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C15–C18 Isomers Synthesized from [^{13}C]-2,3,7,8-Tetrachlorodibenzofuran via Surface Chlorination

R. G. Peterson

California Analytical Laboratories, Inc., 2544 Industrial Blvd.,
West Sacramento, CA 95691

At present, the identification and quantitation of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) (see Figure 1) is performed with GC/MS/DS systems and is facilitated by using relative retention times and relative response factors based on [13C]-isotopically labelled 2,3,7,8-TCDD, 2,3,7,8- TCDF, and OCDD (Esposito et al. 1980; Teirnan et al. 1980; Lamparski and Nestrick 1982). These compounds are used as internal standards for the analysis for TCDD, TCDF, and OCDD respectively and as surrogates for the analysis for penta, hexa, hepta chlorinated dibenzo-p-dioxin and dibenzofuran isomers and octachlorodibenzofuran.

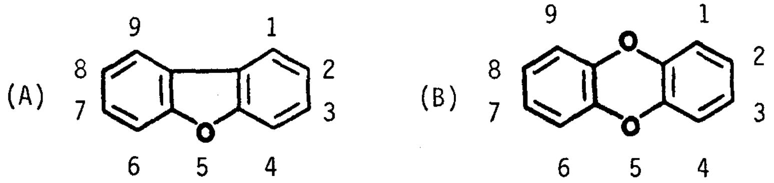

Figure 1. Structure and numbering of (A) dibenzofuran and (B) dibenzo-p-dioxin.

The use of isotopically labelled internal standards for all chlorination levels of dibenzo-p-dioxin and dibenzofuran would greatly improve the quality of currently generated analytical data from the analysis of various matrices for tetrachloro- through octachlorodibenzo-p-dioxins and dibenzofurans. However these compounds are difficult to obtain commercially and are quite expensive (about $1500 per ml of a 50 ppm solution) to be adding to every sample prior to extraction.

To circumvent these drawbacks in the scheme of analysis for PCDFs, the polychlorination of [13C]-2,3,7,8-TCDF was attempted and accomplished using a method based on previously published work that started with [13C]-2,3,7,8-TCDD (Lamparski and Nestrick 1982). After synthesis and quantitation of the labelled products, they can be added to a matrix before extraction and work up and used as true internal standards as opposed to surrogates.

MATERIALS AND METHODS

Due to the toxic and carcinogenic nature of the starting material and its isomers, only those persons with experience in hazardous substance handling should attempt the procedures described. Care must be taken in all steps so as not to contaminate personel or the laboratory. Proper waste diposal must be observed.

The methylene chloride and hexane used were J.T.Baker "Resi-Analysed" grade. The nitrogen and chlorine gas used were Amerigas UN 1066 and Linde UN 1017 (high purity), respectively. The basic alumina was Bio-Rad AG-10 (held at 130 C after being kilned at 600 C for 24 hrs) and the silica gel was Kieselgel 60 (EM Reagents #7734, held at 130 C). Tetradecane (Aldrich #17,245-6) was used a keeper. The [13C]-2,3,7,8-tetrachlorodibenzofuran was from Cambridge Isotope Laboratories,Inc., EF-904, Radian Corp. #R00203. Unlabelled 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, and OCDF were obtained from C. Rappe, University of Umea, Sweden. [13C]-2,3,7,8-tetrachlorodibenzo-p-dioxin, [13C]-octachlorodibenzo-p-dioxin, and unlabelled 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD were obtained from KOR Isotopes.

A Varian Model 3700 gas chromatograph equipped with an electron capture detector (GC-ECD) and a Hewlett-Packard Model 5970B GC/MS system (both with 30m DB-5 capillary columns) were used for various screening, identification, and quantitation tasks.

The surface chlorination was conducted in a Kontes #K-420000 chromatographic column fitted with a stainless steel tee to allow addition of chlorine and/or nitrogen (see Figure 2). A glasswool plug was placed in the column followed by 1.5g of sodium sulfate and 3g of silica gel. A solution of [13C]-2,3,7,8-TCDF was added to the column and washed on with a portion of methylene chloride to disperse the material. Another glasswool plug was then placed about 5cm above the packing. The nitrogen was turned on briefly to evaporate the solvent. Chlorine gas was then bubbled through the column. Reaction times and temperatures were varied to produce differing isomer mixes (heating tape was used to adjust column temperature above ambient). At the end of the reaction period, the chlorine gas was turned off and nitrogen was used to purge the column of residual chlorine. The column was removed from the tee and eluted with 100ml of methylene chloride. To the eluate was added 100ul of tetradecane and the mixture rotory evaporated down to the tetradecane. After transferring and adjusting to a final volume with hexane, the product was ready for use.

