Natural Formation of Chlorinated Phenols, Dibenzo-p-dioxins, and Dibenzofurans in Soil of a Douglas Fir Forest

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The natural formation of 4-MCP, 24/25- and 26-DCP, and 245-TrCP was detected in four selected areas of a rural Douglas fir forest where the humic layer was spiked in situ with a solution of Na^{37}Cl and covered by an enclosure, after 1 year of incubation. Chlorinated phenols (CP) can be formed naturally from organic matter and inorganic chloride by either de novo synthesis or chloroperoxidase (CPO)-catalyzed chlorination. The natural CP congeners were found to be present in high concentrations in soil compared to the other congeners, except for 245-TrCP which was present in a relatively low concentration. This study did not reveal which source, natural or anthropogenic, caused the observed concentrations. Some 20 chlorinated dibenzo-*p*-dioxins and dibenzofurans (CDD/F) were found to be formed naturally in soil of the Douglas fir forest; the formation of three 2,3,7,8-substituted congeners, 2378-TeCDD, 12378-PeCDD, and 123789-HxCDD, deserves special attention. A formation mechanism has been proposed which starts from naturally formed CP congeners and which probably involves peroxidase mediation. Chlorination of CDD/F congeners by the CPO-mediated reaction cannot be ruled out, but seems to be less likely due to the absence of several predicted congeners.

Introduction

The presence of low concentrations of (poly)chlorinated compounds in soil is, no doubt, primarily due to anthropogenic activity, i.e., environmental pollution. However, it is known today that quite a number of chlorinated compounds can also be formed naturally by either biochemical (*1–3*) or geochemical (*4–6*) processes. Some of these compounds, such as chloromethane (*7*) and polychlorinated acetones (*1*), can have both natural and anthropogenic sources. The combustion of natural organic material, such as wood and fuels, which contains appreciable amounts of inorganic chloride (*8*), has already been suggested as a source

of polychlorinated dibenzo-*p*-dioxins and dibenzofurans two decades ago (*9*). Therefore, natural (forest) fires have been suspected to contribute significantly to the global CDD/F emission (*10*). Harrad and Jones (*11*), on the other hand, argued that this is not true for the United Kingdom.

Actually, next to fires, there could be another natural source for CDD/F in the environment. Öberg et al. (*12*) showed the formation of several CDD/F congeners, including some of the 2,3,7,8-substituted congeners, in the horseradish peroxidase-mediated reaction of 2,4,5-trichlorophenol (245-TrCP) and hydrogen peroxide. In addition, Wagner et al. (*13*) observed PeCDD/F and HxCDD/F formation in a mixture of 245-TrCP, hydrogen peroxide, and a culture filtrate of the soil fungus *Phanerochaete chrysosporium* which showed peroxidase (PO) activity. PeCDD/F and HxCDD/F formation was also demonstrated in a mixture of 245-TrCP, hydrogen peroxide, and a fresh milk extract.

Öberg et al. (*14*) reported the formation of ^{13}C-CDD/F, mainly ^{13}C-HpCDD and ^{13}C-OCDD, from ^{13}C-PCP in activated sewage sludge. Öberg et al. (*15*) observed an up to 3-fold increase of CDD/F congeners expressed as international toxic equivalents (I-TEQ) during the composting of uncontaminated garden waste. De novo formation of CDD/F was held responsible because no contamination could have occurred and loss of biomass could not explain the increase of toxicity. However, PCP did not appear to be a precursor for CDD/F in ^{13}C-PCP-spiking experiments with garden waste. The role of PO in the synthesis of CDD/F during composting is not clear yet either. Although several bacteria and white-rot fungi that excrete PO are active in composting processes, chemical oxidation could also occur at the reported temperatures of 60–70 °C.

The above references all suggest the formation of CDD/F from anthropogenic chlorinated phenol congeners by natural processes. However, CP congeners can also be formed biogenically, and so real natural formation of CDD/F comes into perspective. Nine tick species are known to produce 26-DCP (*16*). Eisner et al. (*17*) reported the identification of 25-DCP in the froth of a grasshopper. However, they ascribed this to the uptake of 2,4-dichlorophenoxyacetic acid (2,4-D) or its degradation products in the plants which are eaten by the grasshopper. Ando et al. (*18*) reported the de novo synthesis of 24-DCP by a soil fungus of the group of *Penicillium*. CP congeners can be formed from phenol (*19*) and from compounds present in natural waters (*20*) in the chloroperoxidase-mediated reaction. The omnipresence of PO activity suggests that the natural formation of CDD/F from CP congeners can also occur in soil.

In this paper, we report the natural formation of CP and CDD/F congeners in the humic soil layer of a Douglas fir forest which was spiked in situ with a solution of Na^{37}Cl. The isotope ratios of the CP and CDD/F congeners were compared to those in nonspiked soil samples from the Douglas fir and a beech forest. The possible formation mechanisms are discussed.

