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# POLYCHLORINATED DIBENZO-*p*-DIOXINS

## SEPARATION AND IDENTIFICATION OF ISOMERS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Attempts were made to synthesize all polychlorinated dibenzo-*p*-dioxin isomers containing six to eight chlorine atoms by micro-scale pyrolysis of different polychlorophenates. Eight of the ten possible hexachlorodibenzo-*p*-dioxins, the two hepta- and the octachlorodibenzo-*p*-dioxin were observed and separated by gas chromatography using glass capillary columns. Without actual isolation of these toxic materials, isomers were characterized by gas chromatography and mass spectrometry. Commercial chlorinated phenols were analyzed for the presence of these isomers. The major hexachlorodibenzo-*p*-dioxin observed in two commercial products was the unexpected 1,2,3,7,8,9-substituted isomer, which was not formed as the main dioxin component in any of the pyrolysis experiments. The same isomer was reported to be isolated from toxic fat and identified by X-ray crystallography.

## INTRODUCTION

Chlorinated phenols are used as starting materials for a series of agricultural and industrial chemicals. Many have been shown to contain polychlorinated dibenzo-*p*-dioxins (PCDDs) and other contaminants[1–3]. The highly toxic PCDDs are produced from chlorinated phenols at high temperatures under alkaline conditions during the manufacturing process. Outbreaks of chick oedema disease in the poultry industry may have been due to the presence of PCDDs in animal feeds[4,5]. Several PCDDs have been identified in fats and grease from hides where chlorinated phenols were used as preservatives; one of the compounds first identified as a toxic factor in feed was 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin[6]. The highly toxic hexachlorodibenzo-*p*-dioxins (hexa-CDDs) have been found in pentachlorophenol and other chlorinated phenols[1,3] and their presence in these products gave rise to concern[7]. Hepta- and octachlorodibenzo-*p*-dioxin (hepta- and octa-CDD), usually also present in these samples, are considered to be less toxic and dangerous[8]. The extremely toxic 2,3,7,8-tetrachlo-

rodibenzo-*p*-dioxin (tetra-CDD) may be present in 2,4,5-trichlorophenol[1] and in 2,4,5-T herbicides[9] but was usually not found in tetra- and pentachlorophenol.

A total of 75 different PCDDs exist, including ten hexa- and two hepta-CDD isomers and octa-CDD. Different isomers of the same PCDD vary significantly in their toxicological properties[5,8], and it therefore becomes important to identify them. Many papers have dealt with the analysis for these contaminants[1–3,10] but isomers have usually not been individually identified.

In this paper we describe investigations on the preparation of different isomers of PCDDs containing six to eight chlorine atoms by micro-scale pyrolysis of potassium chlorophenates. Gas chromatographic and mass spectrometric properties of the different isomers were determined using glass capillary columns without the extensive purification, isolation and handling of the bulk of highly toxic materials required when using other spectroscopic methods. Commercial chlorinated phenols were analyzed for the presence of these PCDD isomers.

## EXPERIMENTAL

### *Preparation of potassium chlorophenates*

2,3,4-, 2,3,5-, 2,4,5- and 2,3,6-tri-, 2,3,4,5- and 2,3,5,6-tetra- and pentachlorophenol were obtained in analytical quality from Fluka (Buchs, Switzerland). Technical-grade 2,3,4,6-tetrachlorophenol containing 15% of pentachlorophenol was purified by multiple sublimation (110°, 0.05 torr) in order to obtain a purity of >99%. All compounds were analyzed by mass spectrometry, by capillary gas chromatography after methylation and by checking their melting points.

Potassium salts were prepared by dissolving 2 mmoles of tetra- and pentachlorophenol in 2–3-ml portions of methanol and adding a 1 *N* solution of potassium hydroxide in methanol dropwise (1–2 ml) to a pH of about 12. The solutions were immediately vacuum flash evaporated at 30° and kept overnight in a vacuum desiccator. Mixtures of penta- with tri- or tetrachlorophenates in a 1:1 molar ratio and also 1:1 mixtures of the isomeric tetrachlorophenates were prepared by dissolving 1 mmole of each phenol in methanol and adding potassium hydroxide. After drying the potassium salts, each sample was ground and pulverized.

### *Pyrolysis of potassium chlorophenates and analysis for PCDDs*

Approximately 10 mg of a potassium chlorophenate sample were placed in the tip of a glass reaction tube (150–200 mm × 5 mm I.D.). Pyrolysis of the samples was carried out either under vacuum (evacuation of sample tubes to 0.05 torr and closure with a flame) or at atmospheric pressure with the top of the glass tubes plugged with glass-wool and a 5-cm layer of alumina. The reaction tubes were placed vertically with the tips just immersed into a silicone oil-bath. About two thirds of each tube should be kept cool by shielding it from hot oil vapour with aluminium foil. The tubes are heated for 1–4 h at 290°. A white sublimate is usually formed with a brownish residue in the tip. The pyrolysis experiments should be carried out in a well ventilated hood with appropriate safety precautions.

After cooling, the reaction tubes were opened and the contents extracted with small portions of methylene chloride (5–10 times, total volume 8–10 ml) using ultrasonics. The extracts were passed through a micro-column containing 1 g of basic

alumina (Woelm, Eschwege, G.F.R.) in a disposable Pasteur pipette[3]. The column was washed with further portions of methylene chloride and the extracts (colourless to light brownish) were concentrated to 2–5 ml in a stream of nitrogen.

