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Note

Synthesis and gas chromatographic–mass spectroscopic determination of polychlorinated dibenzo-*p*-dioxins and related compounds in the technical chlorophenol formulation Ky-5

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Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and diphenyl ethers (PCDEs) are well known contaminants of technical chlorophenol formulations[1–3]. Their concentrations vary greatly in different formulations, being typically 1–2000, 50–200 and 100–1000 mg/kg for PCDDs, PCDFs and PCDEs, respectively[4]. Owing to the lack of commercially available reference substances only few studies have been reported on isomer specific identification methods for PCDDs[5], PCDFs[6], and PCDEs[7]. However, in view of the great differences in the toxic and biological effects among different PCDD and PCDF isomers it is of great importance to know their exact structures.

Most studies concerning chlorophenol impurities have been focused on 2,3,7,8-tetraCDD, which is a contaminant of 2,4,5-trichlorophenol and its derivatives. Less attention has been paid to the PCDDs and related compounds of 2,4,6-tri- and 2,3,4,6-tetrachlorophenols, which have been used widely for wood preservation. Particularly polychlorinated phenoxyanisoles (PCPAs) are rather unfamiliar species among chlorophenol impurities[1,8].

The purpose of this work was to determine the major neutral impurities of Ky-5 chlorophenol formulation, which has been one of the most widely used wood preservations in Finland. The investigation was carried out with the aid of model compound synthesis and using gas chromatography with electron capture detection (GC–ECD) and gas chromatography–mass spectrometry (GC–MS) for identification and verification of the components. The major monomeric components of Ky-5 are 2,4,6-tri-, 2,3,4,6-tetra- and pentachlorophenols, and the results obtained may therefore be applied to other chlorophenols of similar composition.

EXPERIMENTAL

The general structures of the compounds studied in this work are shown in Fig. 1. The compounds were synthesised as follows:

Polychlorinated 2-phenoxyanisoles (PC2PAs, 21 isomers), 3-phenoxyanisoles (PC3PAs, 3 isomers) and 4-phenoxyanisoles (PC4PAs, 7 isomers) were obtained by methylation of the corresponding phenoxyphenols, whose syntheses and methylation procedures have published elsewhere[9,10].

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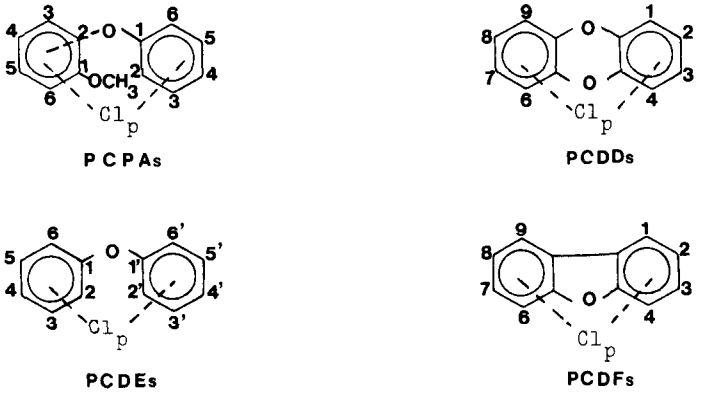
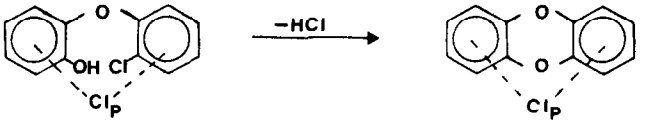


Fig. 1. Structures and abbreviations of the compounds studied.

Polychlorinated diphenyl ethers (PCDEs, fourteen isomers) were synthesised by coupling reaction of chlorinated diphenyl iodonium salts with chlorinated phenols[11].

Polychlorinated dibenzofurans (PCDFs, fourteen isomers) were obtained by cyclization of the above PCDEs with palladium(II) acetate[12].

Polychlorinated dibenzo-*p*-dioxins (PCDDs, twelve isomers) were synthesised by *in situ* cyclization of different polychlorinated 2-phenoxyphenols:



In the procedure the parent PC2PP was heated in N,N-dimethylformamide with potassium carbonate. Identification of the PCDDs formed (one to four isomers) in each experiments was based on the MS properties and GC elution order[13]. The detailed synthetic procedure is given below.

