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Photolytic Method for Destruction of Dioxins in Liquid Laboratory Waste and Identification of the Photoproducts from 2,3,7,8-TCDD

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Analytical and other research laboratories that generate small volumes of dioxin-containing wastes have no convenient method for their disposal. We have used ultraviolet photolysis with a low-pressure mercury lamp to destroy dioxin-like compounds, both as individual congeners and in actual waste analytical samples, down to nondetect levels. Photolysis promises to be an efficient, safe, and inexpensive method for on-site treatment of liquid laboratory wastes that are contaminated by dioxin-like compounds, allowing the treated materials to be discarded as regular organic solvent waste. Experiments with 1,6-[^{3}H]-2,3,7,8-TCDD revealed that the principal photolytic pathway involves cleavage of C−O bonds rather than C−Cl bonds, giving chlorinated hydroxydiphenyl ethers as the initial products and accounting for the low material balances of reductive dechlorination products previously found upon photolysis of PCDDs. The photolysis products from 2,3,7,8-TCDD do not bind to either the Ah receptor or the estrogen receptor in vitro, making it unlikely that the products from UV treatment of PCDD/PCDF in laboratory waste will show either Ah or estrogen receptor-mediated toxicological effects.

Introduction

Halogenated aromatic compounds (HACs), including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs), are well-known toxic pollutants that are the subject of intense analytical and toxicological research interest (*1*, *2*). Numerous laboratories around the world generate small amounts of waste containing these compounds, creating a need for an inexpensive, safe, simple, and efficient method of on-site detoxification.

PCDDs and PCDFs decompose under UV irradiation (*3*, *4*), a reaction that is more efficient in hydrogen donor solvents (*5*, *6*) than in acetonitrile/water mixtures (*7*−*10*). Although this suggests that photodegradation may occur by sequential reductive dechlorination, the yields of dechlorination products are low (*5*, *12*), and the "missing material" is hitherto unidentified. Photolysis in sunlight was used to attempt to destroy 2,3,7,8-TCDD following its accidental release in Seveso, Italy, in 1976 (*13*); photolysis in isopropyl alcohol was used to decontaminate 2,3,7,8-TCDD residues at a former trichlorophenol manufacturing plant in Verona, MO (*14*). Ritterbusch et al. (*15*, *16*) proposed UV treatment of laboratory waste containing PCDD/PCDF in hexane, isooctane, and toluene, using a 150-W mercury lamp but did not characterize the products completely. No remediation technology can be put into practice, however, unless all the products are identified and shown to be less problematic than the original contaminants. In the present study, we developed a photolytic method for the destruction of dioxin-like compounds in liquid laboratory waste using inexpensive and easily available equipment, identified the photolysis products from 2,3,7,8-TCDD, and carried out preliminary toxicity assays.

Experimental Section

Chemicals. Solvents were from Fisher Scientific. OCDD (98% purity; *17*), 2,3,7,8-TCDD, 2,3,7-TrCDD, 2,3-DiCDD, 2-MCDD, and dibenzo-*p*-dioxin (all >99% purity) were previously synthesized in one of our laboratories; 1,2,3,4-TCDD and decachlorobiphenyl (99% purity) were purchased from Accu-Standard. 1,6-[^{3}H]-2,3,7,8-TCDD (specific activity 37 Ci/mmol, >98% purity) was purchased from ChemSyn Laboratories, 2,4,6,7-[^{3}H]estradiol was from Amersham (specific activity 87 Ci/mmol), and Cytoscint ES scintillation fluid was from ICN Pharmaceuticals.

Analytical Methods. Calibration curves for dibenzo-*p*-dioxin, 2-MCDD, 2,3-DiCDD, 2,3,7-TrCDD, 1,2,3,4-TCDD, 1,3,6,8-TCDD, 2,3,7,8-TCDD, OCDD, and DCB were constructed on a Varian Saturn 3 ion trap MS using hexachlorobenzene as the internal standard. The MS was coupled to a Varian Star 3400CX gas chromatograph, the latter being equipped with SPI injector and DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The samples were injected in hexane, with the volumes of injection not exceeding 1.0 μL. The mass spectra were analyzed using Saturn 4D software Version 5.2, instrument detection limit for TCDD 10 pg on-column.

