

## FORMATION OF PCDD/PCDF's VIA AN ENZYMATICALLY MEDIATED CHLORINATION OF HUMIC ACIDS

Jørgen Vikelsøe, Pia Lassen, Elsebeth Johansen and Lars Carlsen\*  
National Environmental Research Institute, Department of Environmental Chemistry,  
DK-4000 Roskilde, Denmark

### 1. Introduction

Previous studies on the PCDD/PCDF congener distribution in a series of Dutch top soils revealed only minor differences in the distribution of tetra- to octachlorinated congeners, and at all investigated sites the distribution resembled that of PCDD/PCDF's in emissions from waste incineration<sup>1)</sup>. A comprehensive survey of background levels of PCDD/PCDF's in UK soils reported values ranging from 6.6 ng/kg for PCDD to 191 ng/kg for OCDD and 23 ng/kg for PCDF to 41 ng/kg for HxCDF<sup>2)</sup>. Although some variations in the distribution of the mono-, di- and trichlorinated congeners were observed, indicating that sources other than waste incineration were operating, no evidence that enzyme mediated reactions would be responsible for PCDD/PCDF's in soils were found.

Recently Carlsen and Lassen<sup>3)</sup> demonstrated that humic acids were chlorinated by chloride ions in the presence of the enzyme chloroperoxidase and hydrogen peroxide. It appeared that the chlorine moieties in chlorinated humics to some extent was labile<sup>3)</sup>, leading to the formation of a wide variety of low molecular organochloro compounds such as chloroform as well as chlorinated acetic acids, acetones, pyruvic acids, maleic- and fumaric acids, acetonitriles and acetamides<sup>5)</sup>. Thus, trichloro acetic acid was generated from a commercially available humic acid (Aldrich) in amounts up to 260 ng/mg of humic acids. The formation from a 'natural' humic acid was approximately one order of magnitude less. It was concluded that since soils apparently contain all the necessary precursors, enzymatically mediated chlorination appears as a plausible mechanisms for the formation of organochloro compounds in the terrestrial environment.

Since a production of low molecular weight organic compounds with a substantial chlorine content as a results of an enzymatically mediated chlorination of humic acids apparently prevails, it appeared appropriate to further study the reaction in order to reveal any formation of PCDD/PCDF's, as even a small natural production of the latter may be of interest due to its apparent omnipresence.

### 2. Experimental

A filtered humic acid (Aldrich) solution (1 g/L) in a 0.1 M disodium hydrogenphosphate

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was adjusted to pH = 3.5 with concentrated phosphoric acid, stirred for 2 hours followed by filtration. To 600 mL of the resulting solution 6 mL 0.1 M sodium chloride and 0.2 mL 30.000 e.u./mL chloroperoxidase were added. The solution was cooled (ice) and stirred during addition (1.5 h) of 10 x 0.6 mL 1 M hydrogen peroxide. The reaction mixture was left over night at 5°C and subsequently freeze-dried.

The freeze-dried reaction mixtures were Soxhlet extracted in toluene for 20 h. The extract was cleaned-up using liquid chromatography on silica/sulphuric acid, alumina oxide and carboxypack C. The analyses were performed by high resolution GC/MS at 1000 resolution, using a Kratos Concept 1 S instrument.

Control samples were prepared and treated as above, however, omitting a) the enzyme or b) the chloride. For comparison 600 mL of a humic acid solution (1g/L) were freeze-dried without prior treatment and analyzed as above.

### 3. Results and Discussion

Preliminary investigations revealed that the enzymatically mediated chlorination of humic acids lead to the formation of small amounts of PCDD/PCDF's as visualized in Figures 1 and 2 elucidating the formation of 1,2,3,4,6,7,8-HpCDD/1,2,3,4,6,7,8-HpCDF and OCDD/OCDF, respectively. It is noted that OCDF apparently is absent.

