Micro Chlorination Procedure for Synthesis of Higher Chlorinated Dibenzo-*p*-dioxins from [^{13}C]-2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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An efficient microscale chlorination procedure termed "surface chlorination" is described. By use of this technique with a silica supporting matrix, 7.4 μg of [^{13}C]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin is converted to [^{13}C]-octachlorodibenzo-*p*-dioxin in ~60 min at 100 °C. Following liquid chromatographic product purification and identification and confirmation of component identity using instrumental techniques, an 80% yield is achieved. The unique advantages and other possible applications of this reaction matrix are also discussed.

The determination of trace organic species in complex sample matrices is often approached by instrumental applications of gas chromatography/mass spectrometry (GC-MS). For many compound classes, GC-MS determinations are routinely accomplished at low part-per-trillion (pptr = pg/g) levels. Because of very low analyte concentrations, the infinite variety of sample matrices, and the relatively high cost of these analyses, the more classical forms of analytical quality assurance (i.e., method validation for each matrix, replicate analyses, cross-checks by method of standard addition, and determination by an alternate technique) are not always feasible. By virtue of component mass specificity, GC-MS techniques can often use an isotopically labeled analyte introduced into each sample as an internal standard to reduce systematic analytical errors and improve both the accuracy and reliability of the determination (*1*). The GC-MS determination of trace chlorinated dibenzo-*p*-dioxins (CDDs) is one area where the use of isotopically labeled internal standards is rapidly growing (*2–5*).

It is only very recently that nonradioactive, isotopically labeled (i.e., ^{37}Cl or ^{13}C) CDD isomers of suitable quality for GC-MS analytical applications have become commercially available. Although ^{13}C- and ^{37}Cl-labeled 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD), [^{13}C]-1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (1234678-H_7CDD), and [^{37}Cl]-octachlorodibenzo-*p*-dioxin (OCDD) can be purchased from KOR Isotopes (56 Rogers St., Cambridge, MA), their cost, which ranges between $1000 and $3000 per 50 μg, is prohibitive for many laboratories. In addition, isotopically labeled pentachlorodibenzo-*p*-dioxins (PCDDs) and hexachlorodibenzo-*p*-dioxins (HCDDs) are not yet commercially available. To a large extent, limited availability of these labeled CDDs is primarily associated with their high biological activity which necessitates extreme laboratory precautions for macrosyntheses. Also, the impending need for isomerically pure, labeled CDDs severely limits macrosynthetic approaches for their preparation (*6, 7*).

In view of the fact that a typical GC-MS CDD determination requires <5 ng of isotopically labeled CDD internal standard per sample, approximately 5–10 μg of the labeled material should be sufficient for hundreds of analyses. Therefore, we have developed a simple, safe, and efficient microchlorination procedure applicable to ^{13}C-2378-TCDD (the least expensive labeled CDD) which can be used to produce ^{13}C-12378-PCDD, ^{13}C-123678-HCDD, ^{13}C-123789-HCDD, ^{13}C-123478-HCDD, ^{13}C-1234678-H_7CDD, and ^{13}C-OCDD. This synthetic approach is unique with respect to other microscale chlorination procedures (*8–11*) because it employs a gas–solid reaction system which we have termed "surface chlorination". Beginning with ~1–10 μg of ^{13}C-2378-TCDD, product recoveries of ^{13}C-enriched higher CDD congeners exceed 80%. We have optimized the reaction conditions to produce ^{13}C-OCDD. However, modification of the reaction conditions to reduce temperature or exposure time should produce equally high yields of species having intermediate degrees of chlorination.

EXPERIMENTAL SECTION

Caution. Persons attempting to prepare these substances should be experienced in the handling procedures for extremely toxic materials. Appropriate precautions should be taken so as to minimize the chances of either personal or environmental exposure to CDDs. All waste materials, to include equipment washing solvents, should be carefully packaged and isolated until destroyed by appropriate techniques.

Reagents. The silica reactant bed (*6*), 10% silver nitrate on silica, basic alumina (*12*), and purified nitrogen (Femtogas) (*13*) have been previously described.