Experimental Section

^{37}Cl Isotope Spiking. Four sites (B1, C1, F2, F3) in a Douglas fir forest in the rural area Speulderbos in The Netherlands were selected because they showed high concentrations of chloroform in soil air and natural chloroform formation was proven (*21*). At each site a soil surface of about 120 cm^2 was spiked with a solution of sodium chloride-37 (95% Na^{37}Cl; CIL CHLM-1225) at a concentration of 10 g of Na^{37}Cl per m^2. An enclosure was put over the spiked area and the natural

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formation of chloroform was studied during 1 year *(21)*. After 1 year of incubation the soil under the enclosure was sampled in glass jars for CP and CDD/F analysis.

Sampling. Two nonspiked soil cores were taken from the Douglas fir and a beech forest in Speulderbos. The soil top layer was sampled with a stainless steel tube ($l = 30$ cm; $\phi_{int} = 8$ cm) after removing the litter layer. The soil core was pressed from the tube by a plunger with a PTFE head and put on a stainless steel plate. The soil core was divided into subsamples based on their color, and the subsamples were put in glass jars (800 mL) which were closed by Bakelite caps with PTFE inner layers. Litter (dead leaves) of the beech forest was sampled in the same type of jars. The samples were stored at -20 °C.

Extraction and Cleanup. The samples were dried for 24 h at 105 °C and, after grinding, sieved through a 1 mm sieve. An internal standard (100 μL) which contained $^{13}C_6$-labeled CP congeners (4-MCP, 24/25-DCP, 245- and 246-TrCP, 2345-TeCP, and PCP) and $^{13}C_{12}$-CDD/F congeners (2378-TeCDD/F, 12378-PeCDD, 23478-PeCDF, 123478-HxCDD, 123678-HxCDF, 123789-HxCDD/F, 1234678-HpCDD/F, 1234789-HpCDF, and OCDD/F) in a concentration range of $40-500$ μg L^{-1} nonane was added to about 100 g of non-Na^{37}Cl-spiked soil. The internal standard was not added to the Na^{37}Cl-spiked soil samples since it would give interference with the native congeners when analyzing ^{35}Cl and ^{37}Cl ions. The samples were Soxhlet extracted with toluene for about 20 h; 90% of the extract was used for the CDD/F analysis and the rest for the analysis of the CP congeners.

The extract for the CDD/F analysis was passed successively through an activated carbon, a modified silica, and an aluminum oxide column. The cleaned extract was evaporated to dryness, and 50 μL of an injection standard of a 250 μg L^{-1} solution of $^{13}C_{12}$-1234-TeCDD in nonane was added. The injection standard was not added to the extracts of Na^{37}Cl-spiked soil samples since it would give interference as mentioned above.

The extract for the analysis of the CP congeners was reduced by evaporation to 1 mL of nonane which had been added beforehand. The extract was diluted with 10 mL of hexane, and the CP congeners were extracted into a 0.1 M K$_2$CO$_3$ solution. After phase separation, 1 mL of acetic acid anhydride was added to the aqueous solution, and the CP acetates formed were extracted three times with 25 mL of hexane. The hexane extracts were dried on anhydrous Na$_2$SO$_4$ and concentrated by evaporation to 1 mL. After the addition of 100 μL of an injection standard of a 1 mg L^{-1} solution of 1,2,3,4-tetrachloronaphthalene (TCN) in hexane, the extract was diluted to 2 mL.

GC–MS Analysis. Separation was performed by gas chromatography (Carlo Erba GC8000). One microliter was injected at 260 °C in the split–splitless mode. The CP congeners were separated on a DB5MS column ($l = 60$ m; $\phi_{int} = 0.25$ mm; film thickness = 0.1 μm) using a temperature program from 50 °C (1 min) to 75 °C (25 °C min^{-1}; 0 min) to 200 °C (3 °C min^{-1}; 0 min) to 300 °C (15 °C min^{-1}; 7 min). The CDD/F were separated on a SP2331 column ($l = 60$ m; $\phi_{int} = 0.25$ mm; film thickness = 0.2 μm) using a temperature program from 130 °C (1 min) to 180 °C (25 °C min^{-1}; 0 min) to 250 °C (2.5 °C min^{-1}; 15 min) to 260 °C (2 °C min^{-1}; 35 min). The GC effluent was transported to the mass spectrometer through a direct coupling interface kept at 275 °C, and the analytes were detected using a Fisons TRIO1000. Detection in the positive electron ionization (PEI) mode was done by detecting the mass ions either in the full scan ($m/z = 34-475$) or in the selected ion mode. Detection in the negative chemical ionization (NCI) mode using methane as ionization gas was done by detecting the mass ions either in the full scan mode ($m/z = 34-475$) or by detecting the mass ions 35 and 37.

Identification and Quantification. The CP and CDD/F congeners were identified on the basis of their retention time and the isotope ratio of their molecular ion. 24- and 25-DCP coeluted on the GC column used. Each CP congener was quantified by means of the corresponding $^{13}C_6$-labeled internal standard in the congener group, i.e., 4-MCP, 24/25-DCP, 245- and 246-TrCP, 2345-TeCP, and PCP, and by the response factor obtained using an external standard containing the $^{12}C_6$- and $^{13}C_6$-labeled congeners and 1234-TCN as mentioned above. The recoveries of the internal standards were calculated on the basis of the results obtained with the external standard and the injection standard in the sample extract. The recovery data were not used for quantification and only give information about the performance of the extraction and cleanup procedure. The recoveries were in an acceptable range (see Table 2).