About 1 mg of the parent PC2PP was placed in a 10-ml vial equipped with a Teflon-lined cap containing 1 ml of N,N-dimethylformamide and 100 mg of potassium carbonate. Then the tube was heated in a oil-bath at 120–130°C for 2–3 h. After cooling, 4 ml of water was added and the PCDD precipitate formed was extracted with chloroform. The crude product (mixture of one to four isomers) was obtained by evaporation of the solvent. Further purification was not needed.

Pretreatment of the Ky-5 sample

The neutral components of Ky-5 were extracted and chromatographed into two fractions, the dioxin one (PCDDs + PCDFs + PCPAs) and the ether one (PCDEs) by the procedure of Buser and Bosshardt[2]. Both fractions were analysed by GC–ECD and GC–MS with the aid of authentic model substances. In the GC–ECD experiments OV-1, OV-1701 and SE-54 fused-silica capillary columns were

used. The ECD responses of PCDDs and PCDFs were taken to be those of PCDEs of the same chlorine number because quantitative PCDD and PCDF standard solutions of individual components were not available.

The Ky-5 used in this work consisted of a mixture (*ca.* 1:1) of 2,4,6-tri and 2,3,4,6-tetrachlorophenol sodium salts.

Apparatus and operating conditions

An Orion Analytica Micromat HRGC dual column gas chromatograph equipped with ^{63}Ni electron capture detectors was used for GC experiments. Fused-silica capillary columns, one 25 m × 0.3 mm I.D. SE-54 (film thickness 0.10 μm, chemically bonded) the other 20 m × 0.3 mm I.D. OV-1701 (0.10 μm, bonded) were used. The temperature programme was 10°C/min from 60°C to 260°C; this final temperature was then held for 30 min. The carrier gas was helium at a flow velocity of *ca.* 22 cm/sec. Detector and injector temperatures were 320 and 250°C, respectively.

Some GC–ECD runs were done with a Carlo Erba Series 4160 gas chromatograph equipped with cold on-column injector. Both a 25 m × 0.3 mm I.D. OV-1701 (0.25 μm non-bonded) and a 50 m × 0.3 mm I.D. OV-1 (0.52 μm, bonded) fused-silica capillary columns were used. The temperature programme was 15°C/min from 80°C to 200°C, and then 5°C/min to 260°C (with OV-1 to 280°C), with this final temperature held for 25 min. Hydrogen was used as carrier gas at a flow velocity of *ca.* 35 cm/sec.

MS investigations were carried out using Varian MAT 212 mass spectrometer equipped with Varian Series 3700 gas chromatograph and a 20 m × 0.3 mm I.D. fused-silica SE-54 capillary column. The capillary interface and ion source temperatures were 230 and 300°C, respectively. The mass spectra were recorded at 70 eV from mass number 50 up to 550.

RESULTS AND DISCUSSION

Synthesis of PCDDs

The PCDD model compounds prepared and the starting PC2PPs are shown in Table I. In previous studies, PCDD model substances have been prepared by direct chlorination of dibenzo-*p*-dioxin[20] or its lower chlorinated homologues[15,17], by pyrolytic dimerization of sodium or potassium salts of *o*-halophenols[14,16,21,22], and by condensation of chlorocatecols (*o*-dihydroxybenzenes) with polychloro- or polychloronitrobenzenes[15,17]. The most favoured method has been the the pyrolytic dimerization of chlorophenols because the starting compounds are readily available and the reaction itself is simple to carry out. However, this method gives low yields and complicated mixtures of PCDD isomers.

In the present work, the various PCDD isomers were synthesized via cyclization of their PC2PP precursors. Due to the Smiles rearrangement[18] four isomeric PCDDs (two, if phenoxy group is symmetrically substituted) were formed in this reaction. In cases where the cyclization leads to the formation of a PCDD with 2,3- or 1,2,3,4-chloro-substitution, the Smiles and normal cyclization gives the same PCDD(s) (one or two isomers). The advantages of this method lie in the minimal handling of the highly toxic PCDDs during the preparation procedure, and the almost quantitative yield. This method is therefore well suited for safe preparation of semiquantitative PCDD standards.