Chemicals and Solvents. All solvents used were Burdick and Jackson, distilled-in-glass quality. Silver nitrate and anhydrous iron(III) chloride were ACS reagent grade. Chlorine was Matheson High Purity Grade. These materials were tested by subjecting them to the analytical procedure to verify the absence of contamination.

Dioxin Standards. The primary standards of 2378-TCDD, 1234678-H_7CDD, OCDD, and ^{13}C-2378-TCDD (86 atom % ^{13}C) have been described (*3*).

Apparatus. The surface chlorination reactor consisting of a 13 mm i.d. × 700 mm Pyrex glass tube equipped with 24/40 ground glass joints, a temperature-controlled tube furnace, and chlorine and nitrogen gas inlets is shown in Figure 1.

Packed Column Gas Chromatography/Electron Capture Detection (GC-EC). Chlorination reaction products were monitored by using a Varian Model 3700 gas chromatograph equipped with a ^{63}Ni pulsed electron capture detector (ECD). A 1-μL on-column injection of each sample, appropriately diluted in isooctane, was made under the following instrumental conditions: column, 2 mm i.d. × 210 cm silylated glass; packing, 0.6% Poly S-179 on 80/100-mesh Permabond Methyl Silicone-10 cycle (HNU Systems, Inc., Newton, MA); column temperature, 230–300 °C at 6 °C/min and held at maximum; injection port temperature, 250 °C; detector temperature, 320 °C; carrier gas, nitrogen at 20 cm^3/min; attenuation, 1280×–2560×.

Capillary Column Gas Chromatography/Electron Capture Detection (HRGC-EC). Verification of product purity was accomplished on a Varian Model 3700 gas chromatograph equipped with a ^{63}Ni pulsed ECD and two different modified surface coated open tubular Pyrex glass capillary columns. The preparation of these columns has been described previously (*7*). A 2-μL injection of each sample, diluted in isooctane, was made under the following instrumental conditions: column, 0.5 mm i.d. × 30 m Pyrex modified SCOT column coated with a 60/40 (w/w) mixture of OV-17 and Poly S-179 or a 0.5 mm i.d. × 25 m Pyrex modified SCOT column coated with Poly S-179; column

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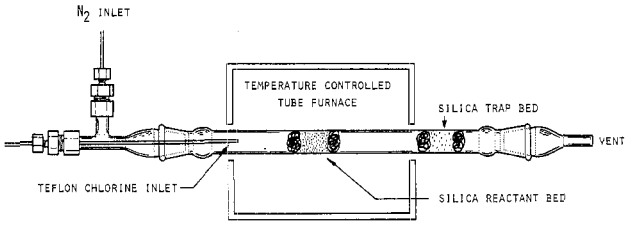

Figure 1. Surface chlorination apparatus for preparation of ^{13}C-OCDD. Entire setup located in a fume hood.

temperature, 280 °C; injection port, splitless direct-injection coupling constructed from Pyrex glass tubing and packed with 0.60% OV-17 and 0.40% Poly S-179 on 80/100-mesh Permabond Methyl Silicone-10 cycle; injection port temperature, 250 °C; detector temperature, 320 °C; carrier gas, nitrogen at 42 cm/s linear velocity; attenuation, 256×.

Packed Column Gas Chromatography/Low-Resolution Mass Spectrometry (GC-LRMS). The isotopic purity of the final surface chlorination product was determined by GC-LRMS using a Hewlett-Packard Model 5992-A operating in the selected ion monitoring (SIM) mode at unit resolution under the following conditions: column, 2 mm i.d. × 210 cm silylated glass; packing, 0.60% OV-17 + 0.40% Poly S-179 on 80/100-mesh Permabond Methyl Silicone-10 cycle; column temperature, 300 °C; injection port temperature, 280 °C; carrier gas, helium at 14 cm^3/min; separator, single-stage glass jet operating at column temperature; electron energy, 70 eV; specific ions were monitored from m/z 460 to 479. Multiple SIM analyses were necessary to reconstruct the molecular-ion isotope cluster. In each case m/z 470 was monitored, and the ion abundances were normalized to the m/z 470 response.