The non-2,3,7,8-substituted CDD/F congeners were identified by comparing our chromatograms of a fly ash sample containing all congeners with those published by Professor Ballschmiter's group *(22–24)*. After the assignment of all the congeners in the chromatogram of our fly ash sample, the congeners present in the soil samples could be identified based on their retention time. Not all DCDF and TrCDF congeners could be identified from data in the quoted references. Therefore, we could not properly quantify 34-DCDF and 126-, 128-, 129-, 134-, 139-, 146-, 147-, 148-, 168-, 178-, 237-, 267-, 346-, and 347-TrCDF. It should be noted that Bacher et al. *(22, 23)* mentioned 148-TrCDD in their list which is, however, identical with 147-TrCDD, while 139-TrCDD was missing. From correspondence with G. Schreiner of Professor Ballschmiter's group it appeared that the systematic numbering of CDD congeners has to be corrected as follows: D22 = 139-TrCDD, D23 = 146-TrCDD, and D24 = 147-TrCDD.

Each CDD/F congener was quantified using the corresponding $^{13}C_{12}$-labeled internal standard in the congener group, i.e., 2378-TeCDD/F, 12378-PeCDD, 23478-PeCDF, 123478-HxCDD, 123678-HxCDF, 123789-HxCDD/F, 1234678-HpCDD/F, 1234789-HpCDF, and OCDD/F, and by the response factor obtained with an external standard containing the $^{12}C_{12}$- and $^{13}C_{12}$-labeled congeners as mentioned above. The MCDD/F, DCDD/F, and TrCDD/F congeners were quantified via $^{13}C_{12}$-27/28-DCDD, because this was the only $^{13}C_{12}$-labeled standard available for the lower chlorinated CDD/F. The recoveries of the internal standards were in an acceptable range (see Table 4).

Results and Discussion

To verify whether natural production of CP and CDD/F congeners can occur, four sites in a rural Douglas fir forest were spiked with a solution of Na^{37}Cl. Natural chlorinated organic compounds are formed from a natural carbon source and inorganic chloride. The detection of a compound containing a ^{37}Cl isotope percentage higher than the natural percentage of 24% implies that this compound is formed naturally in the Na^{37}Cl-spiked soil. At the selected sites, the natural production of chloroform was observed for a period of 1 year *(21)*; after that study the soil under the enclosure was sampled and analyzed by GC–PEI-MS and GC–NCI-MS for the presence of CP and CDD/F congeners with a deviating ^{37}Cl isotope percentage.

CP Congeners. The ^{37}Cl isotope percentage in the various CP congeners was calculated from two isotope masses of the molecular ion in the PEI-MS analysis and from m/z 35 and 37 in the NCI-MS analysis. The reason for analyzing the sample extracts by two ionization methods was that the signals of 245-TrCP and 2346- and 2356-TeCP were disturbed in PEI-MS. All analytes except the MCP congeners show excellent detectability in NCI-MS at the concentrations present in the samples, and the natural ^{37}Cl isotope percent-

TABLE 1. ^{37}Cl Isotope Percentage in the Molecular Mass Ion of CP Congeners[a-c]

	nonspiked CE1638-1645 ($x \pm$ sd)	spiked with Na^{37}Cl				
		B1		C1	F2	F3
analytes		CH057 humus 0-5 cm	CH058 black 5-10 cm	CH059 humus 0-5 cm	CH060 humus 0-5 cm	CH061 humus 0-5 cm
2 MCP	23 ± 2	25	25	24	24	25
4 MCP	24 ± 2	**61**	**32**	30	**32**	27
24/25 DCP	23 ± 0.4	**27**	25	**26**	**26**	25
26 DCP	22 ± 1	**28**	**28**	**26**	24	25
245 TrCP	23 ± 0.7	**29**	nd	22	26	**30**
246 TrCP	24 ± 0.5	26	26	**28**	25	25
2345 TeCP	24 ± 0.4	25	24	24	24	26
2346 TeCP	23 ± 0.8	26	26	26	26	25
PCP	23 ± 0.9	23	24	25	23	26
1234 TCN	23 ± 0.8	24	24	24	24	23

[a] Abbreviations: x, average; sd, standard deviation; nd, not detected. [b] Bold values: isotope percentage of ^{37}Cl higher than isotope percentage of ^{37}Cl + 3 sd in sample extract of nonspiked soil. [c] Data based on NCI-MS except for MCP (PEI-MS).