Photolyzed dioxin waste samples were analyzed using a Micromass VG70SE high-resolution mass spectrometer (HRMS), coupled with a Hewlett-Packard 5890 series II gas chromatograph, equipped with a DB-5 capillary column (J&W, 60m × 0.25 mm × 0.25 μm). The instrumental detection limits were 0.25 pg for tetra congeners; 1.0 pg for penta, hexa, and hepta congeners; and 2.0 pg for OCDD and OCDF. HRMS analyses were performed according to U.S. EPA Method 1613 protocols (*18*).

Qualitative mass spectral analyses of the 2,3,7,8-TCDD photoproducts were performed using a VG Quattro II (Fisons UK Ltd.) triple quadrupole mass spectrometer equipped with an atmospheric pressure ion source and MassLynx software package. The sample solutions were introduced into the mass spectrometer via a 10-μL Rheodyne 7010 injection valve. The mobile phase [50/50 v/v Nanopure water (Barnstead) and acetonitrile (Caledon)] was delivered using a Hewlett-Packard 1090 series II/L binary LC pump at 15 μL/min. The mass spectra were acquired and averaged over at least 8 scans in multichannel analysis (MCA) data acquisition mode by scanning the first quadrupole in 0.1 amu increments from m/z 100 to 800 in 1.2 s.

Apparatus. The reaction vessel was a standard 100-mL three-neck round-bottom flask equipped with a magnetic

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TABLE 1. Photolysis of High-Strength Dioxin Waste

	concentration, mol L^{-1} (conversion, %)				
time, h	Cl_5 PCB, 326[a]	TriCDF, 272[a]	TCDD, 322[a]	PeCDF, 340[a]	PeCDD, 356[a]
0	1.53×10^{-4} (0.0)	3.68×10^{-4} (0.0)	1.88×10^{-4} (0.0)	1.18×10^{-4} (0.0)	1.40×10^{-5} (0.0)
1.5	6.61×10^{-6} (95.7)	3.62×10^{-4} (1.6)	1.75×10^{-4} (6.4)	1.02×10^{-5} (91.3)	8.35×10^{-6} (40.5)
4	2.36×10^{-7} (99.8)	9.37×10^{-5} (74.6)	8.07×10^{-5} (57.0)	ND[b] (100)	2.33×10^{-6} (83.4)
24	ND (100)	1.13×10^{-6} (99.7)	1.35×10^{-6} (99.3)	ND (100)	ND (100)
27	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)

[a] Compound and ion monitored, *m/z*. [b] ND, not detected.

TABLE 2. Yields of Dechlorination Products in Photolysis of 2,3,7,8-TCDD

	concentration, mol L^{-1}			
dioxin congener	0[a,b]	4[a]	10[a]	20[a]
2,3,7,8-TCDD	1.18×10^{-5}	7.44×10^{-6}	1.84×10^{-6}	2.16×10^{-7}
2,3,7-TriCDD	4.22×10^{-8}	1.97×10^{-7}	1.13×10^{-7}	5.63×10^{-8}
2,3-DiCDD[c]	1.44×10^{-8}	ND	ND	ND
2-MCDD	1.64×10^{-8}	1.64×10^{-8}	ND	1.64×10^{-8}
dibenzo-*p*-dioxin	1.56×10^{-8}	1.56×10^{-8}	ND	ND
total concn, M	1.19×10^{-5}	7.67×10^{-6}	1.95×10^{-6}	2.88×10^{-7}
conversion, %	0	36.8	84.4	98.2
total dechlorination products, M (%)	8.86×10^{-8} (0)	2.29×10^{-7} (1.95)	1.13×10^{-7} (0.96)	7.27×10^{-8} (0.62)

[a] Irradiation time, min. [b] 0.75% of less chlorinated dioxins were present originally. [c] Includes 2,6-DiCDD and 2,7-DiCDD, which were assumed to have the same detector response as 2,3-DiCDD.

stirrer. The three necks were provided with a reflux condenser sealed with a rubber septum and a balloon and needle to relieve gas pressure, a rubber septum for taking samples during the irradiations, and a quartz sleeve attached to a commercially available T24 quartz male ground joint. A PenRay 4.6-W low-pressure mercury lamp was placed in the quartz sleeve.

