05 and 0.1% Gibco) and seeded at 200 cells per 60 mm tissue culture dish in 5 ml of medium to determine the cloning efficiency and 10^5 cells per 10 mm dishes. 6 days later the cells were dissociated and reseeded for cloning efficiency, 200 cells per 60 mm dish, and 2 x 10^4 cells per 60 mm dish in 4 ml of medium for determination of the number of 6-thioguanine resistant mutants. 6-thioguanine (Sigma, St. Louis, USA) final concentration 40 µM, was added in 1 ml of medium. The colonies were counted after Giemsa staining. Cloning efficiency was determined by counting the number of colonies in five dishes per point 7-8 days after cell seeding. The frequency of 6-thioguanine resistant mutants was determined by counting 16 dishes per point 12-14 days after cell seeding.

As a positive control we used MNNG, (N-methyl-N'-nitro-N-nitrosoguanidine, Sigma).

In the cell-mediated method (22, 23) the chemicals were cocultivated with rat fibroblasts and V79 cells. 3 x 10^5 V79 cells were seeded on a monolayer of 2 x 10^6 5000R irradiated fibroblasts and the test compound was added and the experiment conducted as above. As a positive control we used DMBA, (7,8-dimethylbenz(a)anthracene, Fluka 98% pure). 26DCP, 2346TeCP, 35DCC and KY-5 were studied in this assay.

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