TABLE I

SYNTHESIS OF CHLORINATED DIBENZO-*p*-DIOXINS

Starting PC2PP	*Corresponding PCDD(s)*	*Ref. for previous synthesis*
46-246	1368 +	14,15
	1379	14
46-2346	12468 and/or 12479 +	21
456-246	12368 + 12379	21
45-2346	12478 + 12378	17
45-23456	123478	15,17
456-2346	123679 and/or 123689 +	16
	123678 + 123789	16,18,19,21
456-23456	1234678	16,17,21

TABLE II

RELATIVE RETENTION TIMES (RRT) OF PCDDs AND PCPAs ON SE-54 AND OV-1701 QUARTZ CAPILLARY COLUMNS

Retention times are normalized to that of 5-chloro-2-(2,4-dichlorophenoxy)-anisole (absolute retention times 18.14 and 17.54 min on SE-54 and OV-1701, respectively), which was defined as 1.000. For conditions see Experimental.

Compound	*RRT*		*Compound*	*RRT*	
	SE-54	*OV-1701*		*SE-54*	*OV-1701*
PCDDs			*PC2PAs*		
1368	1.075	1.052	5-35	0.980	0.968
1379	1.082	1.063	5-34	1.018	1.019
2378	1.125	1.106	5-246	1.052	1.045
12468 and/or			45-24	1.102	1.107
12479	1.192	1.170	5-234	1.128	1.133
12368	1.215	1.189	46-246	1.093	1.078
12478	1.220	1.196	0-23456	1.156	1.144
12379	1.225	1.203	5-2346	1.163	1.155
12378	1.253	1.229	456-24	1.171	1.163
123679 and/or			456-34	1.194	1.192
123689	1.377	1.338	46-2346	1.205	1.188
123678	1.409	1.363	456-246	1.227	1.210
123478	1.411	1.370	3456-24	1.254	1.237
123789	1.432	1.395	5-23456	1.289	1.271
1234678	1.659	1.588	456-345	1.294	1.286
PC4PAs			456-234	1.331	1.319
0-246	0.980	0.968	3456-345	1.351	1.325
2-246	1.081	1.077	456-2346	1.383	1.354
0-2346	1.096	1.086	45-23456	1.435	1.411
26-246	1.112	1.096	3456-234	1.458	1.426
0-23456	1.213	1.195	456-23456	1.597	1.546
26-2346	1.233	1.212	*PC3PAs*		
26-23456	1.383	1.349	0-23456	1.181	1.167
			46-23456	1.405	1.404
			246-23456	1.528	1.445

TABLE III

RELATIVE RETENTION TIMES (RRT) OF PCDFs AND PCDEs ON SE-54 AND OV-1701 QUARTZ CAPILLARY COLUMNS*

PCDFs	*RRT*		*PCDEs*	*RRT*	
	SE-54	*OV-1701*		*SE-54*	*OV-1701*
2468	1.064	1.061	22'44'	0.978	0.967
2378	1.111	1.114	33'44'	1.023	1.018
12468	1.158	1.138	22'44'5	1.063	1.053
12478	1.190	1.168	23'44'5	1.083	1.076
23468	1.206	1.206	22'344'	1.107	1.105
12378	1.215	1.193	33'44'5	1.114	1.109
23478	1.243	1.242	233'44'	1.124	1.124
123468	1.318	1.279	22'44'55'	1.142	1.133
124678	1.324	1.287	22'344'5	1.183	1.172
124689	1.348	1.311	22'3'44'5	1.193	1.186
123678	1.371	1.330	2'33'44'5	1.219	1.211
234678	1.406	1.400	22'33'44'	1.253	1.249
1234678	1.567	1.495	22'344'55'	1.277	1.260
1234689	1.598	1.526	22'33'44'5	1.353	1.335

* Calculation and conditions as in Table II.

On the basis of the above discussion, it would be of particular interest to check the possible formation of dioxin from PC2PPs during sample preparation for PCDD analysis. The cyclization of PC2PPs is monomolecular and the conversion into PCDDs is therefore independent of concentration. Soxhlet extraction with high boiling solvent, for example, might thus to some extent generate PCDDs.