Photolyses. For single congeners, a hexane solution (25.0 mL) of concentration ~100 μM was irradiated at 254 nm; 0.5-mL aliquots were withdrawn at appropriate intervals, mixed with 0.5 mL of a hexane solution of the internal standard, and analyzed by GC−MS.

Two laboratory waste samples were processed; one containing high levels and the other containing low levels of PCDD and PCDF. The 'high' concentration waste sample (5−100 μg/mL) contained a mixture of waste standards in isooctane, principally 3,3′,4,4′,5-pentachlorobiphenyl, 1,4,7,8-TCDD, 1,2,3,7,8-PeCDD, an unknown trichlorodibenzofuran, and an unknown pentachlorodibenzofuran (Table 1). This solution was irradiated at 254 nm, with 0.5 mL withdrawn after 1.5, 4, 24, and 27 h.

The 'low' concentration waste sample (in isooctane) was prepared by combining approximately 600 cleaned-up extracts of environmental samples that had been archived awaiting disposal. It contained native PCDD/PCDF (mostly from incineration/combustion sources; variable concentrations with maximum 1 ng/mL) and also $^{13}C_{12}$-labeled PCDD/PCDF (≈10 ng/mL). A total of 30 mL of the waste sample was photolyzed, with 1.0-mL aliquots removed after 1.5, 3.5, 6, and 27 h for analysis by HRMS. The concentrations of PCDD/PCDF (congener group totals) were determined by peak area comparison to U.S. EPA Method 1613 calibration standards using the external standard method.

Syntheses. (a) 2-Hydroxy-4,4′,5,5′-tetrachlorodiphenyl ether (1). 1 was synthesized according to the method of Humpi (*19*) by condensation of 2,4-dichloronitrobenzene with 3,4-dichlorophenol (78% yield) followed by reduction of the resulting 2-nitro-4,4′,5′-trichlorodiphenyl ether with Sn/AcOH/HCl to 2-amino-4,4′,5′-trichlorodiphenyl ether (95% yield). The amino compound was converted in 25% yield by the Sandmeyer reaction to 2-hydroxy-4,4′,5′-trichlorodiphenyl ether, which gave **1** in 90% yield (20 mg) as a colorless solid upon overnight stirring over SO_2Cl_2: mp 71 °C, purity 98% by GC−MS. ^{1}H NMR (δ, $CDCl_3$), 5.58, s, 1H; 6.90, dd, J = 8.8 Hz, ^4J = 2 Hz, 1H; 6.95, s, 1H; 7.14, *d*, ^4J = 2 Hz, 1H; 7.17, s, 1H; 7.44, d, J = 8.8 Hz, 1H. ^{13}C NMR (δ, $CDCl_3$), 117.6, 118.2, 120.0, 120.2, 123.6, 128.2, 128.6, 131.5, 133.8, 142.0, 146.5, 154.8. MS (EI): 326 (33), 324 (100), 322 (69), 254 (16), 252 (26) 223 (6), 177 (8), 146 (25), 109 (14).

(b) 2,2′-Dihydroxy-4,4′,5,5′-tetrachlorobiphenyl (2). 2 was obtained in four steps from 3,4-dichlorophenol, which was treated with iodine in boiling water in the presence of NaOH to give 2-iodo-4,5-dichlorophenol (33% yield). This was converted to 2-iodo-4,5-dichloroanisole by reflux with methyl iodide in acetone in the presence of K_2CO_3 (90% yield), followed by Ullmann coupling in a sealed ampule at 220 °C in the presence of 40 molar excess of copper-bronze powder to give 2,2′-dimethoxy-4,4′,5,5′-tetrachlorobiphenyl in 14% yield (isolated). Treatment of the latter compound with BBr_3 in dry CH_2Cl_2 at −20 °C gave 2,2′-dihydroxy-4,4′,5,5′-tetrachlorobiphenyl (**2**) in 70% yield (8 mg) as a colorless solid, mp 178−179 °C (decomp), no impurities detected by ^{1}H NMR: (δ, CD_3OD), 7.05, s, 2H; 7.33, s, 2H. ^{13}C NMR (δ, CD_3OD), 118.4, 123.0, 125.7, 132.7, 133.6, 155.5.