TABLE 2. Concentrations (μg kg^{-1} dw) of CP Congeners in Soil of a Beech and a Douglas Fir Forest[a]

	beech				Douglas fir				
analyte	CE1638 leave litter	CE1639 humus 0-5 cm	CE1640 1st black 5-10 cm	CE1641 2nd black 10-15 cm	CE1642 humus 0-5 cm	CE1643 black 5-10 cm	CE1644 gray 10-15 cm	CE1645 yellow 15-20 cm	recovery (%) ($x \pm$ sd)[b]
2 MCP	0.93	0.55	0.21	0.08	0.25	0.09	0.06	0.05	
3 MCP	<0.3	0.07	0.04	<0.01	<0.04	<0.04	<0.02	<0.02	
4 MCP	45	25	6.5	1.4	18	2.2	0.70	0.55	110 ± 20
23 DCP	<0.09	<0.2	<0.04	<0.01	<0.1	<0.05	<0.06	<0.03	
24/25 DCP	7.1	10	4.7	1.1	22	3.8	0.90	0.45	90 ± 10
26 DCP	12	6.3	1.5	0.37	2.8	1.1	0.32	0.14	
34 DCP	<0.09	<0.2	<0.04	<0.01	<0.1	<0.05	<0.06	<0.03	
35 DCP	<0.4	<1	<0.1	<0.01	<0.1	<0.06	<0.07	<0.04	
234 TrCP	<0.2	<0.1	0.11	<0.01	<0.09	<0.05	<0.02	<0.03	
235 TrCP	<0.3	<0.1	<0.04	0.02	<0.1	<0.06	<0.03	<0.04	
236 TrCP	<0.2	<0.08	<0.03	<0.01	<0.07	<0.04	<0.02	<0.03	
245 TrCP[c]	0.22	0.33	0.20	0.03	0.32	0.14	0.04	0.04	110 ± 10
246 TrCP	9.6	9.4	9.5	5.7	19	17	5.4	1.4	100 ± 10
345 TrCP	<0.3	<0.1	<0.05	<0.02	<0.1	<0.06	<0.03	<0.04	
2345 TeCP	0.32	0.57	0.49	0.04	0.54	0.17	0.03	<0.03	120 ± 20
2346 TeCP[c]	0.44	0.47	0.32	0.10	0.55	0.20	0.14	0.12	
2356 TeCP[c]	0.10	<0.09	0.12	0.06	0.09	0.09	0.04	<0.03	
PCP	2.3	2.1	1.0	0.36	4.9	0.60	0.35	0.33	100 ± 10

[a] <, below detection limit indicated. Detection limits are seen to differ for different sample types. [b] x, average; sd, standard deviation. [c] Interferences observed in the quantifying mass. Quantification was done by correcting the response area of the quantifying mass by means of the natural chlorine isotope ratio, ^{35}Cl:^{37}Cl = 76:24, and the response area of the next highest m/z value in the molecular mass ion on the assumption that this m/z was undisturbed.

age of 24% could generally be determined with a lower standard deviation by NCI than by PEI, i.e., about 1%.

Formation of CP congeners from Na^{37}Cl was considered to have been demonstrated if the ^{37}Cl isotope percentage in the sample extracts of the spiked soil was higher than the percentage plus 3 times the standard deviation in the sample extracts of the nonspiked soil. Table 1 shows the average ^{37}Cl isotope percentages and the standard deviations for the nonspiked soils from the beech and Douglas forests (CE1638–1645) and the ^{37}Cl isotope percentages for the Na^{37}Cl-spiked soils in the Douglas forest (CH057–061). The formation of 4-MCP (32–61% ^{37}Cl), 24/25-DCP (26–27% ^{37}Cl), and 26-DCP (26–28% ^{37}Cl) from Na^{37}Cl was detected in three out of five samples. Two humic layers distinctly show the formation of 245-TrCP (29–30% ^{37}Cl) from Na^{37}Cl because the use of NCI-MS rather than PEI-MS eliminated the interference problem and considerably reduced the standard deviation of the ^{37}Cl isotope percentage of this congener. The data for the humic layer of site C1 suggests the natural formation of 246-TrCP (28% ^{37}Cl). However, one should be careful here, because an increase of the ^{37}Cl isotope percentage was not observed in PEI-MS although the standard deviations of both methods were essentially the same in this case.

The natural CP congeners, 4-MCP, 24/25-DCP, 26-DCP, and 245-TrCP, might be present naturally in soil due to de novo synthesis by, e.g., fungi (18) or the chloroperoxidase (CPO)-catalyzed chlorination of aromatic structures (RH) such as phenol (19) or humic material (20):

$$RH + Cl^- + H^+ + H_2O_2 \xrightarrow{CPO} RCl + 2H_2O \qquad (1)$$
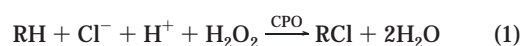
CPO activity has been observed in several soil extracts, and the chlorination activity in the organic layer was about 1000-fold higher than in deeper soil layers (25). The CPOs are most probably excreted by fungi similar to Caldariomyces fumago, a deuteromycete, which is known to produce CPO. Penicillium species that produce 24-DCP (18) also belong to the deuteromycetes, but CPO production has not been studied. Basidiomycetes do not show CPO activity; however, in pure cultures they produce de novo large amounts of chlorinated 4-methoxybenzaldehydes which constitute up to 55% of the total amount of organohalogens (TOX) (26). Basidiomycetes may therefore be responsible for the very high concentrations of chlorinated 4-methoxybenzaldehydes (0.04–20 mg of organic Cl kg^{-1} dw) found in rotting wood,