GC and MS properties of PCDDs, PCDFs, PCDEs and PCPAs

The relative GC retention times of the compounds studied are given Tables II and III. Fig. 2 shows examples of the typical mass spectra of PCDDs and PC2PAs.

Table II shows that PC2PAs eluate within the same retention time range as PCDDs with one chlorine atom fewer. For example, 12379- and 12378-pentaCDDs have almost same retention time on an SE-54 stationary phase as 456-246- and 3456-24-hexaC2PAs, respectively.

As can be seen in Fig. 2, PC2PAs have in their mass spectra an intense fragment ion $(M-CH_3Cl;\ M-50)^{+\cdot}$ with exactly the same m/z value and chlorine number as $M^{+\cdot}$ of PCDDs with one chlorine atom fewer. The further fragment ions of PCDDs are overlapped by those of PC2PAs. Therefore, the presence of PC2PAs interferes in the mass fragmentographic determination of PCDDs. The possibility of the false positive determination of dioxins is increased by the fact that PC2PAs track completely through all stages of sample purification with PCDDs[23]. Recent findings of PC2PAs in environmental[8] and biological samples[24] give rise to further investigations for developing new analytical methods of PCDDs.

In the case of PCDFs, the corresponding interference in their MS detection is caused by the fragment ion $(M-2Cl,\ M-70)^{+\cdot}$ of PCDEs with two more chlorine

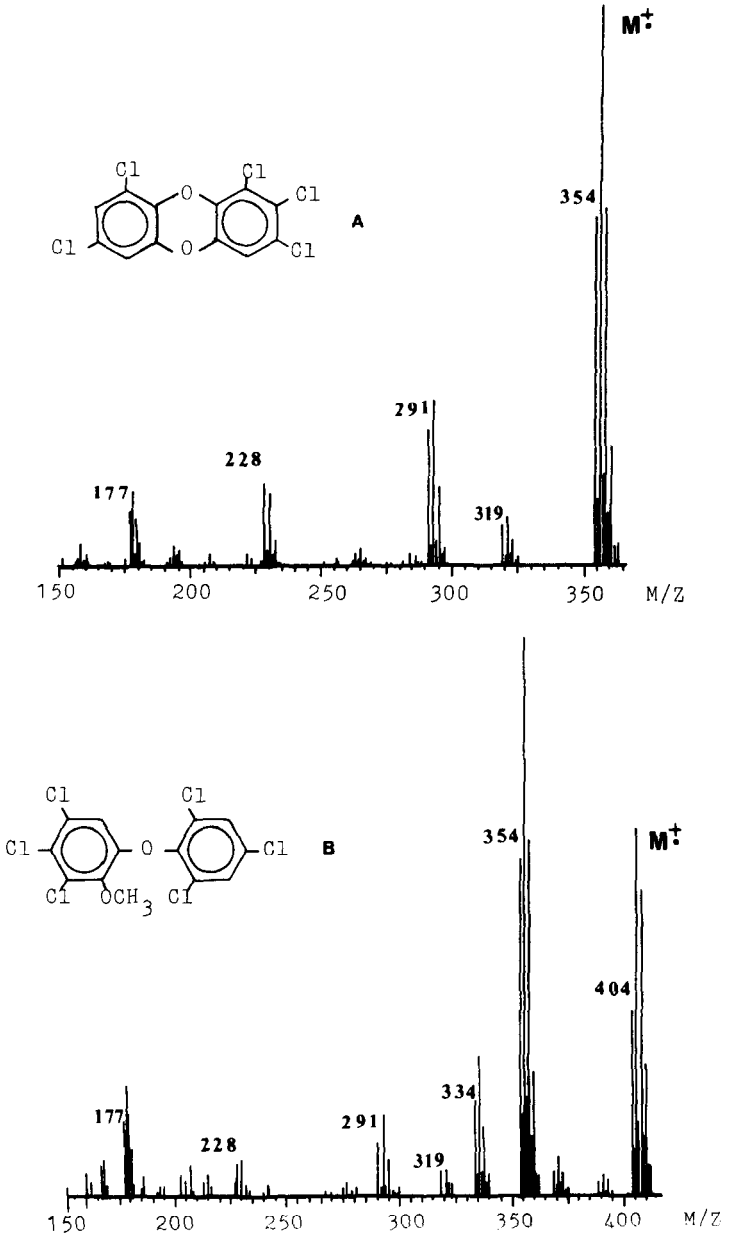


Fig. 2. Electron impact mass spectra of 1,2,3,7,9-pentaCDD (A) and 4,5,6-2,4,6-hexaC2PA (B).