RESULTS AND DISCUSSION

The high cost, low availability, and expected usefulness of [13C]-labelled polychlorinated dibenzofurans as internal standards for the determination of native polychlorinated dibenzofurans by GC/MS prompted this work. Since it is the 2,3,7,8-backbone pattern of chlorination that exhibits the greatest adverse effects (eg. toxicity, carcinogenicity, etc.), [13C]-2,3,7,8-TCDF was choosen as the starting material in order to produce [13C]-labelled internal standards corresponding to the isomers of most concern for each level of chlorination.

The solution of [13C]-2,3,7,8-TCDF obtained from Cambridge Isotope Laboratories, Inc. was actually a mix of [13C]-2,3,7,8-TCDF (46ug/ml) and [13C]-1,2,7,8-TCDF (3ug/ml). These isomers were separated by using a 5g basic alumina column eluted with 3% (v/v) methylene chloride/hexane. Portions of the eluate were collected, evaporated to dryness with a stream of nitrogen and the residues redissolved in hexane and screened by GC-ECD. Those fractions containing only [13C]-2,3,7,8-TCDF (identified by retention time using an authentic 2,3,7,8-TCDF) were combined and used for the surface chlorination work.

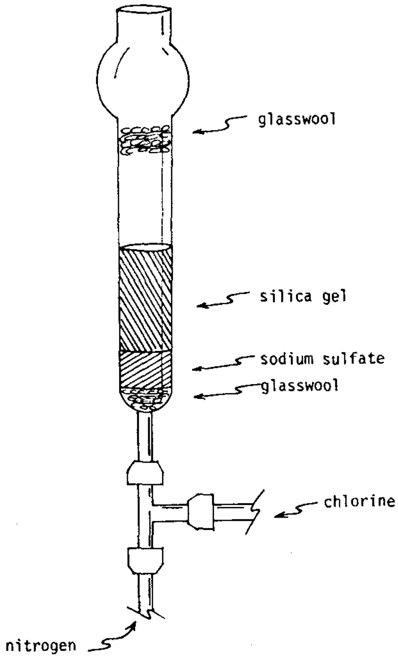

Figure 2. Surface chlorination apparatus.

Attempts to chlorinate 2,3,7,8-TCDF (unlabelled) using procedures described by Hutzinger et al. (1973) were unsuccessful in that they lead to mixtures that required cleanup by column chromatography before GC-ECD screening could be done for the expected products.

The procedure of Lamparski and Nestrick (1982) was modified by using a vertical reaction bed (Figure 2). Since the reaction bed was not restricted, the chlorine and nitrogen could bubble through, thereby exposing the deposited starting material continuously. Combinations of reaction times (30 min to 2 hrs) and temperatures (ambient to 150 C) produced as the major products : [13C]-labelled-1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, and OCDF (Figure 3).

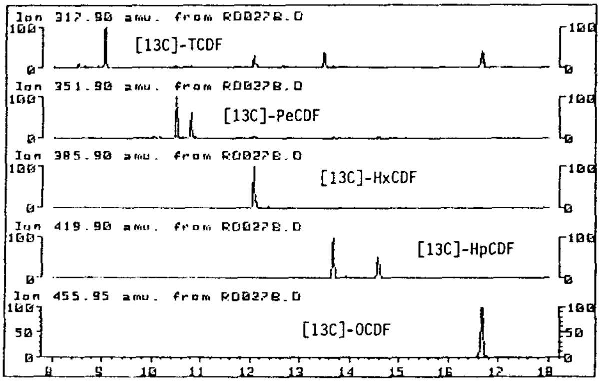

Figure 3. Ion chromatograms for tetra through octa chlorination levels of [13C]-dibenzofuran.

