

## Natural-Abiotic Production of TCDD/Fs

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### Introduction

Identification and characterization of all sources of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in the environment is important. Most sources are of anthropogenic origin but some are not. The ubiquity of PCDD/Fs in the environment was argued to be indicative of their production by natural processes 25 years ago<sup>1</sup>. Natural sources of PCDD/Fs are biotic (of, relating to, or resulting from living organisms) and/or abiotic (physical rather biological; not derived from living organisms; devoid of life; sterile). Natural-biotic sources include certain terrestrial and aquatic plants<sup>2</sup>, as well as mammals, including cows<sup>3</sup> and even humans<sup>4</sup>. Natural-abiotic sources include volcanic activity (thermo chemical), forest fires<sup>1</sup> / burning of wood contaminated with chloride<sup>5</sup>, and biomass burning<sup>6</sup> (chemical combustion). Natural production of other environmental pollutants also has been described as abiotic<sup>7</sup>. Certain natural sources may be of mixed biotic/abiotic origin, including peat<sup>8,9</sup>, compost, and sewage sludge<sup>10</sup>, ball clay and kaolin from specific regions in the U.S., Germany, and Spain<sup>11,12</sup>, and topsoil, river sediments and kaolinite clay from Queensland, Australia<sup>13</sup>.

For some time it has been known that peroxidase enzyme (*i.e.* biotic) coupling of chlorophenols *in vitro* can produce PCDD/Fs and can be found in activated sewage sludges and during composting<sup>10</sup>. Enhanced PCDD/F levels are found in forest soils compared to grassland soils<sup>14</sup>. The ability of some fungi to produce chlorophenolic structures suggests that environmental biotransformation can occur to form, for example, PCDD/Fs, some evidence of which has been obtained in peat bogs<sup>8</sup>, more recently in forest litter<sup>15</sup>, and perhaps, in the human body after exposure to chlorophenols<sup>4</sup>. There also may be a natural-abiotic chlorophenol coupling component to some of these processes.

A tetrachlorinated homologue profile of 2,4,6,8-TCDF, 1,3,6,8-TCDD and 1,3,7,9-TCDD congeners was observed in peat from Eastern Canadian bogs at depths up to 6 metres<sup>8</sup>. A tetrachlorinated homologue profile dominated by 2,4,6,8-TCDF and 1,3,7,9-TCDD was determined in peat from a 3 to 4 metre depth in a UK peat bog<sup>9</sup>. TCDFs are also prominent in the congener profile of peat, peat ash and smoke from burning peat<sup>6</sup>. These homologue profiles do not correspond to those of any known sources and do not result from contamination from such sources.

This study investigated the abiotic oxidative coupling of 2,4-dichlorophenol to produce PCDD/Fs such as the TCDD/F profile found in peat.

### Materials and Methods

#### Reactions

Reagents, including 2,4-dichlorophenol (2,4-DCP), ferric chloride, sodium chloride, were reagent grade purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada), and were used as received. Authentic standards of 2,4,6,8-TCDF and 1,3,6,8-TCDD were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). 2,4-DCP (0.324 g), FeCl<sub>3</sub> (0.13 g), and NaCl (0.29 g), were combined in water (30 mL) and magnetically stirred for 24 hours.

#### Sample Preparation

The crude reaction mixture was quantitatively transferred to a separatory funnel, rinsing with methanol (60 mL). The sample was spiked with  $^{13}\text{C}_{12}$ -labelled surrogates. A 2.5 N solution of LiOH (30 mL) was added, followed by high purity water (270 mL). Partitioning was performed by addition of petroleum ether (180 mL) followed by vigorous shaking. The aqueous phase was drained. The organic phase was washed sequentially with 2.5 N LiOH and high purity water. The petroleum ether layer was quantitatively transferred to a boiling flask and solvent exchanged to hexane by rotary evaporation and concentrated to approximately 5 mL. The crude extract was subjected to cleanup on a multi-layer silica column and a basic alumina column. The eluant was evaporated to just dryness using a ZymarkTurboVap LV. The final extract was quantitatively transferred to a 0.1 mLQsert vial using dichloromethane, followed by toluene. Following evaporation to just dryness under a stream of nitrogen, the final extract was reconstituted in 20  $\mu\text{L}$  of  $^{13}\text{C}_{12}$ -labelled internal standard in toluene. An analytical batch included reaction mixture containing all reagents, reagent blanks (reaction mixtures with either the oxidizing reagent or the phenol substrate omitted), procedural blank, and glassware blank.

### Instrumental Analysis

A Leap Technology CTC A200S autosampler was used to make a 1  $\mu\text{L}$  injection into an HP 5890 series II gas chromatograph (GC). The GC was operated in splitless mode with a head pressure of 175 kPa He through a Restek Rtx-Dioxin2 column (60 m x 0.25 mm i.d. x 0.25  $\mu\text{m}$  film thickness). Selected congeners were identified by mass spectrometry (MS) using multi-group selected ion recording (SIR) at 10,000 resolving power (10% valley) on a VG AutoSpec magnetic sector field mass spectrometer operated in the electron impact (EI) mode at 40 eV.