Analysis of the neutral components in these extracts was carried out on a thin-film, Grob-type glass capillary column (silicone OV-61, 18 m × 0.3 mm I.D.) mounted in a Carlo Erba 2101 gas chromatograph equipped with an inlet splitter. A flexible platinum capillary (50 cm × 0.15 mm I.D.) heated to 275° served as the interface to a Finnigan 1015D quadrupole mass spectrometer. The system has been previously described[11]. Aliquots of 1–2 $\mu$l of sample were injected, without splitting, with the vaporizer at 300° and the column at 140°. 30 sec after injection, the splitter was opened and 90 sec later the column temperature was increased to 180° and programmed to 240° at the rate of 2°/min. A helium carrier gas pressure of 1.1 atm resulted in an average linear velocity of 28 cm/sec at 140°. The column was silanized daily with a few injections of N,O-bis(trimethylsilyl)trifluoroacetamide at 140°. The column developed 35,000 theoretical plates for hepta-CDD at 240°.

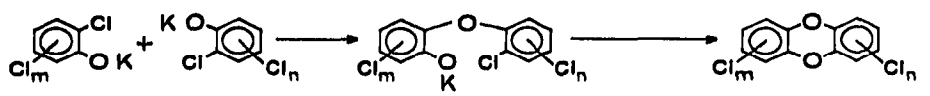
Adjustments and operating conditions for the mass spectrometer were as reported previously[3]. For mass specific detection (mass fragmentography) of PCDDs, their molecular ions at *m/e* 388 (hexa-CDD), *m/e* 422 (hepta-CDD) and *m/e* 456 (octa-CDD) were used. Recorder sensitivities ranged from 2 to 100 mV as required.

## RESULTS AND DISCUSSION

### *Pyrolysis of chlorophenates and formation of PCDDs*

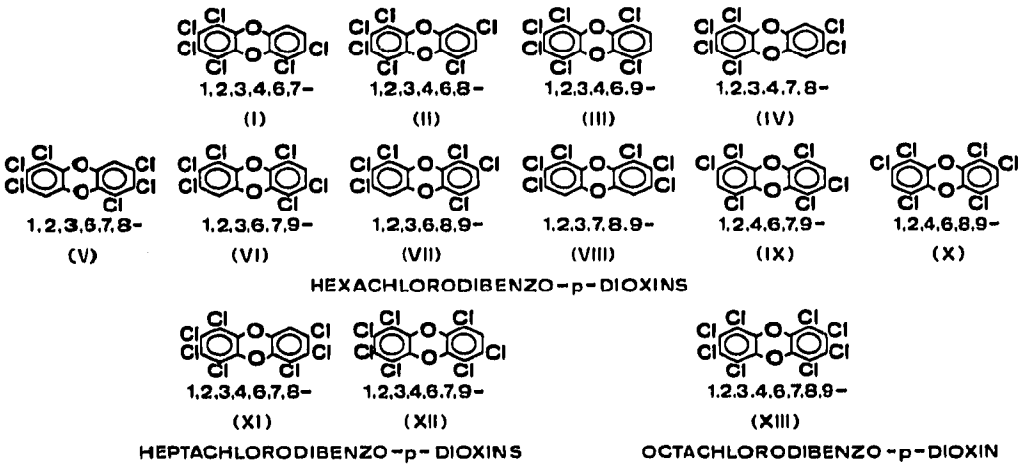
Several papers have reported the pyrolysis of chlorophenates and the formation of PCDDs[5,12,13] but structural assignments of individual isomers were usually not made. Because pyrolysis experiments were found to be simple to carry out even on a micro-scale and the starting materials were readily available, we decided to investigate this reaction and to attempt to prepare all of the isomers of the higher chlorinated members of the PCDD series (hexa- to octa-CDD). The analysis and identification of these isomers by gas chromatography–mass spectrometry (GC–MS) using glass capillary columns proved to be fast and did not require extensive clean-up, purification, isolation and handling of the bulk of highly toxic materials.

PCDDs can be formed in a two-step condensation process with *ortho*-halogen-substituted phenoxy anions (aromatic nucleophilic substitution). The reaction is best carried out by heating alkali metal salts of chlorophenols to about 300°; it probably proceeds via polychlorophenoxyphenates as intermediates and may continue with the formation of tri-, tetra- or polymers.




From the reaction of tetrachlorophenates, we can expect the formation of hexa-CDD isomers that contain three chlorine substituents on each side of the molecule (hexa-CDD isomers V–X). The reaction of tri- with pentachlorophenate should yield hexa-CDD isomers that contain four and two chlorine substituents, respec-

tively, on each side of the molecule (hexa-CDD isomers I–IV) in addition to tetra-CDDs and octa-CDD. Similarly, we can expect hepta-CDDs to be formed from tetra- and pentachlorophenate in addition to hexa-CDDs and octa-CDD. Different isomers should form when reacting isomeric polychlorophenates.




Polychlorophenates that contain Cl-substituents in both *ortho* positions (2 and 6 positions) may form several isomers of the same PCDD, with some being preferred owing to steric and electronic effects resulting in different reactivities of Cl-substituents. In addition, the product distribution can be influenced by rearrangement reactions before, during and after PCDD formation and possibly by dechlorination of higher PCDDs.