Photodechlorination of 2,3,7,8-TCDD. Two milliliters of 1.2×10^{-5} mol L^{-1} solution of 2,3,7,8-TCDD in hexane in an 8 mm Pyrex tube, sealed with a rubber septum, were photolyzed at 300 nm in a Rayonet photoreactor. Aliquots (200 μL) were removed after 4, 10, and 20 min; mixed with 200 μL of HCB (5×10^{-6} mol L^{-1} in hexane); and analyzed by GC−MS to give the results shown in Table 2.

In the photolysis of 2,3,7,8-TCDD spiked with 1,6-[^{3}H]-2,3,7,8-TCDD, the stock solution of 1,6-[^{3}H]-2,3,7,8-TCDD in hexane (10 μL) had activity $2.21 \pm 0.11 \times 10^4$ DPM (nominal concentration 3.7×10^{-8} mol L^{-1}). A solution of 2,3,7,8-TCDD (9.32×10^{-5} mol L^{-1}, 0.9 mL) was placed into each of two 8 mm Pyrex tubes. Hexane (0.1 mL) was added to one tube, and 0.1 mL of the stock solution of 1,6-[^{3}H]-2,3,7,8-TCDD in hexane was added to the other. The tubes were capped with rubber septa, placed in a Rayonet merry-go-round photo-

reactor, and irradiated at 300 nm. Three 10-μL aliquots were taken with a gas-tight 25-μL syringe through the rubber septum from the tube containing 1,6-[^{3}H]-2,3,7,8-TCDD before and after 3 h irradiation, by which time the conversion of unlabeled 2,3,7,8-TCDD had reached 99.9% (GC−MS of the unlabeled sample). Each aliquot of the radiolabeled sample was analyzed by scintillation counting after adding 2.0 mL of scintillation cocktail. The radiolabeled samples were also separated by HPLC (Perkin-Elmer model 250 isocratic LC pump, Rheodyne model 7010 injector equipped with 20-μL sample loop, Waters μ-Bondapack C_{18} 3.9 × 300 mm column, and Gilson model 202/204 fraction collector, pure methanol as the mobile phase). The fractions were collected directly into scintillation vials and counted (Beckman LS 7000 scintillation counter). Chromatograms were obtained by plotting the radioactivity of each fraction vs elution time. Wipe tests were performed after all experiments with radiochemicals.

Hydroxylapatite Assay with the Products of Photolysis of 2,3,7,8-TCDD. A solution of 2,3,7,8-TCDD in hexane (2.40 mL, 7.8×10^{-5} mol L^{-1}) was placed in an 8 mm Pyrex tube and irradiated at 300 nm in the merry-go-round photoreactor. Aliquots (200 μL) taken after 0, 0.5, 1, 2, 4, 8, 12, 20, 35, and 60 min were placed in 1-mL sample vials, then 25 μL was drawn from each aliquot and mixed with 25 μL of 5×10^{-6} HCB in hexane prior to analysis by GC−MS (triplicate injections). The remaining 175 μL of hexane was carefully removed under a slow stream of nitrogen, and each residue was redissolved in 50 μL of DMSO (Fisher, Spectranalyzed Grade) and then diluted 1000-fold before use.

The assay procedure followed that of Gasiewicz and Neal (*20*) with only minor modifications. Hepatic cytosol from immature Sprague−Dawley rats was prepared as described previously (*21*). Aliquots of rat liver cytosol were thawed and diluted to 2.0 mg/mL with HEGD buffer (1 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 1 mM EDTA sodium salt, 1 mM dithioerythritol, 10% v/v glycerol, pH 7.6). One milliliter of diluted cytosol was added in quadruplicate to 10 μL of 1×10^{-7} mol L^{-1} 1,6-[^{3}H]-2,3,7,8-TCDD and 10 μL of each (diluted) photolyzed solution and incubated for 45 min at 23 °C. Then 0.20 mL was withdrawn and incubated for 10 min on ice with 0.25 mL of freshly washed hydroxylapatite slurry in ice-cold HEGD buffer. One milliliter of ice-cold HEGD buffer containing 1% Triton X-100 surfactant was added; after mixing, the mixture was centrifuged at 2000*g* for 2 min, and the supernatant was discarded. After two more similar washings, the HAP pellet was transferred quantitatively to a plastic 20-mL scintillation vial with 3 × 0.75 mL of ethanol. Ten milliliters of scintillation cocktail was added, and the radioactivity was counted. Aliquots of cytosol incubated with [^{3}H]TCDD alone gave a value for "total binding", while samples incubated with [^{3}H]-TCDD and a 200-fold excess of TCDF were used to determine "nonspecific" binding. Quadruplicate values of the activities of the samples (sample binding), measured by scintillation counting, were averaged, and the percent specific binding of [^{3}H]TCDD to the Ah receptor was calculated using

