

USE OF CARBON ISOTOPE COMPOSITIONS OF OCTACHLORINATED DIBENZO-*P*-DIOXIN (OCDD) AS TRACERS OF NATURAL FORMATION OF DIOXINS IN BALL CLAY

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Abstract

Elevated concentrations of dioxins found in ancient ball clays from Mississippi Embayment in the USA, suggested natural formation of dioxins in the environment. The evidence for natural formation of dioxins in ball clay was based on unique congener profiles in undisturbed ball clay deposits and the lack of other anthropogenic contaminants in those deposits. In this study, we provide new evidence of natural formation of dioxins, which is based on congener-specific carbon isotopic analysis (CSIA- $\delta^{13}\text{C}$) of octachlorinated dibenzo-*p*-dioxin (OCDD) in ball clays from the United States and Japan (e.g. Gaerome and Kibushi). The analyses were performed using a combination of double column high performance liquid chromatography (HPLC) clean-up and two dimensional gas chromatography-isotope ratio mass spectrometry (2DGC-IRMS). Values of CSIA- $\delta^{13}\text{C}$ of OCDD and bulk- $\delta^{13}\text{C}$ were used to distinguish dioxins from natural sources (clay), anthropogenic sources (fly ash and pentachlorophenol; PCP), and environmental samples (sediment and soil). This is the first study to show high concentrations of OCDD in Japanese ball clays.

Introduction

Ball clay in the United States is in sedimentary beds laid down in Mississippi Embayment (e.g., Tennessee, Kentucky, and Mississippi States) during the early to middle Eocene Epoch (approximately 40-45 Ma)¹. An estimated 1.2 million metric tons of ball clay were mined in 2006 by the ceramic industry². Dioxins in the ball clay were characteristic of dominant OCDD (>130,000 pg/g dry weight-dw) and decreasing concentrations with a corresponding reduction in chlorination level³. Concentrations of polychlorinated dibenzofurans (PCDFs) are very low or nondetectable, and 1,2,3,7,8,9-hexachlorinated dibenzo-*p*-dioxin (HxCDD) predominates over the other 2,3,7,8-substituted HxCDD isomers, which is different from what is found in known anthropogenic sources of contamination. In a recent study, bulk radiocarbon and chlorine isotope analysis of OCDD were investigated in ball clay⁴ and revealed a poor correlation between black carbon content and polychlorinated dibenzo-*p*-dioxin (PCDD) concentrations. Stable chlorine isotope composition of OCDD yielded -0.2‰, which is within the range known for abiotic organochlorines (-6 to +3‰) rather than the postulated range for biotic chlorination by perchlorooxidase enzymes (-12 to -11‰). The chlorine isotope study indicated a natural abiotic and non-pyrogenic origin of dioxins in ball clay. High concentrations of dioxins were found not only in American ball clay, but also in kaolin from Germany and Spain^{3,5}, which are similar in PCDD isomer composition to those found in American ball clay. Based on this, the hypothesis of natural formation of dioxins in kaolin was suggested and *in situ* mineral-catalyzed synthesis was shown as a mechanism of formation. However, the chemical pathway and precursors of dioxin formation are not clearly understood. Development of alternative approaches is required to complement the existing methods for understanding the sources and/or formation mechanism of dioxins in kaolin. In recent years, stable carbon isotopic analysis, in particular congener-specific carbon isotope analysis (CSIA- $\delta^{13}\text{C}$), is becoming popular in understanding the sources and fate of POPs in the environment. We developed 2DGC-IRMS and demonstrated its performance in the CSIA of PCBs and PCNs⁶. In the present study, in order to provide a new evidence of natural formation of dioxins, CSIA- $\delta^{13}\text{C}$ of OCDD in kaolin (ball clays from the United States and Japan, and Spanish kaolin), anthropogenic dioxin source materials (fly ash and pentachlorophenol - PCP), and contaminated environmental samples (soil and sediment) was performed using a combination of double column HPLC clean-up and 2DGC-IRMS. Bulk- $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and concentrations of OCDD were also measured to provide additional evidences to support our conclusions. This is the first study on the occurrence of dioxins in Japanese ball clays (called 'Gaerome' and 'Kibushi'),

which resemble in mineral constituents and sedimentary pattern to American ball clays. The objectives of this study were to determine the carbon isotope signature of OCDD in samples from natural and anthropogenic sources and to distinguish the dioxin sources isotopically.