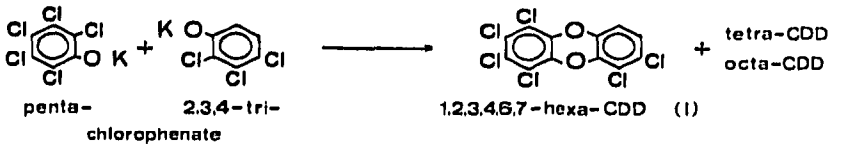
Initial experiments established the feasibility of the present study and the analytical conditions required. Pyrolyzate extracts were passed through an alumina microcolumn in order to remove possibly interfering phenolic compounds. PCDDs were found to be the only neutral components that were eluted in the retention time range investigated and therefore no further fractionation from polychlorinated dibenzofurans (PCBFs) or polychlorinated diphenyl ethers (PCDPEs) was required. For convenience, methylene chloride was used as the eluent; all isomers were also completely eluted in the 50 % methylene chloride–*n*-hexane fraction of the previously described elution system[3].

A high-resolution glass capillary column was used because difficulties were anticipated in separating some of the isomers. Semi-polar methyl phenyl silicone OV-61 showed better selectivity than non-polar methyl silicone OV-101 towards the separation of PCDD isomers. A standard temperature programme (180–240°) was used with these columns in order to permit the analysis of all PCDDs. Tetra-CDDs were found to be eluted from the OV-61 glass capillary column between 200 and 215°, penta-CDDs between 215 and 230° and hexa-CDDs between 230 and 240°, followed by hepta- and octa-CDD. An amount of sample pyrolyzed of 10 mg was not exceeded in order to prevent saturation of extracts with some PCDDs, which would have increased the apparent concentration of minor components and may have caused difficulties in identifying the major products.

Preliminary pyrolysis of tetrachlorophenates was carried out at temperatures ranging from 220° to 330°. It was found that a temperature of 290° and reaction times of 1–4 h would result in yields of 3–10% of hexa-CDD and very little other neutral by-products. At lower temperatures (≤250°), the yields decreased significantly (<0.5%); no increase in the yield or change in the isomeric distribution was observed at higher temperatures.

*Hexa-CDD isomers from tri- and pentachlorophenates*

Pyrolysis of tri- or pentachlorophenate gives primarily tetra- or octa-CDD, respectively. In addition to these PCDDs, pyrolysis of a mixture of both chlorophenates should lead to the formation of hexa-CDD isomers containing four and two Cl-substituents, respectively, on each side of the molecule:




penta- 2,3,4-tri-chlorophenate

1,2,3,4,6,7-hexa-CDD (I)

From the pyrolysis of penta- with 2,3,4-trichlorophenate, we expect the formation of 1,2,3,4,6,7-hexa-CDD (I). Similarly, 2,3,5- and 2,4,5-trichlorophenate would form 1,2,3,4,6,8- (II) and 1,2,3,4,7,8-hexa-CDD (IV), respectively. 2,3,6-Trichlorophenate is expected to form both 1,2,3,4,6,7- (I) and 1,2,3,4,6,9-hexa-CDD (III) because two Cl-substituents are available in the *ortho*-position for reaction with pentachlorophenate. These reactions should produce all four possible isomers in this series of hexa-CDDs.

Chromatograms of pyrolyzed samples showed the presence of hexa-CDDs in addition to tetra- and octa-CDD. Hexa-CDDs were not obtained from the pyrolysis of tri- or pentachlorophenate alone. Fig. 1 shows partial mass fragmentograms of these samples, showing the elution range of the hexa-CDD isomers between 230 and 240°. Gas chromatographic peaks with four different retention times were observed. Fig. 1a

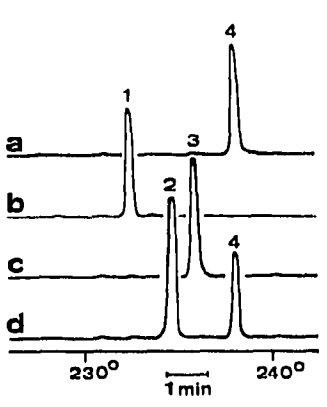



Fig. 1. Partial mass fragmentograms (OV-61 glass capillary column, 230–240°, *m/e* 388) showing elution of hexa-CDD isomers produced from penta- and (a) 2,3,4-, (b) 2,3,5-, (c) 2,4,5- and (d) 2,3,6-trichlorophenate. Peak assignments: 1 = 1,2,3,4,6,8- (II); 2 = 1,2,3,4,6,9- (III); 3 = 1,2,3,4,7,8- (IV); 4 = 1,2,3,4,6,7-hexa-CDD (I).

shows a single hexa-CDD isomer formed from penta- with 2,3,4-trichlorophenate and eluting at the longest retention time. This peak was assigned to 1,2,3,4,6,7-hexa-CDD (I). Figs. 1b and 1c show single peaks obtained from penta- with 2,3,5- and 2,4,5-trichlorophenates, assigned to 1,2,3,4,6,8- (II) and 1,2,3,4,7,8-hexa-CDD (IV), respectively, and eluting at different retention times prior to isomer I. As indicated in Fig. 1d, 2,3,6-trichlorophenate formed two peaks, the first to be eluted assigned to the structure of 1,2,3,4,6,9-hexa-CDD (III) and the second to 1,2,3,4,6,7-hexa-CDD (I), the isomer formed with 2,3,4-trichlorophenate. These results established the elution order and the retention times for the first series of hexa-CDD isomers containing four and two Cl-substituents, respectively, on the two sides of the molecule. The mass spectrum of 1,2,3,4,6,8-hexa-CDD (II) is presented in Fig. 3a; the spectra for the remaining isomers of this series (I, III and IV) were almost identical with that spectrum, with minor deviations only in the relative intensities of some peaks in the lower mass range.