atoms[23]. However, as Table III shows, PCDFs eluate with the same retention time range as PCDEs with *one* more chlorine. In addition, the most PCDEs can be separated from PCDFs by alumina chromatography[2]. PCDFs are therefore not readily confused in their MS determination with PCDEs.

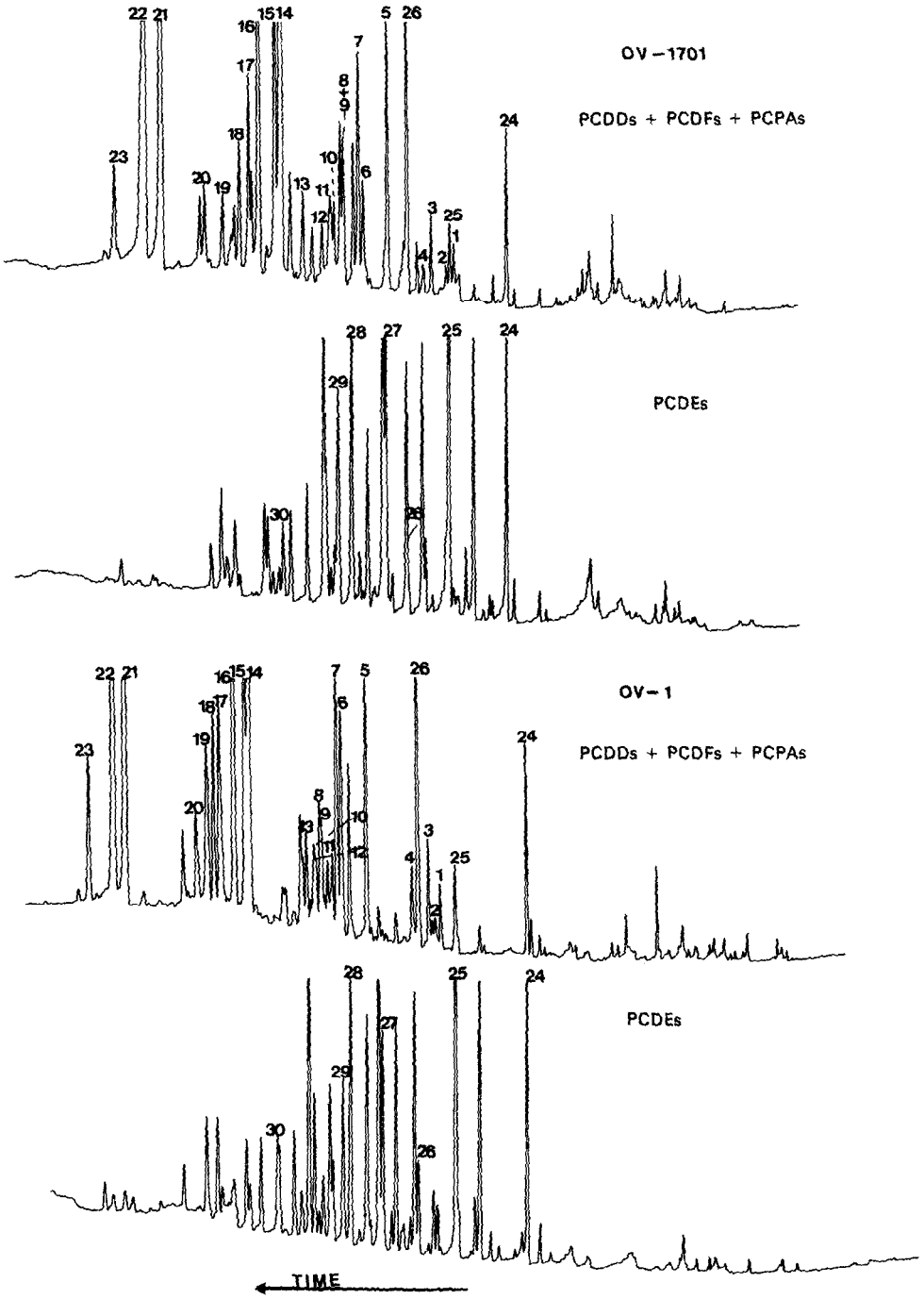


Fig. 3. GC–ECD chromatograms of a Ky-5 sample recorded on an OV-1 and an OV-1701 quartz capillary column. The instrument used was Carlo Erba Series 4160. For GC conditions see Experimental.