Materials and Methods

Samples. The types of samples analyzed in this study are as follows. OCDD/F standards (n=4 each) were purchased from four different companies. Ball clays (n=5) collected from mines located in Tennessee and Kentucky, USA and Spanish kaolin (n=1) were kindly provided by Dr. Joseph Ferrario. Two kinds of Japanese ball clays, Gaerome and Kibushi, which are used as materials of ceramic industry, and Japanese bentonite (as a control) were obtained from clay industries located in Japan. As samples contaminated heavily with dioxins (n=21), reference materials of river sediment (JSAC0432) and fly ash (JSAC0502) with certified concentrations of PCDD/Fs were purchased from The Japan Society for Analytical chemistry (JSAC). Two fly ash samples, three sediment samples, and a soil sample from 7th to 11th rounds of international intercalibration exercises for PCDD/Fs analysis and two other fly ash samples produced by fluidized bed and stoker type incinerators in Japan were also obtained. Surface sediment and sediment core samples from Tokyo Bay, Japan, were collected from 2000 to 2004. Five pentachlorophenol (PCP) standards from different producers and lot numbers were obtained.

Chemical analysis. The sample extraction and clean-up procedure of OCDD were based on the method described earlier^{7,8}, with some modifications. Briefly, dried sample (sediment, soil, and fly ash) was extracted using a mixture of acetone and *n*-hexane (1:1, v/v) and toluene in accelerated solvent extractor (ASE-200, DIONEX Co.). The extracts were first pre-cleaned by passing through a multi layer silica gel column. Target chemicals were eluted with 200 mL of *n*-hexane. The analytes were then cleaned up and fractionated using 10 g of activated basic alumina column chromatography. Further, the target chemicals were fractionated using double column HPLC, porous activated graphite carbon column (Hypercarb) and pyrenyl silica column (PYE). The aliquot from alumina column was manually injected into Hypercarb-HPLC which was eluted forward using 5% dichloromethane (DCM) in *n*-hexane (12 mL), 50% toluene in *n*-hexane (33 mL) and back flushed using toluene (37.5 mL) by heating the column at 50°C. Due to the need for separation of OCDD and OCDF, the toluene fraction was further sub-fractionated using PYE-HPLC column. The analytes from the PYE-HPLC column were eluted first with 10% DCM in *n*-hexane (27 mL) and then with DCM (48 mL). OCDD fraction (57-64 min) was collected separately, and was micro-concentrated to 100 μ L under a gentle stream of nitrogen, and 2 μ L aliquot was taken for measuring concentrations of OCDD using high-resolution gas chromatography and high-resolution mass spectrometry (HRGC-HRMS). The remaining extract was concentrated to 10-50 μ L depending on OCDD concentration, and was used for CSIA- $\delta^{13}\text{C}$ of OCDD. The details of analysis and validation of OCDD concentrations are given in previous reports⁸.

CSIA- $\delta^{13}\text{C}$ and bulk- $\delta^{13}\text{C}$ analysis. CSIA- $\delta^{13}\text{C}$ of OCDD was carried out using a 2DGC equipped with Finnigan MAT252 IRMS with a combustion furnace⁶. The 2DGC system was built from a Trace GC 2000 (Thermo Electron Co.) equipped with a moving capillary stream switching (MCSS) (CE Instruments). OCDD were separated from interferences by a less-polar capillary column, DB-5 (30 m x 0.25 mm i.d. x 0.25 μ m; J&W Scientific), cut by MCSS and separated again on another column (Rtx-200, 15 m x 0.32 mm i.d. x 0.25 μ m, Restek Corp.). The column oven temperature was programmed from 70 °C (1 min) to 200°C at a rate of 20°C/min, and to 280°C at 5 °C/min, which was held for 10 min, then finally to 300°C (5 min) at 20°C/min. Bulk isotope ratio analysis was carried out using an elemental analyzer (EA1110 CHNS) equipped with Finnigan ConFlo III and Delta plus IRMS. Isotope ratios of carbon and nitrogen were reported in parts per thousand, per mil (‰), and all values reported are relative to the international standard by conventional delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). The details of isotope ratio analysis and instrumental conditions were described in previous reports^{6,9}.