*Hexa-CDD isomers from tetrachlorophenates*

The pyrolysis of tetrachlorophenates produced almost exclusively hexa-CDDs as neutral components with very little lower or higher chlorinated PCDDs. The hexa-CDD isomers formed by this reaction are expected to contain three Cl-substituents on each side of the dioxin ring system:


Six isomers (V–X) belong into this group. As indicated, 2,3,5,6-tetrachlorophenate should form 1,2,4,6,7,9-hexa-CDD (IX), whereas 2,3,4,5-tetrachlorophenate is expected to form predominantly 1,2,3,6,7,8-hexa-CDD (V). The mass fragmentograms in Figs. 2a and 2b indicate that both tetrachlorophenates each form a major hexa-CDD isomer, both isomers with retention times different from those obtained with

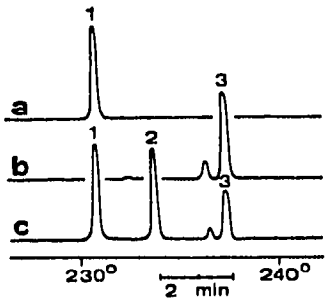



Fig. 2. Partial mass fragmentograms (OV-61 glass capillary column, 230–240°, *m/e* 388) showing elution of hexa-CDD isomers produced from (a) 2,3,5,6-, (b) 2,3,4,5-, (c) 2,3,5,6- and 2,3,4,5-tetrachlorophenate. Peak assignments: 1 = 1,2,4,6,7,9- (IX); 2 = 1,2,3,6,8,9- (VII); 3 = 1,2,3,6,7,8-hexa-CDD (V).

tri- and pentachlorophenate. The pyrolysis of a mixture of 2,3,5,6- and 2,3,4,5-tetrachlorophenate is expected to give in addition 1,2,3,6,8,9-hexa-CDD (VII) containing both tetrachlorophenate moieties. As indicated in Fig. 2c, the new 1,2,3,6,8,9-hexa-CDD (VII) observed is eluted at a retention time between that of 1,2,4,6,7,9-(IX) and 1,2,3,6,7,8-hexa-CDD (V). The mass spectrum of 1,2,3,6,7,8-hexa-CDD (V) is presented in Fig. 3b; the spectra of 1,2,4,6,7,9- (IX) and 1,2,3,6,8,9-hexa-CDD (VII) are very similar to that of isomer V but all three differ significantly in the lower mass range from those of the first series prepared from tri- and pentachlorophenate (Fig. 3a).

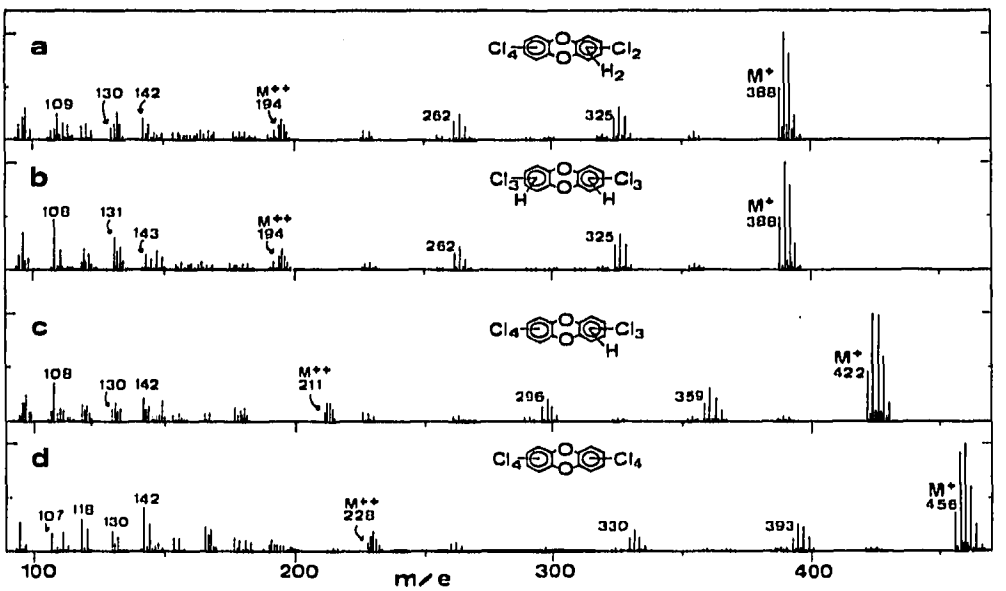



Fig. 3. Mass spectra (*m/e* 90–500) of (a) 1,2,3,4,6,8-hexa-CDD (II), (b) 1,2,3,6,7,8-hexa-CDD (V), (c) 1,2,3,4,6,7,9-hepta-CDD (XII) and (d) octa-CDD.

