NATURAL FORMATION OF CHLORINATED PHENOLS, DIBENZO-*P*-DIOXINS AND DIBENZOFURANS IN SOIL OF A DOUGLAS FIR FOREST

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Introduction

The formation of several chlorinated dibenzo-*p*-dioxin and dibenzofuran (CDD/F) congeners, including some of the 2,3,7,8-substituted congeners, in the horseradish peroxidase (HRPO)-mediated reaction of 2,4,5-trichlorophenol (245-TrCP) and hydrogen peroxide were reported [1,2]. Öberg *et al.* [3] reported the formation of ^{13}C-CDD/F from ^{13}C-PCP in activated sewage sludge. The same research group observed an up to 3-fold increase of CDD/F concentration during the composting of uncontaminated garden waste[4]. *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 above references all suggest the formation of CDD/F from anthropogenic chlorinated phenol (CP) congeners by natural processes. However, CP congeners can also be formed biogenically and so real natural formation of CDD/F comes into perspective. CP congeners can be formed from phenol [5] and from compounds present in natural waters[6] in the chloroperoxidase (CPO)-mediated reaction. The females of nine tick species are known to produce 26-DCP as sex pheromone[7]. 25-DCP was identified in the froth of a grasshopper[8] and 24-DCP was reported as a *de novo* product of a soil fungus [9]. However, the experimental conditions in the latter two studies cannot exclude that the 24- and 25-DCP are degradation products of 2,4-dichlorophenoxyacetic acid.

Silk et al. [10] found that 2468-TeCDF, 1368-TeCDD and 1379-TeCDD were predominantly present in peat bog samples from Canada. These congeners were also formed during CPO-mediated coupling of 24-DCP. The omni-presence of peroxidase (PO) activity and the natural formation of CP congeners suggest that the natural formation of CDD/F congeners can occur in soil.

Methods and materials

Four sites in a Douglas fir forest in the rural area Speulderbos in The Netherlands were selected because natural chloroform formation was observed to take place[11]. At each site the soil surface was spiked with a solution of Na^{37}Cl. An enclosure was put over the spiked area and the natural formation of chloroform was studied during one year. After one year of incubation the soil under the enclosure was sampled in glass jars for CP and CDD/F analysis. The samples were stored -20°C. The sample preparation and analysis are described here shortly [12]. After drying grinding and sieving the samples were spiked with an internal standard which contained $^{13}C_6$-labelled CP and $^{13}C_{12}$-CDD/F congeners. The internal standard was not added to the Na^{37}Cl spiked soil samples since it would give interference with the native congeners when analysing the ^{35}Cl and ^{37}Cl ions. The samples were Soxhlet extracted with toluene; 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 aluminium oxide column. The cleaned extract was evaporated to dryness and an injection standard containing $^{13}C_{12}$-1234-TeCDD was added. The injection standard was not added to the extracts of $Na^{37}Cl$ spiked soil (see above).

The extract for the analysis of the CP congeners was reduced by evaporation. The extract was diluted with hexane and the CP congeners were extracted into an aqueous K_2CO_3 solution. After phase separation, acetic acid anhydride was added to the aqueous phase and the CP-acetates formed were extracted with hexane. The hexane extracts were dried on anhydrous Na_2SO_4 and concentrated by evaporation. An injection standard containing 1,2,3,4-tetrachloronaphthalene was added.

The CP and CDD/F congeners were separated on a DB5-MS and a SP2331 column, respectively. The congeners were detected by PEI-MS either in scan mode or SIM. CP congeners were also detected by NCI using methane scanning mass ions 35 and 37. 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 co-eluted on the GC column used. 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 [13]. 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.

Results and discussion

Formation of CP congeners from $Na^{37}Cl$ was considered to have been demonstrated if the ^{37}Cl isotope percentage of the congeners in the sample extracts of the spiked soil was higher than the percentage plus three times the standard deviation in the sample extracts of the non-spiked soil ($23\pm1\%$ ^{37}Cl). 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 $Na^{37}Cl$.

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 $\mu g\ kg^{-1}$ dw, 10–22 $\mu g\ kg^{-1}$ dw and 2.8–6.3 $\mu g\ kg^{-1}$ 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 (0.32-0.33 $\mu g\ kg^{-1}$ dw).

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 synthesised 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 [14] 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.

Natural formation for CDD/F was demonstrated in the same way as for CP congeners. 12378-PeCDD showed a significantly increased ^{37}Cl isotope percentage in two out of five samples (28-29% ^{37}Cl) and 2378-TeCDD (33% ^{37}Cl) and 123789-HxCDD (35% ^{37}Cl) in one sample. Of the non-2,3,7,8-substituted congeners, 4-MCDF (46-110% ^{37}Cl), 17-DCDF (29-34% ^{37}Cl) and 1247/1248/1369-TeCDD (33-46% ^{37}Cl) were found to be formed naturally in all the $Na^{37}Cl$-spiked soils and 1-MCDD (29-34% ^{37}Cl), 12346-PeCDD (34-37% ^{37}Cl) and 1234689-HpCDF (27-28% ^{37}Cl) 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 (>10 ng kg^{-1} dw) in soil appear not to lead to one of the natural CDD/F congeners found. Other than the natural CP congeners, the natural CDD/F congeners are not present in the highest concentrations but 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 PO-mediated coupling of CP congeners [1]. The formed CDD/F congeners were interpreted as being direct coupling products or indirect products from chlorine rearrangements, abstractions and additions. The Öberg *et al.* 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 chlorophenoxy radical reaction mechanisms, which are proposed to occur in gas-phase pyrolysis [15] and fly ash-catalysed oxidation [16,17]. However, both mechanisms appeared not to fit with our results.

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 [1]. Taking these observations into account, we adapted the fly ash-mediated mechanism. For the formation of CDD congeners it was assumed that hydrogen can also be a leaving group [18] and for the formation of CDF congeners we suggest a completely new mechanism that closely resembles the CDD formation mechanism.

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-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. As regards 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.

The formation of 1368- and 1379-TeCDD as found in the CPO-mediated reaction of 24-DCP [10] can also be explained by our mechanism, however not the formation of 2468-TeCDF. We confirm that the concentrations of these congeners in soil are high (>10 ng kg^{-1} dw) but we did not have indications that the pattern was significantly different of that of a fly ash.

In summary, the mechanism proposed above provides a rather satisfactory explanation of our experimental results 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. 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 natural formation mechanism of CP and CDD/F congeners.

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