TABLE 3. ^{37}Cl Isotope Percentage in the Molecular Mass Ion of CDD/F Congeners Obtained from PEI-MS[a,b]

analyte	nonspiked CE1638−1645 ($x \pm$ sd)	B1 CH057	B1 CH058	C1 CH059	F2 CH060	F3 CH061
		spiked with Na^{37}Cl				
1 MCDD	24[c]	**29**	**34**	**29**	**31**	27
1247/1248/1369 TeCDD	24 ± 1	**33**	**45**	**33**	**46**	**38**
1378 TeCDD	24 ± 2	30	**38**	26	**32**	29
2378 TeCDD	25 ± 2	**33**	nd	nd	23	21
12346 PeCDD	24 ± 2	**37**	**34**	**35**	30	**35**
12378 PeCDD	24 ± 0.8	**29**	**28**	24	27	26
123478 HxCDD	25 ± 2	27	nd	18	nd	19
123678 HxCDD	25 ± 0.8	25	17	25	27	20
123789 HxCDD	25 ± 2	26	30	25	**35**	24
1234678 HpCDD	24 ± 1	24	24	24	24	24
OCDD	24 ± 2	24	26	24	23	25
1 MCDF	26 ± 2[c]	**42**	27	32	**34**	nd
2 MCDF	24 ± 1[c]	**31**	25	22	23	22
3 MCDF	24 ± 1[c]	22	23	24	25	27
4 MCDF	23 ± 3[c]	**50**	**110**	**63**	**54**	**46**
17 DCDF	25 ± 0.6	**30**	**31**	**29**	**31**	**34**
124/167 TrCDF	25 ± 0.8	25	**29**	**33**	**30**	24
1234/1678 TeCDF	25 ± 1	28	**31**	25	26	26
1347 TeCDF	26 ± 2	25	22	**34**	24	25
2378 TeCDF	25 ± 2	24	25	24	23	25
12346 PeCDF	24 ± 1	26	**30**	26	**28**	**29**
12348/12378 PeCDF	24 ± 1	25	35	25	25	27
12489 PeCDF	25 ± 1	**36**	**36**	21	22	24
23478 PeCDF	24 ± 0.2	24	24	24	23	24
123478/123479 HxCDF	24 ± 2	26	24	26	26	26
123678 HxCDF	24 ± 2	26	24	24	28	25
123789 HxCDF	25 ± 2	24	nd	20	nd	nd
234678 HxCDF	24 ± 0.8	23	22	20	20	21
1234678 HpCDF	24 ± 1	26	26	25	26	26
1234679 HpCDF	25 ± 0.7	**29**	25	28	**29**	23
1234689 HpCDF	24 ± 0.4	**27**	27	27	26	**28**
1234789 HpCDF	24 ± 0.4	25	23	24	24	22
OCDF	24 ± 0.8	23	25	24	22	27

[a] 2,3,7,8-Substituted congeners in italics; x, average; sd, standard deviation; nd, one of the two isotope masses in the molecular ion not detected. [b] Bold values: isotope percentage of ^{37}Cl exceeds that in the nonspiked samples + 3 sd. [c] Values obtained using another MS instrument.

moldy leaf litter, and soil from a *Lepista nuda* fairy ring (*27, 28*) compared to the TOX concentration (4−920 mg of Cl kg^{-1} dw (*29*)). How basidiomycetes synthesize chlorinated aromatics is not clear yet.

Since the two nonspiked soil cores contained an internal standard, the concentrations of the CP congeners in these samples could be calculated to get an impression about the order of magnitude (Table 2). Since the internal standard was added after the drying procedure (24 h at 105 °C), the recoveries shown in Table 2 do not take into account the whole treatment procedure, and therefore the results may have been influenced by the experimental setup. We know of only one other study which describes background levels of CP congeners in rural soil. Edelman (*30*) screened 40 different sites spread over The Netherlands by taking 10 cm cores and drying the soil samples for 48 h at 35 °C. He found 24-DCP, 246-TrCP, and PCP concentrations of <0.5−18, <0.2−2.7, and <0.1−4.4 μg kg^{-1} dw, respectively. Since these results are of the same order of magnitude or lower than ours (i.e., the average of our 0−5 cm and 5−10 cm samples in Table 2), the influence of the sample drying can be considered to have been small.

Five congeners were not found in any sample, and three more showed up in one or two samples only. For all 11 remaining analytes, the concentrations are seen to decrease with increasing soil depth, and there are no real differences in the concentration profiles in the soils of the beech and the Douglas forest. The concentrations of the natural CP congeners, 4-MCP, 24/25-DCP, and 26-DCP, in the humic layers were found to be 18−25, 10−22, and 2.8−6.3 μg kg^{-1}

dw, respectively, and were high compared to those for which natural formation was not observed. The concentrations of 245-TrCP observed in soil were invariably low.

From our results it cannot be concluded that the presence of 4-MCP, 24/25-DCP, 26-DCP, and 245-TrCP are only due to natural formation in soil. Emissions from production and use of CP congeners or herbicides synthesized from CPs will also contribute to the presence of CP congeners in soil of rural areas by evaporation, atmospheric transportation, and deposition. Industrial chlorination of phenol to obtain commercial wood preserving agents (*31*) mainly proceeds via 4-MCP, 24-DCP, 246-TrCP, and 2346-TeCP to PCP. Therefore, the presence of high concentrations of CP congeners in rural soils may also be due to anthropogenic sources, and the relative contributions of the different inputs remain to be elucidated.