The pyrolysis of 2,3,4,6-tetrachlorophenate should give the same isomers as a mixture of 2,3,5,6- and 2,3,4,5-tetrachlorophenate. The partial mass fragmentogram obtained from pyrolyzed 2,3,4,6-tetrachlorophenate (Fig. 4a) indicates the formation of 1,2,4,6,7,9- (IX) and 1,2,3,6,8,9-hexa-CDD (VII) and the presence of a smaller amount of the last eluting 1,2,3,6,7,8-hexa-CDD (V). This suggests a preferred reactivity of the chlorine atom in the 2-position during the initial step of the condensation from 2,3,4,6-tetrachlorophenate to polychlorophenoxyphenate. In addition, a fourth hexa-CDD isomer was observed, the same isomer also being a minor by-product in the pyrolysis of 2,3,4,5-tetrachlorophenate and eluting immediately prior to 1,2,3,6,7,8-hexa-CDD (V). Its mass spectrum was identical with that of isomer V (Fig. 3b) and its structure has to be that of 1,2,3,6,7,9- (VI), 1,2,3,7,8,9- (VIII) or 1,2,4,6,8,9-hexa-CDD (X), isomers that have not yet been assigned. The formation of four hexa-CDD isomers by pyrolysis of 2,3,4,6-tetrachlorophenate was reported previously[5,12]. Because the 2,3,4,6-tetrachlorophenate used in the present study had

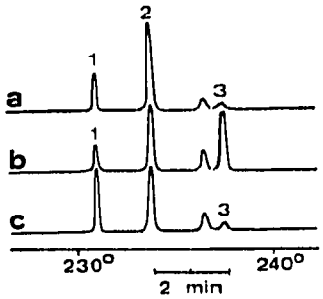
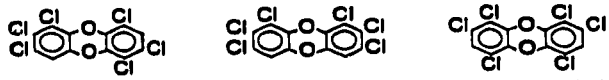



Fig. 4. Partial mass fragmentograms (OV-61 glass capillary column, 230–240°, *m/e* 388) showing elution of hexa-CDD isomers produced from (a) 2,3,4,6-, (b) 2,3,4,6- and 2,3,4,5-, (c) 2,3,4,6 and 2,3,5,6-tetrachlorophenate. Peak assignments as in Fig. 2.

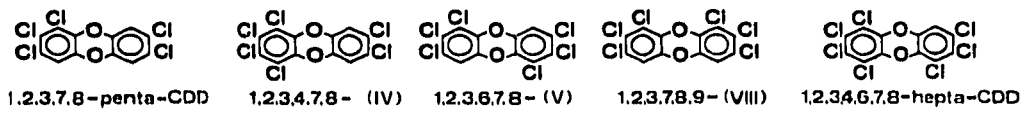
an isomeric purity of >99%, we concluded that the unknown hexa-CDD isomer has to be formed by isomerization during pyrolysis and is not due to the presence of an isomeric tetrachlorophenol impurity as suggested elsewhere[5].




The three remaining, not yet assigned, hexa-CDD isomers should be formed by pyrolysis of mixtures of 2,3,4,6- and 2,3,4,5- or 2,3,5,6-tetrachlorophenate. The results obtained with such pyrolyzates are shown in Figs. 4b and 4c. No hexa-CDD peaks were observed that had not been previously obtained by pyrolysis of the individual polychlorophenates. Several attempts using lithium chlorophenates, addition of copper, potassium carbonate, magnesium oxide and changing the pyrolytic conditions over a wide range failed to produce the additional compounds. It appeared as if 2,3,4,6-tetrachlorophenate would react first, followed by the reaction of the other tetrachlorophenate without the formation of appreciable amounts of isomers that contain different tetrachlorophenate moieties.

*Partial chlorination of 2,3,7,8-tetra-CDD*

Partial chlorination of 2,3,7,8-tetra-CDD should give, in addition to some fully chlorinated octa-CDD, only intermediate PCDD isomers that contain Cl-substituents in all four lateral (2-, 3-, 7- and 8-) positions in the dioxin ring system. The PCDDs that satisfy this limitation include one penta-, three hexa- and one hepta-CDD isomer:



Two of these hexa-CDD isomers (IV and V) have already been obtained in the previous pyrolysis experiments but the third isomer expected, 1,2,3,7,8,9-hexa-CDD (VIII), would be one that has not yet been assigned.

Partial chlorination was carried out by reacting a few hundred micrograms of 2,3,7,8-tetra-CDD with 0.2 ml of antimony pentachloride in a small PTFE-capped reaction vial for 30–60 min at a temperature not higher than 100°. After cooling, excess of antimony pentachloride was destroyed by careful addition of 3 ml of 20% hydrochloric acid and the PCDDs were extracted with 2 ml of methylene chloride. After further washing with hydrochloric acid and distilled water, the extract was passed through an alumina micro-column and analyzed by capillary gas chromatography. The chromatogram in Fig. 5 shows, in addition to octa-CDD and unchanged 2,3,7,8-tetra-CDD, the formation of one penta-, three hexa- and one hepta-CDD isomer, as expected. The first and last eluting hexa-CDD isomers had retention times identical with those of 1,2,3,4,7,8- (IV) and 1,2,3,6,7,8-hexa-CDD (V), respectively. The second eluting hexa-CDD isomer was therefore assigned the structure of 1,2,3,7,8,9-hexa-CDD (VIII). It had a retention time identical with that of the isomer formed as a minor product in the pyrolysis of 2,3,4,6- and 2,3,4,5-tetrachlorophenate.