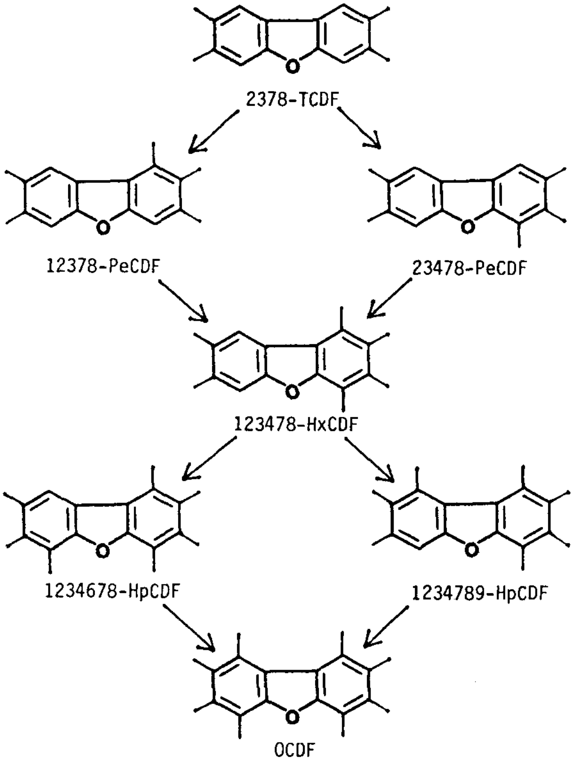

Figure 4. Chlorination sequence from tetra- through octa-chlorodibenzofuran.

The pathways through which the chlorination proceeds are presented in Figure 4. The predominant PeCDF of the pair formed is the 1,2,3,7,8-isomer; thus it appears that the 1-position is favored over the 4-position with regards to surface chlorination. Since the only HxCDF formed is the 1,2,3,4,7,8-isomer, it seems that the remaining opening (the 4-position of 1,2,3,7,8-PeCDF or the 1-position of 2,3,4,7,8-PeCDF) of the first ring is more easily chlorinated than either the 6- or 9- position of the second ring. There are two HpCDFs formed, with the 1,2,3,4,6,7,8-isomer being predominant. By analogy from the TCDF to PeCDF path, the 9-position should be the more active site as compared to the 6-position. But since the 6-position is chlorinated over the 9-position, possibly the 1-position chlorine sterically hinders chlorination at the 9-position. Finally, the remaining position is chlorinated to produce the octachloro-isomer.

As the reaction time and temperature are increased, the distribution of isomers shifts from the lesser to more-chlorinated species (Table I).

Table 1. Comparison of isomer mixture produced versus reaction time and temperature. Values listed are amounts of each isomer produced relative to the amount of 123478-HxCDF produced at a given combination of reaction time and temperature.

TIME (min.)	TEMP (C)	12378 PeCDF	23478 PeCDF	123478 HxCDF	1234678 HpCDF	1234789 HpCDF	OCDF
30	RT	1.06	0.76	1.00	0.12	-	-
30	100	0.04	-	1.00	0.40	0.34	0.30
120	100	0.03	-	1.00	0.51	0.32	0.32
120	150	-	-	1.00	1.67	0.33	8.00

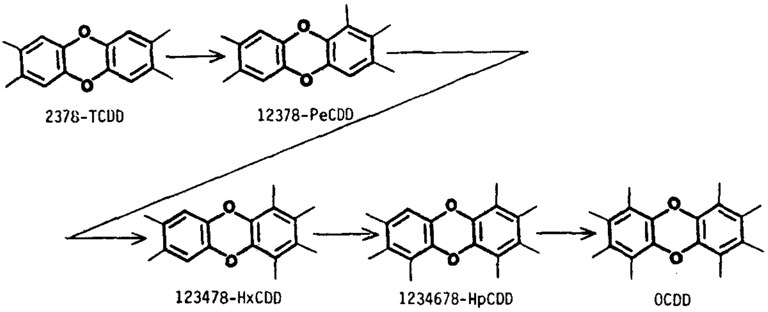

Figure 5. Chlorination sequence from tetra- through octa-chloro-dibenzo-p-dioxin.

[13C]-2,3,7,8-TCDD was also chlorinated by this procedure. The products and pathway are shown in Figure 5. The scheme of chlorination of the [13C]-2,3,7,8-TCDD follows that presented for the [13C]-TCDF; i.e., once a ring has three chlorines on it, another chlorine is added to that ring before any chlorines are added to the other. Because of the symmetry of the dibenzo-p-dioxin system, chlorination from the tetra- to the penta-isomers and from the hexa- to the hepta-isomers leads to only one penta-isomer and only one hepta- isomer, respectively. We did not find the 1,2,3,6,7,8-HxCDD isomer reported by Lamparski and Nestrick (1982).

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