Analysis of Ky-5 sample

GC–ECD chromatograms of the PCDD and PCDE fractions of a Ky-5 sample are presented in Fig. 3. The compounds identified are listed in Table IV. Identification was based on a comparison of the GC retention times with those of authentic model substances. In the GC experiments, OV-1, SE-54 and OV-17101 quartz capillary columns were used. The structures of compounds were confirmed by full-scan GC–MS analysis.

TABLE IV

STRUCTURAL ASSIGNMENT OF GC PEAKS OF FIG. 3

Compound	*Peak No.*	*Amount (μg/g)**	*Compound*	*Peak No.*	*Amount (μg/g)*
PCDDs			*PCDFs*		
1368-TCDD	1	+	12468-PnCDF	5	+ +
1379-TCDD	2	+	12478-PnCDF	6	+
12468- and/or			12378-PnCDF	9	+
12479-PnCDD	7	+ +	23468-PnCDF	11	+
12368-PnCDD	8	+	23478-PnCDF	13	+
12379-PnCDD	10	+	123468-HxCDF	14	+ + + +
123679- and/or			124678-HxCDF	15	+ + +
123689-HxCDD	18	+	124689-HxCDF	16	+ + + +
123678-HxCDD	19	+	123689-HxCDF	17	+
1234678-HpCDD	23	+	234678-HxCDF	20	+
PCDEs			1234678-HpCDF	21	+ + + +
22'44'-TCDE	24	+ +	1234689-HpCDF	22	+ + + +
22'44'5-PnCDE	25	+ + +	*PCPAs*		
22'344'-PnCDE	26	+ +	46-246-PnC2PA	3	+
22'44'55'-HxCDE	27	+ +	26-246-PnC4PA	4	+
22'344'5-HxCDE	28	+ +	456-246-HxC2PA	12	+
22'3'44'5-HxCDE	29	+			
22'344'55'-HpCDE	30	+			

* + < 1, + + = 1–5, + + + = 5–10, + + + + = 10–40.

In this study, altogether 30 neutral dimers were detected in Ky-5. As Fig. 3 shows, the pre-dominant compounds in the dioxin fraction of Ky-5 are hexa- and hepta-CDFs[14,15,16,21,22]. Among pentaCDFs the toxic 1,2,3,7,8- and 2,3,4,7,8-pentaCDFs could also be detected. The most abundant congener was, however, the less toxic 1,2,4,6,8-pentaCDF[5]. These results are in good agreement with those reported by Rappe *et al.*[6].

PCDDs were mainly tetra- to heptachloro isomers and, with respect to PCDFs, were minor constituents of the dioxin fraction. As expected, their structures correspond to those of condensation products (normal and Smiles rearranged) of 2,4,6-tri- and 2,3,4,6-tetrachlorophenols[15]. As reported earlier, some PCPAs constituted[8] the trace species of certain Ky-5 lots. In this study, one additional PCPA[12] was detected.

The major species of the ether fraction were shown to be tetra- to hexaCDEs.

The proposed structures are presented in Table IV. To the best of our knowledge, the structural data for PCDEs in technical chlorophenols have not been reported, with exception of pentachlorophenol[7].

CONCLUSIONS

GC–ECD can be used succesfully as a check identification method for PCDDs and related compounds in technical chlorophenols. The GC retention times and MS properties of the compounds studied are needed for analysis of environmental samples contaminated with chlorophenols and their probable PCDD, PCDF, PCDE and PCPA impurities. The results show, for example, that group analysis of PCDDs by GC–MS is greatly interfered with by PCPAs. Therefore, in analytical examination of PCDDs, the presence of PCPAs must be taken into consideration.

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