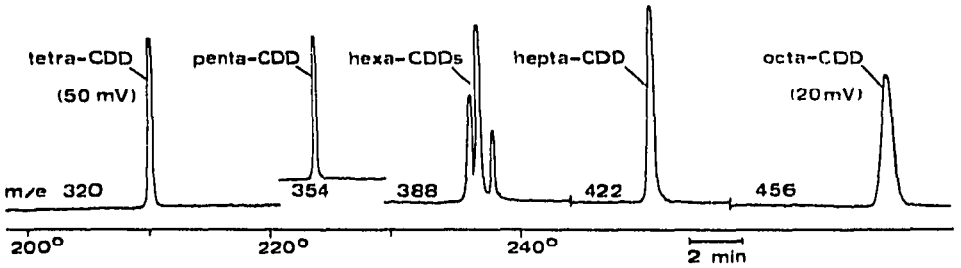
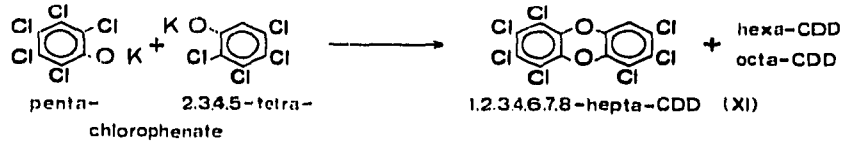



Fig. 5. Partial mass fragmentogram (OV-61 glass capillary column, 200–240°, *m/e* 320, 354, 388, 422 and 456) showing elution of PCDDs obtained by partial chlorination of 2,3,7,8-tetra-CDD. Sensitivity: 5 mV or as indicated.

*Hepta-CDD isomers and octa-CDD*

Whereas the pyrolysis of pentachlorophenate should result in the formation of octa-CDD as the only major product of the PCDD series, mixtures of tetra- and pentachlorophenate could be expected to produce hepta-CDD isomers in addition to hexa- and octa-CDDs:




Penta- and 2,3,4,5-tetrachlorophenate should form 1,2,3,4,6,7,8-hepta-CDD (XI) by this reaction, whereas with 2,3,5,6-tetrachlorophenate the 1,2,3,4,6,7,9-isomer (XII)

would be expected. Finally, both isomers should be obtained when reacting penta- with 2,3,4,6-tetrachlorophenate.

The results in Fig. 6 indicate that the first eluting hepta-CDD isomer is formed as the major product from penta- with 2,3,5,6-tetrachlorophenate, the second eluting as the major product with 2,3,4,5-tetrachlorophenate and both isomers are formed with 2,3,4,6-tetrachlorophenate. The first eluting hepta-CDD was therefore assigned to the 1,2,3,4,6,7,9-hepta-CDD (XII) and the second to the 1,2,3,4,6,7,8-hepta-CDD isomer (XI). These assignments were further substantiated by the fact that, by partial chlorination of 2,3,7,8-tetra-CDD, only the second eluting hepta-CDD isomer was formed, which therefore has to have the 1,2,3,4,6,7,8-hepta-CDD structure (XI). In addition, it should be noticed that very little hepta-CDD is formed with penta-chlorophenate or with pure tetrachlorophenate alone. The mass spectra of both hepta-CDD isomers were identical; the spectrum of 1,2,3,4,6,7,9-hepta-CDD is shown in Fig. 3c and that of octa-CDD in Fig. 3d.

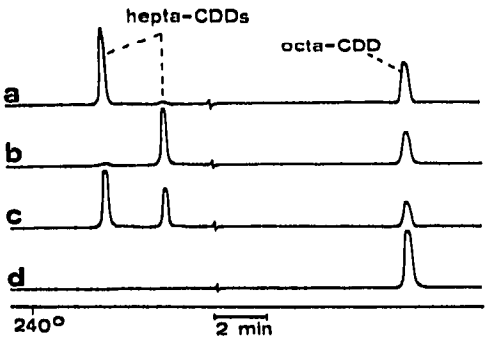
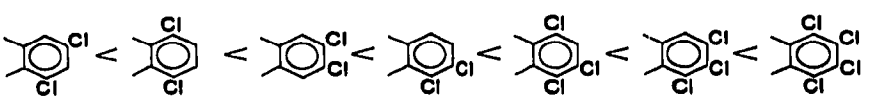



Fig. 6. Partial mass fragmentograms (OV-61 glass capillary column, 240°, *m/e* 422 and 456) showing elution of hepta- and octa-CDDs produced from penta- and (a) 2,3,5,6-, (b) 2,3,4,5-, (c) 2,3,4,6-tetrachlorophenate, and (d) from pentachlorophenate alone.

*Separation and elution order of higher PCDD isomers*

Fig. 7 is a partial chromatogram of a combined pyrolyzate sample, showing the separation achieved by the OV-61 glass capillary column with eight hexa-CDD isomers eluting between 230 and 240°. The elution order of these hexa-CDD isomers as well as of the hepta- and octa-CDDs is presented graphically in Fig. 8, together with the structures previously assigned. In Table I, the elution temperatures and the retention times of these isomers are reported.