### Results and Discussion

Data are presented to show that the same TCDD/F congeners observed in peat<sup>8,9</sup> are also formed when 2,4-dichlorophenol (2,4-DCP) is oxidatively dimerized in the presence of ferric chloride or autoclaved peat *in vitro*.

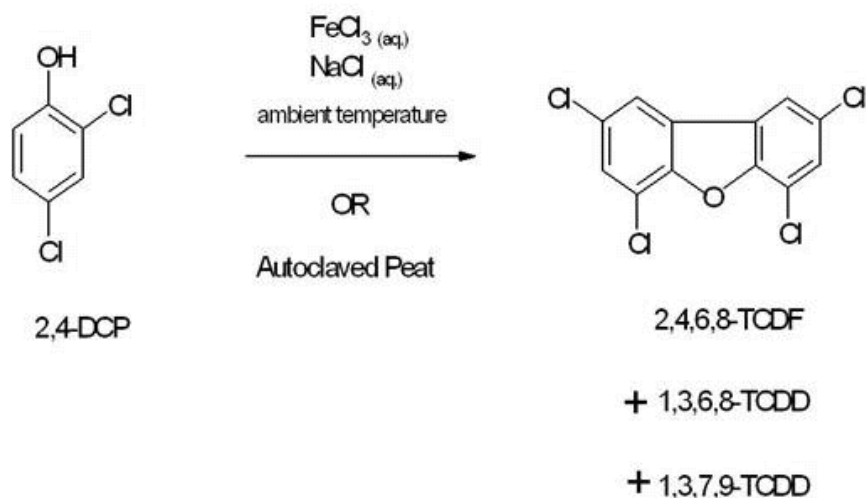


Figure 1. *In vitro* abiotic production of TCDD/Fs via Fe(III) oxidation or residual oxidizing capacity of autoclaved peat.

Although the same TCDD/F congener pattern described above was also formed when 2,4-DCP was oxidatively coupled in the presence of "live peat" *in vitro*, some residual oxidative capacity was retained by the autoclaved peat which is apparently unrelated to biotic processes.

Furthermore, the TCDD/Fs formed in the ferric chloride reaction are not artifacts of the purity of the 2,4-DCP used. Although the 2,4-DCP was shown to contain 2,4,6,8-TCDF, 1,3,6,8-TCDD and 1,3,7,9-TCDD, the concentration of these compounds was two to three times greater in products of the reaction of 2,4-DCP with ferric chloride (see Table 1).

Table 1. Concentrations of 2,4,6,8-TCDF and 1,3,6,8-TCDD measured in 2,4-DCP and in reaction of 2,4-DCP with ferric chloride

Experiment	2,4,6,8-TCDF Concentration (pg/g 2,4-DCP)	1,3,6,8-TCDD Concentration (pg/g 2,4-DCP)
2,4-DCP (control)	768 ± 112	48.3 ± 10.3
2,4-DCP + ferric chloride	2020 ± 437	101 ± 7.5

Note : no quantitative data are reported for 1,3,7,9-TCDD because no standard was available.

HRGC/HRMS analysis was performed by monitoring two exact *m/z* channels and isotopic ratios and retention time matches with authentic standards of 2,4,6,8-TCDF and 1,3,6,8-TCDD.

Decay and transformation of organic substances in soil lead to the formation of a multitude of organohalogenes by purely geochemical / abiotic processes. Soils consist mainly of inorganics and humic substances (HS). HS contain many functional groups including phenols and quinones and, therefore, are involved in many redox processes. One very important oxidant is iron. It is very abundant (4<sup>th</sup> in the earth's crust), is highly redox-sensitive, and thus plays a key role in redox systems in natural environments. It is therefore likely that Fe(III) is involved in natural-abiotic oxidative coupling of chlorophenols to PCDD/Fs. In addition to iron, other redox sensitive metals such as manganese and copper may also contribute to abiotic based dioxin production.

Origins of the chlorinated precursors involved in the coupling reactions also may be anthropogenic and/or natural<sup>2,15</sup>. Natural chlorination leading to formation of PCDD/Fs has been proposed<sup>16</sup>. Natural-abiotic chlorination and/or photochlorination of phenol has been shown to occur in the presence of Fe(III) and chloride, both of which are abundant in acidic aerosols of marine origin<sup>17</sup>. 2,4-DCP is known to be a naturally produced compound<sup>9</sup>.

A phenoxy radical mechanism to account for the formation of 2,4,6,8-TCDF, 1,3,6,8-TCDD and 1,3,7,9-TCDD from 2,4-DCP is proposed.

Based on the results of this study, it is reasonable to conclude that natural-abiotic processes may be responsible for the widespread occurrence of PCDD/Fs that are not of human origin. These natural sources warrant further investigation.

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