OCDD standard (AccuStandard, product no.: D-801N, lot no.: 970325R-AC) was analyzed at the beginning and at the end of sample measurements to check stability of the instrument. The standard deviation of repeated measurements of that was 0.30‰ (1s) throughout the analysis (average of $\delta^{13}\text{C}_{\text{OCDD}}$: -30.64‰, n=46).

Results and Discussion

Concentration of OCDD. High concentrations of OCDD were observed in American ball clays ranging from 237 to 659 ng/g dw. The average concentration of OCDD in American ball clays was above 400 ng/g dw.

The OCDD concentration in Spanish kaolin was 10 times lower than those in American ball clays. High concentrations of OCDD was also found in Japanese clays, Gaerome and Kibushi, at concentrations of 0.26 and 1.5 ng/g dw, respectively, while Japanese bentonite (control) did not contain detectable concentrations (<0.05 ng/g dw). The average concentration of OCDD in Japanese ball clay (0.76 ng/g dw) fell between that of US kaolin (0.19 ng/g dw) and German kaolin (3.2 ng/g dw)³. The high concentrations of OCDD in American and Japanese ball clay and Spanish kaolin found in this study are similar to that reported previously^{1,3}. The OCDD concentration in PCP and fly ash samples varied from 0.17 to 104 µg/g and from 33 to 381 ng/g dw, respectively. The concentration of OCDD in Kibushi was similar to that found in surface sediments from Tokyo Bay in which the concentration ranged from 1.2 to 2.4 ng/g dw. This concentration was 1.5-4 fold lower than the OCDD concentrations in sediment core from Tokyo Bay collected at a depth of 8-32 cm (2.3-5.6 ng/g dw). It is well known that Tokyo Bay is one of the highly contaminated areas with dioxin like compounds in Japan. This is the first study to report high concentrations of OCDD in Japanese ball clays. Both Japanese and American ball clays which have similar mineral constituents, are deposits of lacustrine sediments of Tertiary Era. Thus, significant amount of OCDD exists in the strata of pre-industrial age.

CSIA- $\delta^{13}\text{C}$ of OCDD. $\delta^{13}\text{C}$ values of OCDD from natural (clay), anthropogenic sources (fly ash and PCP), and environmental samples (soil and sediment) were determined, along with $\delta^{13}\text{C}$ values of OCDD/F standards (Table 1, Fig.1). The $\delta^{13}\text{C}$ values in the samples and OCDD/F standards (n=30) varied widely from -32.4 (PCP) to -22.8‰ (fly ash). The $\delta^{13}\text{C}_{\text{OCDD}}$ values in each matrix were $-25.8 \pm 1.1\%$ for clay, $-30.1 \pm 1.6\%$ for soil and sediment, $-23.7 \pm 0.8\%$ for fly ash, -31.9 ± 0.7 for PCP, and $-29.5 \pm 1.0\%$ for OCDD standard. The $\delta^{13}\text{C}$ values of OCDF standards were

$-25.1 \pm 0.8\%$, which had approximately 4‰ of ^{13}C depleted, in comparison with those of OCDD standards. In this study, the highest ^{13}C depletion was observed in PCP. On the other hand, enrichment of ^{13}C was found in fly ash. There exists approximately 10‰ difference in $\delta^{13}\text{C}$ between two major dioxin sources. The exhaust gas from waste incineration processes and several banned agrochemicals such as PCP and 2,4,6-trichlorophenyl-4'-nitrophenyl ether (CNP) have been the most prominent dioxin sources in the environment in Japan. It is noteworthy that $\delta^{13}\text{C}$ values of OCDD from both PCP and fly ash showed discriminant isotope signatures. Therefore, it is strongly believed that $\delta^{13}\text{C}_{\text{OCDD}}$ is a powerful diagnostic tool to identify the sources of dioxins in the environment. Further, the magnitude of contributions from different sources to contamination in the samples can be determined using mass balance calculations, if the isotope signatures of the sources retain their integrity during transport.