$$\%\ \text{specific binding} = \frac{[\text{sample (DPM)} - \text{nonspecific (DPM)}]}{[\text{total (DPM)} - \text{nonspecific (DPM)}]} \times 100$$

Estrogen Receptor Assay with the Products of Photolysis of 2,3,7,8-TCDD. The gel filtration chromatographic method for determining relative estrogenic binding affinities developed by Cox and Bunce (*22*) was used without alterations, using 2,4,6,7-[^{3}H]estradiol (1.5×10^{-8} mol L^{-1}) as the reference radioligand. Five samples of the photolyzed 2,3,7,8-TCDD were used in the assay, using 10-μL aliquots of the (undiluted) DMSO solutions. Rat liver cytosol was obtained from female Long−Evans rats as described previously (*22*). The HPLC analysis was as described previously, except that a Phenomenex Biosap S400 size exclusion column (300 mm × 7.8 mm) was used with flow rate of mobile phase (25 mM phosphate, 1.5 mM EDTA, 100 mM KCl, 10% glycerol, pH 7.1) of 0.5 mL/min. For each chromatogram, 0.5-mL fractions were collected between 6.5 and 12 min after the injection.

Results and Discussion

Photolysis of Waste. Our objective was to develop methodology for the removal of dioxins to below the detection limits of high resolution GC−MS, using inexpensive, commercially available equipment with minimal custom modification. We wanted the equipment to be compact, easily assembled, and able to operate unattended in a laboratory fume hood.

We chose a 254-nm PenRay lamp as the UV source. The advantages of low-pressure mercury lamps over the high- or medium-pressure mercury lamps proposed previously (*15*) include low power (4.6 W), high photon efficiency, and low operating temperature (<60 °C) to avoid the need for external water cooling. Insertion of the light source in a quartz sleeve avoided deposition of materials on the surface of the lamp and completely circumvented heating the reaction mixture (the temperature inside the reactor never exceeded ambient by 3 °C). Evaporative solvent loss during overnight photolyses in hexane was avoided by sealing the reflux condenser with a septum (equipped with a balloon for pressure relief). The fire hazard was minimal, and the equipment could run unattended in a fume hood.

Preliminary photolyses involved individual dioxin congeners in hexane. Decachlorobiphenyl (DCB) was also included in the trials to demonstrate the applicability of the method for detoxification of PCB-containing waste. As shown in Figure 1, irradiation of 1,2,3,4-TCDD (1.0×10^{-5} mol L^{-1} in hexane), 1,3,6,8-TCDD (1.3×10^{-4} mol L^{-1}), OCDD (5.0×10^{-5} mol L^{-1}), and DCB (3.0×10^{-5} mol L^{-1}) gave >99% conversion in <2 h. In every case, the concentrations of starting material and secondary products dropped below the detection limit of Saturn 3 GC−MS within 5 h. Pseudo-first-order kinetics were followed for the disappearance of each compound, consistent with low light absorption [$I_{abs} = I_0$-$(1-10^{-Abs})$, which is directly proportional to substrate concentration at low absorbance]. No attempt was made to determine quantum yields because they were not the objective of this project.