CDD and CDF Congeners. Since analysis of the samples from the Na^{37}Cl-spiked Douglas soils revealed that several MCP to TrCP congeners are formed naturally, the nonspiked CE1638−1645 and the spiked CH057−061 sample series were also analyzed by GC−PEI-MS for the presence of CDD/F congeners. The ^{37}Cl isotope percentage was calculated from the two isotope masses of the molecular ion with the highest intensity, i.e., under the natural nonspiked conditions of samples CE1638−1645. Natural formation was demonstrated in the same way as for CP congeners. Table 3 shows the ^{37}Cl isotope percentage of CDD/F congeners with a ^{37}Cl isotope percentage above the natural level and the data for all 2,3,7,8-substituted congeners. From among the latter group of analytes, 12378-PeCDD showed a significantly increased

TABLE 4. Concentrations of Selected CDD/F Congeners and TEQ Values in Soil of a Beech and a Douglas Fir Forest (ng kg^{-1} Dw)[a]

	beech				douglas fir				recovery (%)
analyte	CE1638	CE1639	CE1640	CE1641	CE1642	CE1643	CE1644	CE1645	$(x \pm sd)$[b]
23/27/28 DCDD	2.2	3.0	1.8	<0.1	5.8	3.5	1.3	0.35	120 ± 20
139/237 TrCDD	0.58	1.3	0.71	<0.08	0.65	0.22	<0.06	<0.08	
1247/1248/1369 TeCDD	3.4	9.5	4.4	0.37	3.3	1.5	0.36	0.10	
1378 TeCDD	2.5	6.6	2.7	0.18	2.1	0.61	0.13	0.05	
2378 TeCDD	0.43	1.1	0.37	0.04	0.46	0.23	0.05	0.05	90 ± 10
12346 PeCDD	<2.2	2.6	1.8	0.34	1.2	0.51	0.20	<0.2	
12378 PeCDD	1.9	3.8	2.1	0.22	2.0	0.81	0.15	0.07	90 ± 20
123478 HxCDD	<6	3.9	2.1	0.31	2.1	1.0	0.19	<0.2	80 ± 20
123678 HxCDD	<6	8.3	3.8	0.31	4.1	1.5	0.17	<0.2	
123789 HxCDD	4.1	8.2	4.2	0.32	5.3	1.8	0.38	<0.3	70 ± 20
1234678 HpCDD	38	95	37	4.2	44	18	2.4	0.80	60 ± 20
1234679 HpCDD	39	86	36	4.4	47	18	2.2	0.76	
12346789 OCDD	140	360	170	33	190	70	12	5.4	60 ± 30
17 DCDF	0.50	0.94	1.6	0.08	0.53	0.31	0.10	0.06	
23 DCDF	0.51	0.94	1.9	0.21	0.57	0.53	0.25	0.08	
27 DCDF	2.5	4.0	4.8	0.53	3.1	1.8	0.69	0.57	
28 DCDF	3.7	6.7	9.2	<0.03	0.20	<0.05	0.15	0.39	
124/167 TrCDF	1.2	1.9	1.8	<0.03	<0.08	0.75	0.18	<0.08	
234/238 TrCDF	4.2	6.9	7.6	0.95	5.8	2.2	0.55	0.19	
1234/1678 TeCDF	6.7	5.3	7.8	3.4	2.3	0.70	<0.1	<0.1	
1347 TeCDF	2.6	6.4	6.8	0.83	4.8	2.6	0.54	0.59	
2378 TeCDF	4.1	11	5.3	0.67	7.4	2.7	0.51	0.20	80 ± 10
12346 PeCDF	2.2	5.0	4.8	0.34	2.1	1.1	0.27	<0.1	
12348/*12378 PeCDF*	5.2	13	14	1.0	6.3	3.6	0.74	0.15	
12489 PeCDF	<2	1.5	1.2	0.14	0.77	0.44	0.11	<0.1	
23478 PeCDF	4.3	10	8.2	0.91	6.2	3.1	0.61	0.16	80 ± 20
123478/123479 *HxCDF*	4.1	13	13	1.4	6.9	4.6	0.93	0.21	
123678 HxCDF	3.7	11	8.8	0.98	5.8	3.3	0.68	0.16	80 ± 20
123789 HxCDF	<7	4.2	6.5	0.70	1.9	1.8	0.53	0.30	90 ± 30
234678 HxCDF	<8	9.8	6.9	0.81	5.0	2.7	0.55	0.15	
1234678 HpCDF	21	58	88	8.5	33	29	6.1	1.4	70 ± 20
1234679 HpCDF	3.9	7.4	6.4	0.87	3.6	2.5	0.54	0.09	
1234689 HpCDF	3.2	9.6	20	1.2	7.2	5.3	0.74	0.15	
1234789 HpCDF	<7	<7	5.1	0.91	3.0	2.6	0.74	0.38	80 ± 30
12346789 OCDF	<60	42	120	16	35	44	12	2.6	80 ± 40
I-TEQ	6.1	18	13	1.4	9.8	4.9	0.98	0.31	
BGA-TEQ	7.3	21	16	1.6	11	5.4	1.1	0.34	

[a] Naturally formed congeners and 2,3,7,8-substituted CDD/F congeners (italics) including DCDD/F and TrCDD/F that are substituted at one or more of the 2,3,7,8 positions were selected. <, below detection limit indicated. Detection limits are seen to differ for different sample types. [b] *x*, average; sd, standard deviation.