The results show a dependence of the retention times on the number and location of the Cl-substituents in the dioxin ring system. The following order was derived from compounds that contain four Cl-substituents on one side of the molecule:



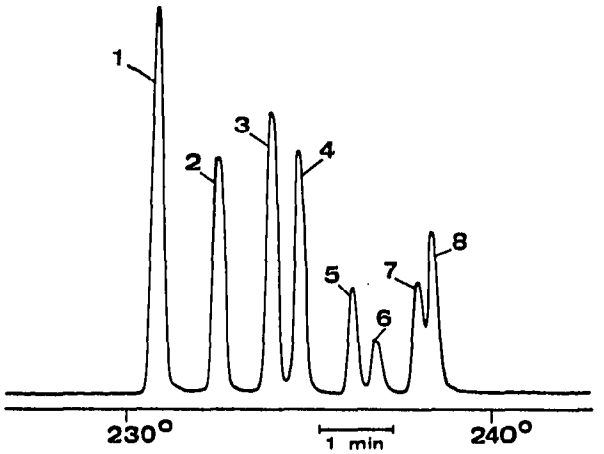



Fig. 7. Partial mass fragmentogram (OV-61 glass capillary column, 230–240°, *m/e* 388) showing separation of eight hexa-CDD isomers in a combined pyrolyzate sample. Peak assignments: 1 = 1,2,4,6,7,9- (IX); 2 = 1,2,3,4,6,8- (II); 3 = 1,2,3,6,8,9- (VII); 4 = 1,2,3,4,6,9- (III); 5 = 1,2,3,4,7,8- (IV); 6 = 1,2,3,7,8,9- (VIII); 7 = 1,2,3,6,7,8- (V); 8 = 1,2,3,4,6,7-hexa-CDD (I).

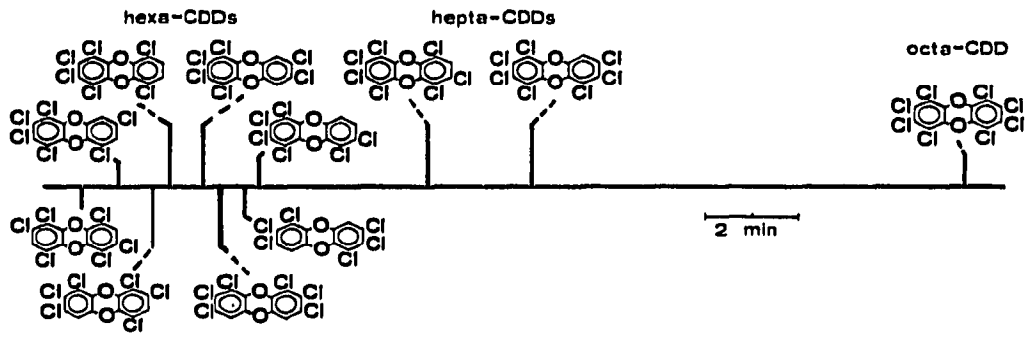



Fig. 8. Graphical presentation of the elution order of hexa-, hepta- and octa-CDDs on the OV-61 glass capillary column.

All hexa-CDDs are eluted prior to the hepta-CDD isomers, which in turn precede the octa-CDD. In the series of dichloro-substitution on one side of the molecule, the elution order is 1,3(*meta*)-$Cl_2$ < 1,4(*para*)-$Cl_2$ < 2,3(*ortho*)-$Cl_2$ < 1,2(*ortho*)-$Cl_2$. The same order was observed with a series of tetra-CDDs prepared by pyrolysis of isomeric trichlorophenates[14]. In the series of trichloro-substitution, the order derived from both hexa- and hepta-CDDs is 1,2,4-$Cl_3$ < 1,2,3-$Cl_3$. In both series, the retention time increases if the Cl-substituents are adjacent (*ortho*) to each other and, to a lesser extent, if they are adjacent to an oxygen atom in the dioxin system. According to these results, the two hexa-CDD isomers, 1,2,3,6,7,9- (VI) and 1,2,4,6,8,9-hexa-CDD (X), which were not observed in the preceding experiments, are expected to be eluted prior to 1,2,3,6,8,9- (VII) and 1,2,4,6,7,9-hexa-CDD (IX), respectively.

*PCDD isomers in commercial chlorinated phenols*

Two commercial chlorinated phenols, Dowicide-6 (technical 2,3,4,6-tetra-

TABLE I

ELUTION TEMPERATURES AND RETENTION TIMES OF HEXA-, HEPTA- AND OCTA-CDDs ON AN OV-61 GLASS CAPILLARY COLUMN

| *Compound* | *Elution temperature (°C)* | *Retention time* (min)* |
|---|---|---|
| 1,2,4,6,8,9-Hexa-CDD (X) | **— | **— |
| 1,2,4,6,7,9-Hexa-CDD (IX) | 230.8 | 27.5 |
| 1,2,3,4,6,8-Hexa-CDD (II) | 232.5 | 28.3 |
| 1,2,3,6,7,9-Hexa-CDD (VI) | **— | **— |
| 1,2,3,6,8,9-Hexa-CDD (VII) | 234.0 | 29.1 |
| 1,2,3,4,6,9-Hexa-CDD (III) | 234.7 | 29.4 |
| 1,2,3,4,7,8-Hexa-CDD (IV) | 236.2 | 30.2 |
| 1,2,3,7,8,9-Hexa-CDD (VIII) | 236.8 | 30.5 |
| 1,2,3,6,7,8-Hexa-CDD (V) | 238.0 | 31.0 |
| 1,2,3,4,6,7-Hexa-CDD (I) | 238.4 | 31.2 |
| 1,2,3,4,6,7,9-Hepta-CDD (XII) | 240 | 34.8 |
| 1,2,3,4,6,7,8-Hepta-CDD (XI) | 240 | 37.1 |
| 1,2,3,4,6,7,8,9-Octa-CDD (XIII) | 240 | 46.0 |