Solvent-Based Chlorination. For the initial solvent-based chlorination, 10 μg of ^{13}C-2378-TCDD was quantitatively transferred into a Pyrex 10-mL volumetric flask, the isooctane solvent was evaporated under a stream of Femtogas nitrogen, and then 10 mL of chlorine-saturated carbon tetrachloride was added. The reaction mixture was exposed to normal laboratory light, and sampled at 15 min intervals by removing a 10-μL aliquot which was evaporated to dryness and redissolved in isooctane for analysis by GC-EC. After 60 min of reaction time, several small crystals of anhydrous $FeCl_3$ (~1 mg total) were added to the reaction mixture. After the mixture was manually shaken, most of the $FeCl_3$ remained undissolved. After an additional 30 min of reaction time, a 10-μL aliquot was removed for GC-EC analysis.

Surface Chlorination (See Figure 1). In the central portion of the Pyrex reactor tube, a 3-g bed of silica was supported by a small plug of Pyrex glass wool. A solution of 10 μg of native 2378-TCDD in 2 mL of methylene chloride was carefully administered to the top of this bed and allowed to soak into the silica support matrix. Via the appropriate inlet adaptor shown in Figure 1, purified nitrogen carrier at 200 cm^3/min was passed through the reactor tube which was positioned in a temperature-controlled tube furnace adjusted to ~60 °C. After several minutes, essentially all of the methylene chloride solvent had been distilled from the system, leaving behind a relatively even distribution of 10 μg of 2378-TCDD on a silica surface having an approximate area of 1000 m^2. The reactor tube was removed from the furnace and cooled to ambient temperature, nitrogen flow was discontinued, and a second ~3-g silica bed was positioned at the exit port (see Figure 1) to act as a secondary trap to prevent possible loss of chlorinated dioxins from the system due to volatilization in the carrier gas. At this point, appropriate fittings were reinstalled, nitrogen carrier was reestablished at ~20 cm^3/min, and the reactor tube was returned to the furnace which was adjusted to 100 °C. Chlorine gas was then admitted into the nitrogen carrier at an approximate rate of 10–20 cm^3/min for a period of 30 min. Following exposure, excess chlorine was vented from the system by the nitrogen carrier. The reactor tube was then cooled to ambient temperature and inverted (inlet end up), and chlorinated dioxins were eluted from the system with 50 mL of methylene chloride.

The experiment was repeated with 7.4 μg of ^{13}C-2378-TCDD as the starting material. The chlorine exposure time was extended from 30 to 60 min in order to effect total perchlorination, and the volume of methylene chloride solvent used for product elution was increased from 50 to 75 mL to ensure maximum recovery. Following reaction, the crude product was eluted and an aliquot removed for analysis by GC-EC. Final purification of the ^{13}C-OCDD was accomplished by evaporation of the methylene chloride under a stream of Femtogas nitrogen. The resulting residue was purified by liquid chromatography on a Macro 1.5 g 10% $AgNO_3$/silica column and a High Aspect 5.0 g basic alumina column which have been previously described (*3*).

RESULTS AND DISCUSSION

The initial goal of this work was to determine a simple and efficient means for converting sub-10-μg quantities of commercially available, stable-isotope labeled 2378-TCDD into labeled OCDD suitable for use as an internal standard in GC-MS analyses. ^{13}C-2378-TCDD was selected as the precursor because it permits the use of inexpensive native chlorine for the preparation of labeled higher chlorinated congeners as opposed to the necessity of generating $^{37}Cl_2$ from a variety of rather expensive reagents.