Some analytical wastes of dioxin-like compounds are generated in toluene, whose high absorbance at 254 nm outcompetes HACs for incident radiation. We considered whether photolysis might still proceed through energy transfer from toluene to the chlorinated substrates [triplet energy of toluene = 347 kJ mol^{-1}, those of chlorinated benzenes (model for PCDDs) are 335−345 kJ mol^{-1}, and that of biphenyl (model for DCB) is 274 kJ mol^{-1} (*23*)]. In practice, toluene was unsatisfactory as a solvent: 1,3,6,8-TCDD (2.3×10^{-5} mol L^{-1}) was unchanged after 3 h of irradiation in toluene, and DCB was almost unreactive. In toluene−hexane mixtures, the photolysis of 1,3,6,8-TCDD slowed progressively with the proportion of toluene: in 1% toluene, 99.8% conversion was achieved after 18 h, while 13% of 1,3,6,8-TCDD remained after 31 h in 10% toluene. An attempt to assist the photolysis by adding triethylamine as an electron donor (*24*) to toluene was unsuccessful. We conclude that PCDD wastes generated in toluene should be solvent-exchanged into an alkane solvent ahead of photolysis; a few percent of residual toluene can be tolerated at the expense of longer irradiation times.

Two types of samples of liquid waste were tested. A "high-concentration" sample contained 5−100 μg/mL each of 1,4,7,8-TCDD and 1,2,3,7,8-PeCDD and unspecified pen-

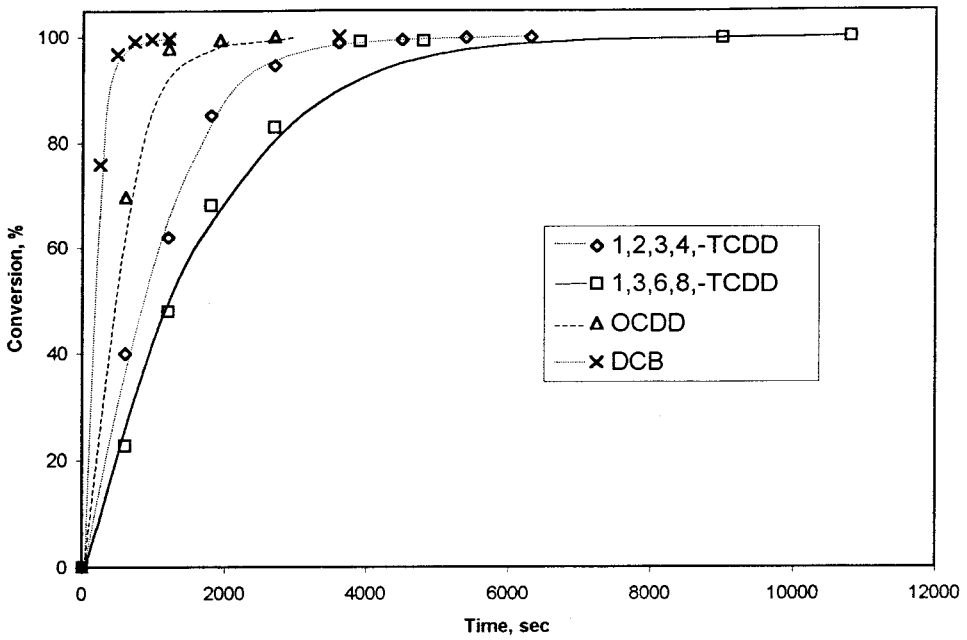


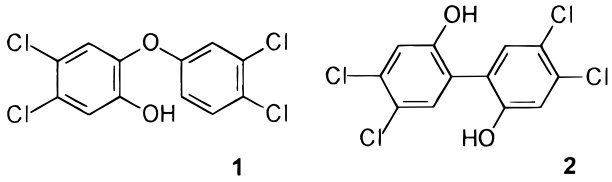
FIGURE 1. Disappearance of 1,2,3,4-TCDD, 1,3,6,8-TCDD, OCDD, and DCB during photolysis at 254 nm.

tachlorobiphenyl, trichlorodibenzofuran, and pentachlorodibenzofuran. The mixture was irradiated at 254 nm; after 27 h, the concentrations of all starting materials and chlorinated intermediates dropped below the level of detection of the Saturn 3 GC−MS (Table 1). The "low-concentration" waste was a mixture of ca. 600 environmental samples, mostly extracts of PCDD/PCDF from fly ash, mixed with ^{13}C-labeled standards. It contained 5−100 ng/mL of PCDD/PCDF, which was below the detection limits of the Saturn 3 GC−MS, so the samples were analyzed by HRMS at 10000 resolution. After 27 h, all analytes were below the instrumental detection limit (Figure 2).