* Temperature programme 180–240° at 2°/min.
** Isomers not observed in this study.

chlorophenol) and Dowicide-7 (pentachlorophenol), were analyzed for individual PCDD isomers. Both samples had previously been shown to contain significant amounts of hexa-, hepta- and octa-CDDs in addition to other contaminants[3]. These samples were subjected to alkaline extraction and fractionation of the neutral components on an alumina micro-column. The fractions that contained PCDDs were re-analyzed on the glass capillary column. The chromatograms of both samples and of a sample of combined pyrolyzates are presented in Fig. 9. The retention times for

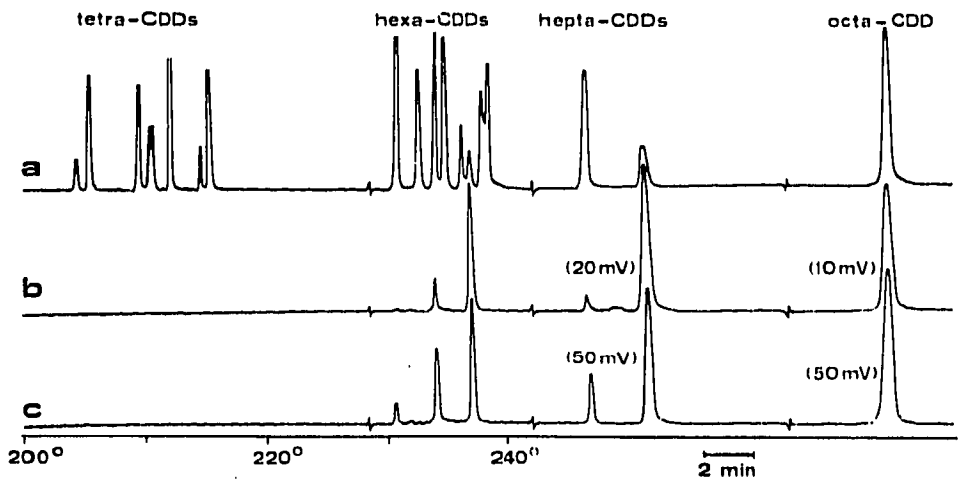



Fig. 9. Mass fragmentograms (OV-61 glass capillary column, 200–240°, *m/e* 320, 388, 422 and 456) showing elution of PCDDs in (a) combined pyrolyzate sample, (b) Dowicide-6 and (c) Dowicide-7. Sensitivity: 2 mV or as indicated.

the hexa-, hepta- and octa-CDD isomers were verified by co-injection and re-analysis.

The analysis of Dowicide-6 (Fig. 9b) shows two hexa-CDD isomers (total amount 6 ppm), with 1,2,3,7,8,9-hexa-CDD (VIII) being the major and 1,2,3,6,8,9-hexa-CDD (VII) the minor component; 1,2,3,4,6,7,8-hepta-CDD (XI, 55 ppm) and octa-CDD (XIII, 39 ppm) were also found to be present. In Dowicide-7 (Fig. 9c), the major hexa-CDD component was again the 1,2,3,7,8,9-substituted isomer (VIII), with smaller amounts of 1,2,3,6,8,9- (VII) and 1,2,4,6,7,9-hexa-CDD (IX) (total amount 9 ppm). Both hepta-CDD isomers (235 ppm) and octa-CDD (250 ppm) were found in this sample. Complete mass spectral analysis confirmed the presence of these compounds in both samples.

The chromatogram of a sample of combined pyrolyzates in Fig. 9a further shows the separation of seven isomeric tetra-CDDs eluting between 200° and 215°; none of these compounds was detected in the two commercial chlorinated phenols.

## CONCLUSIONS

Significant amounts of PCDDs were obtained by pyrolysis of all of the chlorophenates investigated. Eight of the ten possible hexa-, two hepta- and octa-CDD were formed when reacting isomeric tri-, tetra- or pentachlorophenates. Attempts to obtain the remaining two hexa-CDD isomers by pyrolysis failed but their retention times were estimated from those of the other isomers. The Cl-substitution was assigned to all isomers observed. Combined GC–MS using glass capillary columns proved to be a rapid and powerful means of separating and analyzing these isomers. The mass spectra of the two groups of hexa-CDD isomers containing three, four and two Cl-substituents on each side of the molecule showed significant differences in the lower mass range, thus assisting in their identification.

The commercial chlorinated phenols Dowicide-6 and Dowicide-7 showed the presence of the unexpected 1,2,3,7,8,9-hexa-CDD (VIII) as the major hexa-CDD isomer. This isomer was not formed as the main dioxin component in any of the pyrolysis experiments and it does not consist of a single tetrachlorophenol moiety. Its formation from 2,3,4,6-tetrachlorophenol is not easily explained and we may therefore have to look for alternative routes of formation of PCDDs during the synthesis of commercial chlorinated phenols other than the commonly assumed condensation of two molecules of chlorophenol. The isolation of a chick oedema factor from toxic fat and its identification as 1,2,3,7,8,9-hexa-CDD (VIII) by using X-ray crystallography was reported by Cantrell *et al.*[6]. The finding of the same isomer as the major hexa-CDD contaminant in commercial chlorinated phenols should therefore provide further evidence for the suggested link between chlorinated phenols and the chick oedema factor in toxic fats[4].

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