A literature survey indicated that general knowledge concerning the chlorination of aromatic species is extensive; however, few references could be found dealing with quantitative perchlorination of CDDs on a microgram scale. Hutzinger and co-workers described exhaustive chlorination as a technique for the determination of aromatic hydrocarbons (*8*). Although their described perchlorination reagents, including chlorine, sulfuryl chloride, aluminum chloride, trichlorosulfur–tetrachloroaluminate, and antimony pentachloride, were claimed to be successful when applied to CDDs, no data were given. Also, the examples typically employed milligram quantities of starting material. Williams and Blanchfield reported the development of a perchlorination technique for screening corn oil for CDDs (*9*). In this work, quantitites of 2378-TCDD ranging from ~1 to ~100 μg were converted to OCDD by refluxing in CCl_4 containing gram amounts of iron filings and trace iodine with continuous Cl_2 saturation. After reaction times of 4–6 h, OCDD recoveries relative to the 2378-TCDD starting material were shown to be the following: 100 μg of 2378-TCDD gave 60–70% OCDD, 10 μg of 2378-TCDD gave 15–20% OCDD, and 1 μg of 2378-TCDD gave 3–10% OCDD. A modification of this procedure to include the addition of 50 μg of naphthalene was later shown to give 20–30% OCDD with 1 μg of 2378-TCDD starting material (*10*). Although encouraging with regard to OCDD recovery at very low microgram quantities, the necessity of including naphthalene limits its usefulness for the preparation of a labeled OCDD reference material because of the additional product purifications required to remove octachloronaphthalene.

In view of the fact that much of the cited literature dealt with perchlorination reactions, we initially opted to examine the reaction characteristics of 2378-TCDD when subjected to typical solvent-based chlorination conditions. Since low microgram quantities of 2378-TCDD under such conditions were reported to yield only small amounts of OCDD, it seemed reasonable to expect this approach might be useful for producing the intermediate higher chlorinated congeners. When 10 μg of 2378-TCDD was dissolved in chlorine-saturated CCl_4 (10 mL) and allowed to stand at ambient temperature exposed to laboratory light, as described in the Experimental Section, the results were surprising.

GC-EC analysis of the crude reaction mixture at 15 min intervals up to 60 min revealed that no higher chlorinated products were produced; analysis conditions were such that a 1–2% conversion to 12378-PCDD could easily be observed. Addition of ~1 mg of anhydrous ferric chloride as a catalyst also produced a surprising result when the reaction mixture was examined 30 min later. GC-EC results revealed that the 2378-TCDD concentration had decreased to ~40% of its

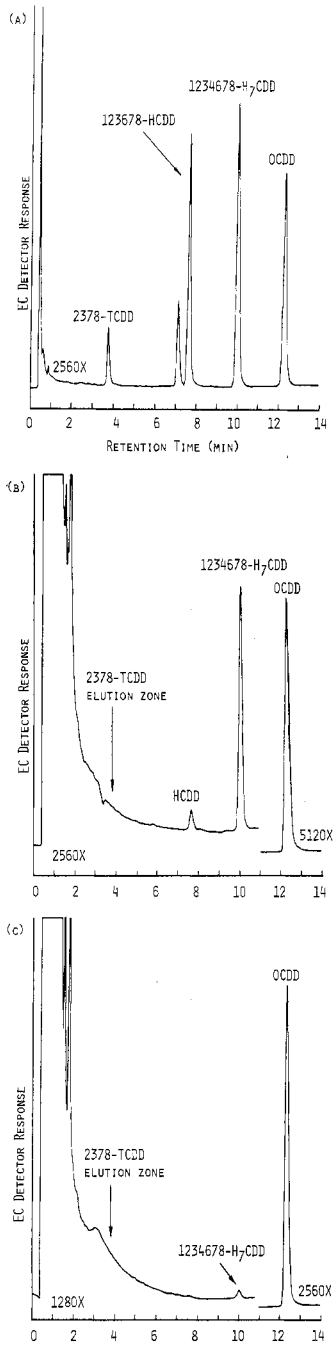

Figure 2. GC-EC chromatograms of (A) mixed CDD calibration standard, (B) crude product from surface chlorination of native 2378-TCDD, and (C) crude product from surface chlorination of ^{13}C-2378-TCDD.

initial level, and yet no significant amounts of higher CDDs were observed. At this point, it was not apparent whether the 2378-TCDD was being destroyed under these rather mild conditions in the presence of $FeCl_3$ or whether it and/or its chlorination products were simply adsorbed on the surface of the remaining undissolved catalyst. Since production of higher CDDs appeared extremely inefficient, the experiment was discontinued. It is interesting to note that 2378-TCDD and several other higher chlorinated congeners were later shown to maintain constant concentration when they were dissolved in CCl_4 that did not contain chlorine, and to which $FeCl_3$ crystals were added as described.