Products and Mechanism of PCDD Photolysis. Practical implementation of photolytic waste treatment requires detailed product studies to ensure that the procedure will not form harmful contaminants. Table 2 shows that PCDD/PCDF give only low yields of photodechlorination products in hydrogen donor solvents, consistent with many previous studies (*4*, *25*−*27*). No other products were observed by GC−MS, casting doubt on the suggestion that the major photolysis pathway of PCDDs involves successive C−Cl homolysis (*28*). Previous explanations for the low material balance include PCDD heterolysis to yield a carbene that reacts with a second molecule of the parent molecule (*29*) and the formation of polychlorobenzenes during photolysis of 1,2,3,7,8-PeCDD in CCl_4 (*30*); neither has any precedent in the photochemistry of haloaromatic compounds (*25*).

After photolysis to 99.9% conversion of 2,3,7,8-TCDD spiked with 1,6-[^{3}H]-2,3,7,8-TCDD, a 10-μL aliquot exhibited almost equal radioactivity as an unirradiated control (3165 ± 110 vs 3380 ± 60 dpm). This showed that the photolysis products remained in solution rather than adsorbing to the reaction vessel, precipitating, or volatilizing. No significant peaks were seen upon HPLC analysis of an irradiated solution of unlabeled 2,3,7,8-TCDD, but scintillation counting of fractions from 20 μL of irradiated 1,6-[^{3}H]-2,3,7,8-TCDD separated by C_{18} reverse-phase HPLC revealed products with short retention times (Figure 3). These more polar products were observed by conventional HPLC analysis of unlabeled TCDD (UV absorbance detector, λ = 227 nm) when the solution was concentrated 500 times.

Fractions of the radiolabeled material eluting after 3−4 min (fraction A) and 4−6 min (fraction B), collected from five injections on the analytical column, had mole ratios 1:3.3 by scintillation counting and accounted for ~90% of the total radioactivity of the starting material. Analysis by negative ion ES−MS, which detects quasi-molecular ions at m/z values corresponding to $(M - H)^-$ showed three major peaks in fraction A (m/z 271, 303, and 321; ratio 1:3:4; with isotope patterns indicating 3, 3, and 4 chlorines, respectively). The parents corresponding to these quasi-molecular ions were $C_{12}H_7Cl_3O$ (m/z 271), $C_{12}H_7Cl_3O_3$ (m/z 303), and $C_{12}H_6Cl_4O_2$ (m/z 321). All these compounds must be phenolic, with the protons lost from hydroxyl groups. Only m/z 321 appeared in the major fraction B, which could be either 2-hydroxy-3′,4,4′,5-tetrachlorodiphenyl ether (**1**) or 2,2′-dihydroxy-4,4′,5,5′-tetrachlorobiphenyl (**2**).



1 **2**

Preparation of authentic samples indicated **1** rather than **2** as the likely major product based on HPLC retention time. C−O rather than C−Cl homolysis is therefore the major pathway in the photolysis of dioxins, explaining the low dechlorination yields. Literature precedents to support C−O bond cleavage include 10% of **2** (as the dimethyl ether, after diazomethane treatment) upon photolysis of 2,3,7,8-TCDD in isooctane; however >80% of the starting material was undetected (*5*). Photolysis of dibenzo-*p*-dioxin (*31*, *32*) gives 2,2′-dihydroxybiphenyl and 4-hydroxydibenzofuran (*33*, *34*); diphenyl ether photorearranges to *o*- and *p*-phenylphenol (*35*).