^{37}Cl isotope percentage in two out of five samples and 2378-TeCDD and 123789-HxCDD in one sample. Of the non-2,3,7,8-substituted congeners, 4-MCDF, 17-DCDF, and 1247/1248/1369-TeCDD were found to be formed naturally in all the Na^{37}Cl-spiked soils and 1-MCDD, 12346-PeCDD,k and 1234689-HpCDF in four out of five. Nine non-2,3,7,8-substituted congeners were formed in less than four spiked soils.

The natural formation of CDD/F congeners in the soil of the Douglas forest may be caused by the chloroperoxidase-mediated chlorination of CDD/F congeners. Since the chloroperoxidase-mediated reaction chlorinates organic material randomly, the CDD/F congeners are expected to be chlorinated according to their relative concentration ratio, and thus the natural CDD/F congeners are expected to be mainly formed from the congeners present at the highest concentrations in soil. This is the constraint of the CPO mechanism because most congeners with a relative high concentration in soil appear not to lead to one of the natural CDD/F congeners found. Table 4 shows the concentrations of the naturally formed congeners and the 2,3,7,8-substituted CDD/F congeners including DCDD/F and TrCDD/F that are substituted at one or more of the 2,3,7,8 positions, in the soil core samples from the Douglas fir and beech forests. All MCDD/F congeners were found to be below their detection limits. Next to the congeners reported in Table 4, 35 non-2,3,7,8-substituted TeCDD/F to HxCDD/F congeners were found to be present in concentrations higher than 10 ng kg^{-1}

dw in the humic layer of the forests. The concentrations of all CDD/F congeners found in both forest soils decrease with increasing depth, and the concentrations in the litter sample are lower than in the humic layer. Other than the natural CP congeners, the natural CDD/F congeners are not present in the highest concentrations but are always below 10 ng kg^{-1} dw in the humic layer.

Another mechanism that could explain the formation of CDD/F congeners enriched with ^{37}Cl is the peroxidase (PO)-mediated coupling of CP congeners as studied by Öberg et al. *(12)* who found the following main reactions:

$$2CP + 2H_2O_2 \xrightarrow{PO} CDD + 4H_2O \qquad (2)$$

$$2CP + H_2O_2 \xrightarrow{PO} CDF + 3H_2O \qquad (3)$$

The formed CDD/F congeners were interpreted as being direct coupling products or indirect products from chlorine rearrangements, abstractions, and additions. The authors argued that CDD/F congeners are formed via chlorophenoxy radicals. However, they did not mention or discuss the reaction pathways which they assumed. Therefore, we studied two reaction mechanisms which are proposed to occur in gas-phase pyrolysis *(32)* and fly-ash-catalyzed oxidation *(33, 34)*.

In the gas-phase pyrolysis of CP congeners, CDF congeners are assumed to be formed via *o*-C—*o*-C coupling of two

chlorophenoxy radicals to a 2,2′-dihydroxybiphenyl, whereas CDD congeners are supposed to be formed via the attack of a chlorophenoxy radical on a CP congener at the ortho position, forming a 2-phenoxyphenol radical *(32)*. The presence of a chlorine atom at the ortho position of the two reacting CP congeners appears to be essential for the formation of CDD congeners. Since, in our study, all the naturally formed CP congeners and all CP congeners with high concentrations in soil have a chlorine atom in the ortho position (except 4-MCP), we expected that combinations of these congeners will most probably cause the formation of CDD/F congeners. However, the mechanism proposed by Born et al. *(32)* for the formation of CDD/F congeners in pyrolysis processes does not predict our results in soil except for the formation of 1378-TeCDD, 12346-PeCDD, and 1234689-HpCDF. We, therefore, tentatively conclude that a gas-phase pyrolysis-like mechanism does not take place in soil.

The formation of CDD congeners from CP congeners on fly ash is thought to follow the same mechanism as in gas-phase pyrolysis and, in parallel, a Smiles rearrangement of the 2-phenoxyphenol anion is suggested *(33)*. The presence of a chlorine atom in the ortho position of the reacting CP congeners is not necessary for the formation of CDD congeners since meta- and para-substituted CP congeners are easily isomerized to ortho-substituted analogues on fly ash *(34)*. No formation of CDF congeners by the fly-ash-mediated reaction is reported, and CP congeners are not expected to be CDF precursors in combustion *(34)*. Therefore, the fly-ash-mediated mechanism cannot explain our results any better than the gas-phase pyrolysis of CP congeners referred to above.

The PO-mediated reaction and the fly-ash-mediated mechanism have in common that ortho substitution is not important while they differ with regard to the products formed. The CDD and CDF congeners formed in the PO-mediated reaction appear to have the same number of chlorine atoms as their reactants *(12)*. Taking these observations into account, we adapted the fly-ash-mediated mechanism. Figure 1 shows our proposed mechanism that proceeds via anions; however, a radical mechanism would lead to the same products *(34)*. For the formation of CDD congeners it was assumed that hydrogen can also be a leaving group *(35)*, and for the formation of CDF congeners we suggest a completely new mechanism that closely resembles the CDD formation mechanism. The hypochlorous acid that can be formed during CDF formation may be responsible for the chlorination reaction that Öberg et al. *(12)* observed in the PO-mediated reaction of CP congeners.