The $\delta^{13}\text{C}$ values of OCDD in all ball clays showed similar values, ranging from -27.1 to -23.5‰. The average $\delta^{13}\text{C}_{\text{OCDD}}$ in ball clay was 2‰ depleted from that found in fly ashes, and 6‰ enriched relative to that in PCP. This suggests that OCDD in ball clay originated from different carbon source, or from a different precursor, and/or a different chemical pathway relative to anthropogenic sources. The $\delta^{13}\text{C}$ values of OCDD in ball clays fell within the range reported for marine crude oil (-30 to -20‰)¹⁰, and similar to that reported for terrestrial plants, especially C_3 plants (Calvin Cycle) ranged around -26‰¹¹. Further, $\delta^{13}\text{C}$ values of OCDD in ball clays were in the ranges reported for other organohalogen compounds such as PCBs (-34.4‰ to -22.0‰)⁶, PCNs (-26.3‰ to -21.7‰) and organochlorine pesticides including Toxaphene (-31.8‰ to -22.4‰)¹², although the organohalogens had a wide range of $\delta^{13}\text{C}$ values. It is possible that naturally formed OCDD could be distinguished from anthropogenic OCDD by using carbon isotope composition, although further studies are needed to elucidate the $\delta^{13}\text{C}$ profiles of other anthropogenic sources of dioxins such as CNP, and steel and chloralkali industries.

Table 1. CSIA- $\delta^{13}\text{C}$ of OCDD (or OCDF) and bulk $\delta^{13}\text{C}$ values in clay, fly ash, soil, sediment, pentachlorophenol, and OCDD/F standards.

Sample	CSIA- $\delta^{13}\text{C}$, ‰		Bulk $\delta^{13}\text{C}$, ‰	
	Ave.	S.D.	Ave.	S.D.
Clay (n=6)	-25.8	1.1	-25.6	1.1
Fly ash (n=5)	-23.7	0.8	-24.8	4.2
Sediment and soil (n=9)	-30.1	1.6	-20.9	2.8
Pentachlorophenol (n=2)	-31.9	0.7	-31.3	0.5
OCDD standard (n=4)	-29.5	1.0	na	na
OCDF standard (n=4)	-25.1	0.8	na	na

Stable carbon isotopic composition is useful to identify and quantify transformation reaction and to examine the reaction mechanisms as well as source apportionment. The $\delta^{13}\text{C}$ value of OCDD reflects the origin of carbon in OCDD and formation mechanisms. The $\delta^{13}\text{C}$ values of bulk and OCDD in the samples were presented in Table. 1. The $\delta^{13}\text{C}$ values of bulk and OCDD were similar in clay, fly ash, and PCP samples, whereas these were different in sediment and soil samples. There was a significant correlation between material carbon ($\delta^{13}\text{C}_{\text{bulk}}$) and OCDD ($\delta^{13}\text{C}_{\text{OCDD}}$) in the samples that is considered to have carbon source of OCDD found in the sample matrices themselves, and that includes PCP, fly ash, and clay; OCDD could be produced *in situ*. Overall, the isotopic data provided in this study agreed well with the hypothesis of natural formation of dioxins in the clays. The carbon source of OCDD might be from organic carbon in the clay itself. Moreover, we found significant concentrations of OCDD in Japanese ball clays, in which mineral compositions resemble to ball clays in the United States, and non-detectable levels of OCDD in bentonite (control) with different mineral composition. This strongly suggests *in-situ* formation of dioxins in clay minerals. Natural formation of dioxins can be associated with the composition of clay mineral, but sedimentation conditions such as temperature, pressure, and pH in the clay may be important. Kaolin occurs widely as products of hydrothermal alteration and weathering and as constituents of sediment. As Rappe et al. (2001) pointed out that high level of dioxins is not limited to the Mississippi Embayment³, and dioxins are ubiquitous in kaolin from several countries. Further studies will focus on the occurrence of dioxins in kaolin from other countries and their relationship to clay mineral composition, their parent rocks and sedimentation processes.

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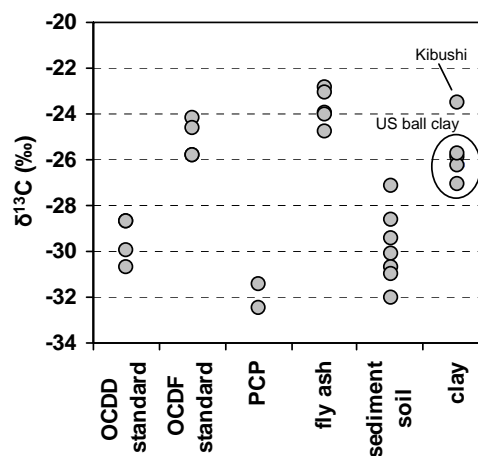


Fig. 1. Profiles of CSIA- $\delta^{13}\text{C}$ of OCDD in natural and anthropogenic sources, and OCDD/F standards.