Surface Chlorination. In response to the need for an efficient chlorination technique for CDDs that could be routinely used for at least very low microgram quantities, we have developed a procedure termed "surface chlorination". The

Table I. GC-EC Analysis Data for Crude ^{13}C-OCDD Product

starting material	7400 ng of ^{13}C-2378-TCDD	22.3 nmol
mol % conversions	8900 ng of ^{13}C-OCDD	19.0 nmol, 85%
	87 ng of ^{13}C-1234678-H_7CDD	0.2 nmol, 0.8%
wt % composition (based on H_7CDD + OCDD total)	^{13}C-OCDD	99%
	^{13}C-1234678-H_7CDD	1%

Table II. GC-EC Analysis Data for Purified ^{13}C-OCDD Final Product

starting material	7400 ng of ^{13}C-2378-TCDD	22.3 nmol
mol % conversion	8400 ng of ^{13}C-OCDD	18.0 nmol, 80%
	53 ng of ^{13}C-1234678-H_7CDD	0.1 nmol, 0.5%
wt % composition	^{13}C-OCDD	99.4%
	^{13}C-1234678-H_7CDD	0.6%

fundamentals of this procedure involve adsorption of the starting material on a high surface area support that is packed into a Pyrex glass tube which serves as a reactor vessel. The closed system is then exposed to chlorine in a flowing stream of nitrogen at elevated temperature. Upon completion of the reaction, excess chlorine is purged via the nitrogen carrier. When cooled to ambient temperature, the products are removed by elution of the reactor bed with an appropriate solvent in a fashion similar to that of a classical liquid chromatographic column. A diagram of this equipment is shown in Figure 1.

The efficacy of surface chlorination for 2378-TCDD was initially tested via the deposition of 10 μg of native material on a 3-g bed of $\sim$350 m^2/g silica as described in the Experimental Section. After a 30-min chlorination period at 100 °C and elution of the reactor bed with CH_2Cl_2, a sample of the crude reaction products was examined by GC-EC, shown in Figure 2. Under these surface chlorination conditions, 10 μg of 2378-TCDD yielded $\sim$80% conversion to OCDD, $\sim$18% conversion to 1234678-H_7CDD, and $\sim$2% conversion to 123678-HCDD (7). No detectable levels of 2378-TCDD or 12378-PCDD were observed.

On the basis of these results, the experiment was modified as described in the Experimental Section using 7.4 μg of ^{13}C-2378-TCDD as the starting material. Following reaction, the crude product was examined by GC-EC. As illustrated in Figure 2C, perchlorination of the ^{13}C-2378-TCDD was essentially complete. Only a minor amount of 1234678-H_7CDD could be detected other than the major component OCDD. Quantitative analysis of the crude product by GC-EC provided the data shown in Table I.

Final purification of the ^{13}C-OCDD product was accomplished by evaporation of the CH_2Cl_2 solvent under a stream of Femtogas nitrogen. The resulting oily-white residue was purified by a liquid chromatographic separation on a dual column system consisting of a Macro 1.5 g 10% $AgNO_3$/silica column followed by a High Aspect 5.0 g basic alumina column (3). Quantitative analysis of the ^{13}C-OCDD product by GC-EC, using an appropriate native H_7CDD and OCDD calibration standard, provided the chromatograms shown in Figure 3. As indicated, the purification procedure removed essentially all of the early eluting species present in the crude product. Composition data from this GC-EC analysis are given in Table II.