Table 5 gives an overview of how the various CDD/F congeners that were formed in our Na^{37}Cl-spiked soil experiments can be explained by specific combinations of naturally formed CP congeners and congeners with a high concentration in soil. 17-DCDF and 1247-/1248-/1369-TeCDD seem to be typical products in soil since they can be formed from *naturally* formed CP congeners by a *natural* reaction. The formation of 12346-PeCDD, 12346-PeCDF, and 1234679- and 1234689-HpCDF in soil cannot be called purely natural because one of the reactants, PCP, originates from anthropogenic sources. 1369- and 1378-TeCDD can be formed from either purely natural CP congeners or a natural congener and anthropogenic 246-TrCP. The formation of 1-MCDD and 4-MCDF cannot be explained on the basis of the above-mentioned CP congeners only: if our mechanism holds true, the involvement of phenol is suggested. Regarding the congeners that were less frequently found in the Na^{37}Cl-spiked soil studies, their formation should probably be explained also by chlorine atom rearrangements.

In summary, the mechanism proposed above provides a rather satisfactory explanation of our experimental results
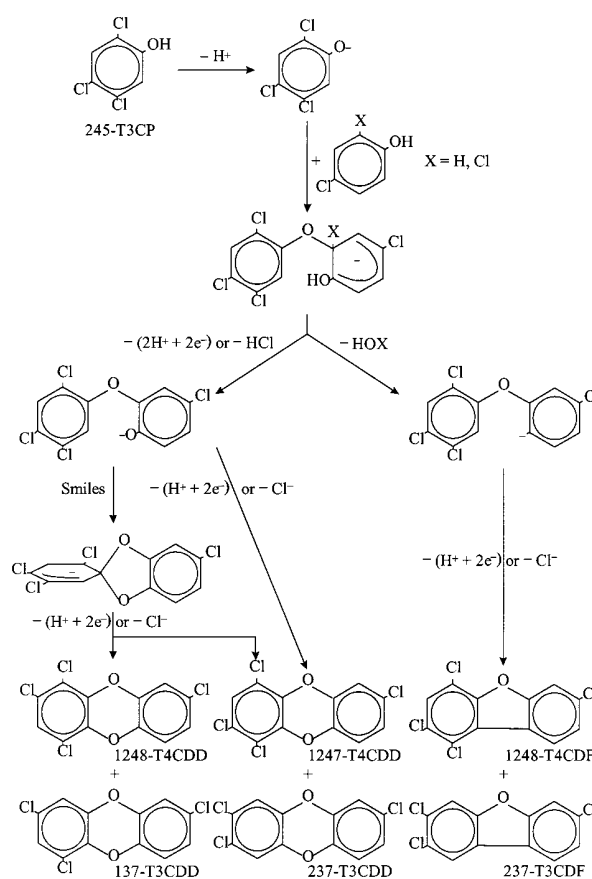

FIGURE 1. Possible formation mechanism of CDD/F congeners mediated by peroxidases.

TABLE 5. Natural Formation of CDD/F Congeners Explained by PO-Mediated Formation from Combinations of CP Congeners[a]

naturally formed CP congeners	CP congeners with highest concentrations in soil					
	4-MCP	24-DCP	25-DCP	26-DCP	246-TrCP	PCP
4-MCP						
24-DCP			1369-D			
25-DCP		1369-D		17-F	1369-D	
26-DCP			17-F			12346-D
						12346-F
245-TrCP	1247-D	1247-D	1247-D		1378-D	1234679-F
	1248-D	1248-D	1248-D			1234689-F
			1378-D	1347-F		

[a] D, CDD; F, CDF.

on the natural formation of CDD/F congeners, even though it must be admitted that 245-TrCP plays a larger role than would be expected on the basis of its rather low concentration in soil. In addition, our mechanism predicts the formation of more congeners than we actually found (see, e.g., Figure 1 where the formation of 137- and 237-TrCDD and 1248-TeCDF is predicted (note that 237-TrCDF could not be identified; cf. Experimental Section)). Although one can, of course, argue that some congeners may well have been formed in too low amounts to permit their detection by the current analytical procedure, this is too unsubstantiated a way out. It is obvious that further work will have to be carried out to validate and/or modify the proposed mechanism. Nevertheless, we feel that the present results, and their tentative interpretation, do contribute to the unravelling of the problem of the natural formation of CP and CDD/F congeners.

Finally, now that it has been demonstrated that natural formation of CP and CDD/F congeners in soil is possible, subsequent research should be directed at evaluating the significance of this natural formation compared to other sources such as atmospheric deposition.

Abbreviations

CDD	chlorinated dibenzo-*p*-dioxins
CDF	chlorinated dibenzofurans
CP	chlorinated phenols
D	di
Hp	hepta
Hx	hexa
M	mono
O	octa
Pe	penta
Te	tetra
Tr	tri

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