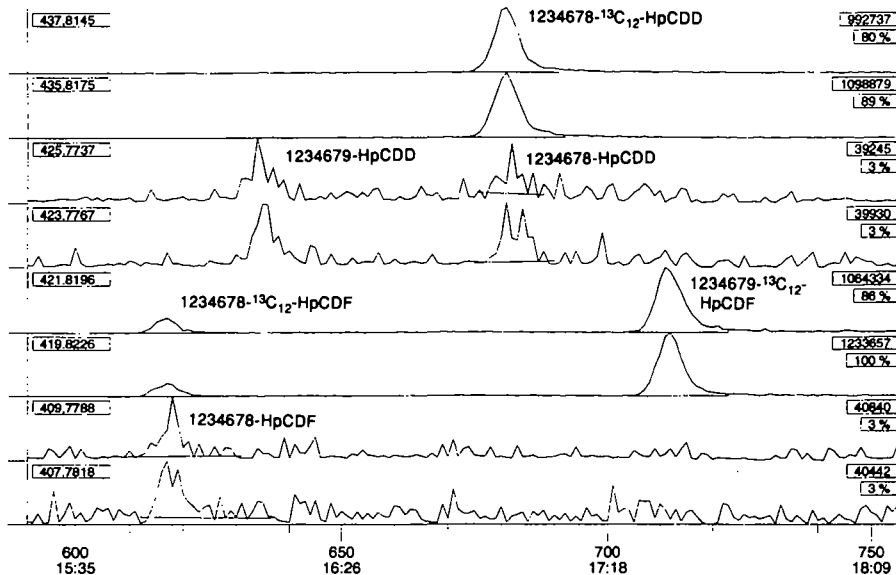


Figure 1. Chromatographic analysis (SIM mode) of HpCDD/HpCDF compared to <sup>13</sup>C and unlabelled HpCDD and HpCDF standards

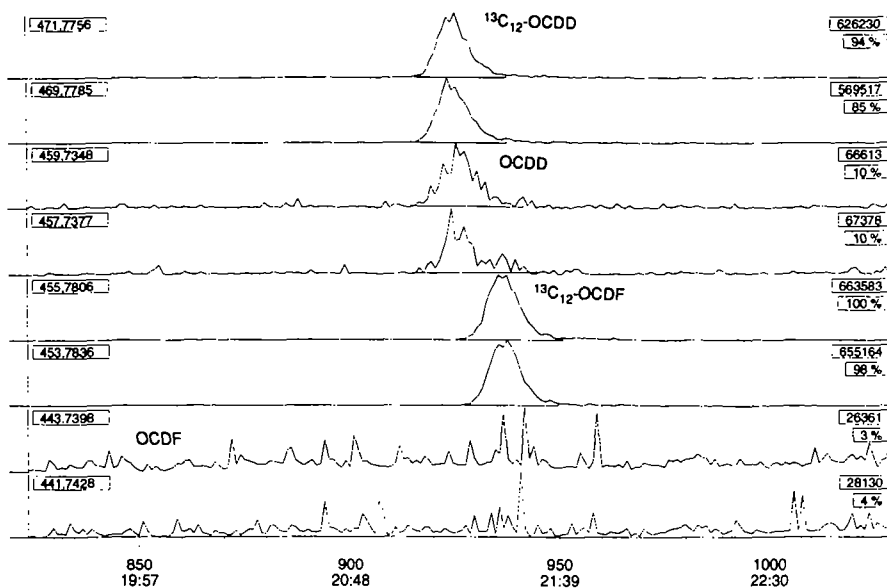


Figure 2. Chromatographic analysis (SIM mode) of OCDD/OCDF compared to  $^{13}\text{C}$  and unlabelled OCDD and OCDF standards.

It appeared that only a few PCDD/PCDF congeners apparently were found as a result of the enzymatically mediated reaction in contrast to e.g. the findings in sewage sludge<sup>6</sup>). Thus, apart from the above demonstrated formation of 1,2,3,4,6,7,8-HpCDD/1,2,3,4,6,7,8-HpCDF only 2,3,7,8-TCDF and OCDD was detected together with the un toxic congener 1,2,3,4,6,7,9-HpCDD, the latter being generated in amounts comparable to 1,2,3,4,6,7,8-HpCDD.

In Table 1 the calculated amounts of the four congeners are given together with the corresponding values found in sewage sludge. In addition value for municipal sewage sludge spiked with pentachlorophenol are given<sup>7</sup>). The predominance of the OCDD component in the sample spiked with pentachloro phenol is obvious. However, it should be noted that OCDD is the major congener in the survey of PCDD/PCDF levels in UK soils<sup>2</sup>).

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*Table 1. Concentrations (ng/kg dw) of PCDD/PCDF congeners found following enzymatically mediated chlorination of humic acids compared to the concentrations of the corresponding congeners in sewage sludge.*

	Humic Acids	Sewage Sludge <sup>6)</sup>	Sewage Sludge + pentachloro phenol <sup>7)</sup>
2,3,7,8-TCDF	17	42	
1,2,3,4,6,7,8-HpCDF	12	44	74
1,2,3,4,6,7,8-HpCDD	19	764	1039
OCDF	nd	131	84
OCDD	120	2649	5224

Obviously the formation of PCDD/PCDF's as a result of an enzymatically mediated chlorination of humic acids may seem somewhat surprising. However, PCDD/PCDF during composting has been reported previously<sup>8)</sup>, a reaction which obviously can be expected to involve naturally occurring organic material probably of a humic like structure. A priori it was to be expected that a broader range of PCDD/PCDF congeners would have been produced. However, the formation of the high chlorine containing congeners are in agreement with the previously observed high degree of chlorination of the low molecular weight organochlorine compounds found among the reaction products<sup>5)</sup>.

## 4. References

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