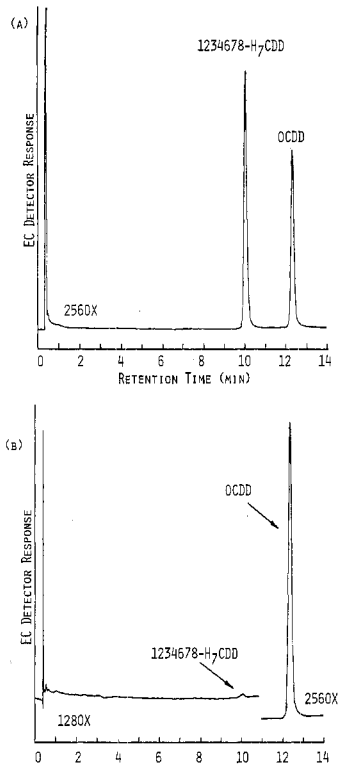

Figure 3. GC-EC chromatograms of calibration standard and purified ^{13}C-OCDD product.

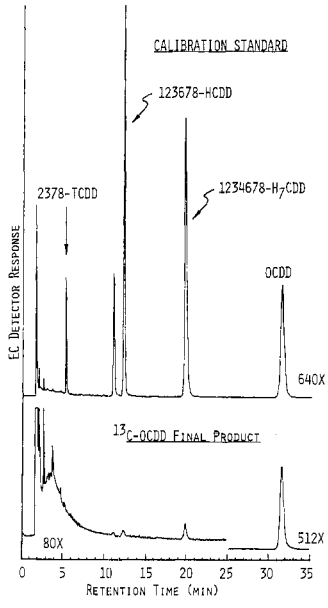

Figure 4. HRGC-EC chromatogram of mixed CDD calibration standard and ^{13}C-OCDD final product on 30 m × 0.5 mm (I.d.) modified Scot column coated with 60% OV-17 + 40% Poly S-179. Conditions are as described in text.

^{13}C-OCDD Product Verification. The identity of the ^{13}C-OCDD product was verified by comparison of retention times via HRGC-EC using two different glass capillary columns as described in the Experimental Section. Analytical reference standards of native HCDDs, 1234678-H_7CDD, and OCDD were used for comparison purposes. The identities of the ^{13}C-OCDD as OCDD, and the ^{13}C-1234678-H_7CDD minor impurity as 1234678-H_7CDD, were confirmed on the basis of correct retention times on both capillary columns. Example chromatograms for one of these comparisons are shown in Figure 4. As indicated, operation at very high sensitivity shows the possible presence of at least two ^{13}C-HCDD isomers in the final product.

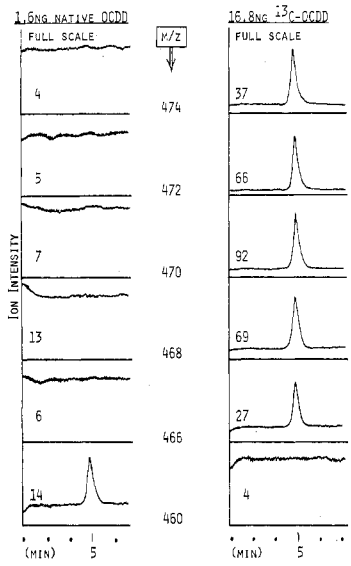

Figure 5. GC-MS SIM chromatogram of (left) native OCDD calibration standards and (right) ^{13}C-OCDD produced by surface chlorination.

The next stage of product verification involved GC-LRMS to determine the isotopic identity as ^{13}C-OCDD by monitoring the most intense parent ion, m/z 470, and other characteristic molecular ions 466, 468, 472, and 474 and to simultaneously determine the apparent response at the most intense parent ion for native OCDD at m/z 460. (Note that the ^{13}C-2378-TCDD used in this work had a ^{13}C-isotopic enrichment of 86 atom %.) These GC-LRMS-SIM chromatograms, shown in Figure 5, indicate that ion responses at the correct GC retention time for OCDD were observed at all denoted characteristic parent ion masses for ^{13}C-OCDD. No response was observed at m/z 460, when the limit of detection is defined as 2.5 times peak to valley noise in a region nearby the expected elution zone. Hence, no more than 0.77% by weight of native OCDD can be present in the ^{13}C-OCDD final product.

The final phase of product verification involved mass spectrometric determination of the ^{13}C isotopic composition. The technique for determining the degree of ^{13}C enrichment involved comparison of the normalized fractional abundances for the observed product parent ions to the theoretical values calculated for a compound containing 8 chlorine atoms and 12 carbom atoms at varying levels of ^{13}C enrichment. In order to minimize the total amount of sample required to obtain these measurements, we conducted repetitive analyses by GC-LRMS-SIM. Monitoring the most intense parent ion m/z 470 during each analysis sequence and then normalizing all responses to its intensity permitted the total spectrum to be compiled independent of absolute species concentration. On the basis of experimental data obtained (see Table III), the ^{13}C-OCDD demonstrated a ^{13}C enrichment of 86–87 atom %.

CONCLUSIONS

In less than 8 h of laboratory time, 7.4 μg of ^{13}C-2378-TCDD provided 8.4 μg of ^{13}C-OCDD (~80% yield) after product cleanup and analytical confirmations. Surface chlorination also was shown to be useful for preparing mixtures of ^{13}C-enriched higher CDD congeners from ^{13}C-2378-TCDD which may include 12378-PCDD, three HCDD isomers, and 1234678-H_7CDD. The resulting CDD congeners can easily be purified and separated in quantities as large as ~100 μg/run using our previously described reversed-phase high-performance liquid chromatography (RP-HPLC) system (*3*). Hence, the combination of surface chlorination and RP-HPLC separation should provide a chemically and economically feasible means for acquiring a variety of ^{13}C-enriched CDD congeners

Table III. Comparison of Observed Fractional Ion Abundances for Synthesized ^{13}C-OCDD to Theoretical Values Based on Various Degrees of ^{13}C Enrichment

m/z	theoretical 86 atom % ^{13}C	measd value	theoretical 87 atom % ^{13}C
464	4.6	3.2	3.6
465	14	11	12
466	32	28	29
467	55	54	52
468	78	72	75
469	95	93	93
470	100	100	100
471	91	92	91
472	78	76	81
473	54	49	55
474	40	41	42
475	21	17	21
476	14	13	15
477	5.2	6.0	5.0

that are not currently commercially available.

Perhaps more interesting is the development of an easily performed microsynthesis technique that permits efficient chlorination reactions to be conducted reasonably quickly on microgram quantities of material. Surface chlorination derives its uniqueness from the matrix in which chlorination is achieved. That is, molecules of starting material are adsorbed onto a high surface area "inert" support via deposition from a dilute solution. Knowledge of the approximate surface area of the support, and the amount and concentration of the coating solution, permits reasonably uniform precursor deposition at levels far below those required for a most densely packed monomolecular layer. Hence, when the coating solvent is removed, the precursor molecules remain behind on the support surface in a dispersed array where they can interact with gaseous reagents essentially as independent molecules. As a synthetic route, surface chlorination appears to have several advantages: (a) Chlorination reactions can be accomplished in the absence of a solvent. (b) Dispersion and immobilization of molecular species should inhibit their intermolecular reaction capabilities; hence side reactions should be minimized and essentially limited to intramolecular pathways. (c) High molecular weight, heavily chlorinated compounds might be more easily prepared since solvent-based chlorinations often terminate when the product reaches a chlorination level where it becomes insoluble in the solvent system. (d) A variety of "inert" supporting surfaces can be used; thus possible catalytic effects and/or surface-molecule geometric alignment effects might be used to affect reaction rates and product formation. (e) A wide range of microscale reactions might be possible under these conditions since the only basic requirements are that one of the reagents is a gas, and the other a low volatility liquid or solid under reaction conditions.

Because surface chlorination permits reasonably high product yields for sub-10-μg quantities of 2378-TCDD under the described perchlorination conditions, a reaction that is known to be difficult for conventional systems (*8–11*), this technique may find application as a portion of analytical procedures designed to screen various materials for the presence of chlorinated biphenyls, chlorinated naphthalenes, chlorinated terphenyls, chlorinated dibenzofurans, CDDs, and other aromatics. In such cases, an appropriate fraction would be isolated from the sample, the residue chlorinated, and a final chromatographic separation performed to determine the perchlorinated products. Because each of the example compound classes would produce a single perchlorinated congener whose separation and quantitation would be greatly simplified, the procedure might provide a rapid and very sensitive technique to monitor the possible presence of lower chlorinated species.

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