Receptor Binding Assays of the Products of Photolysis of 2,3,7,8-TCDD. We carried out two bioassays to investigate whether the products of photolysis of PCDD/PCDF might pose a threat of toxicity. Binding to the Ah receptor protein was studied, since it is well-known that the major toxic responses to dioxins are Ah receptor-mediated (*1*, *2*). Product mixtures from various stages of photolysis of 2,3,7,8-TCDD in hexane were incubated with 1,6-[^{3}H]-2,3,7,8-TCDD and rat hepatic cytosol containing the Ah receptor. The radiolabeled TCDD competed with residual unphotolyzed (un-

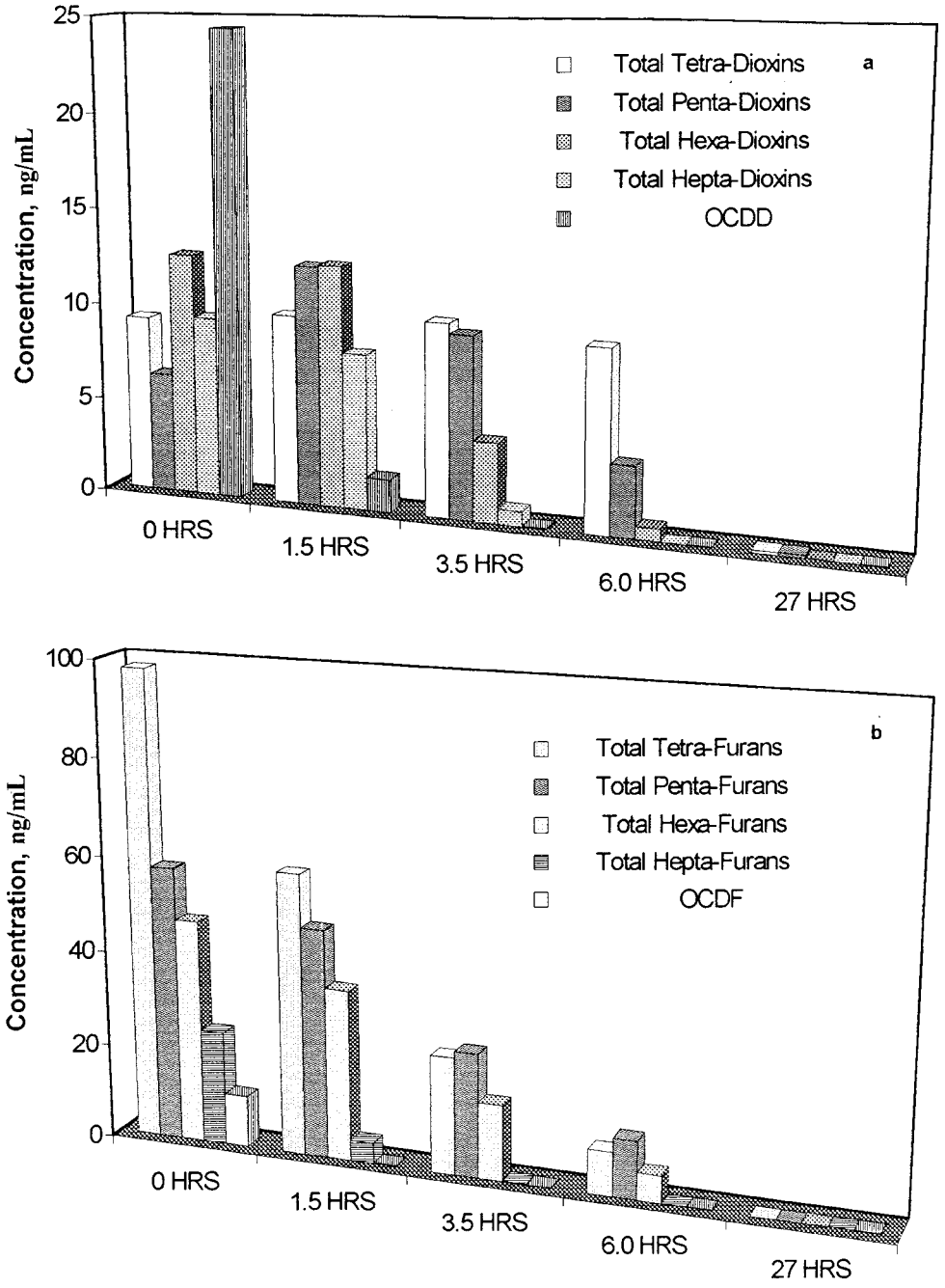


FIGURE 2. Change in concentrations (congener totals) of PCDD (a) and PCDF (b) during photolysis of the low-strength waste at 254 nm.

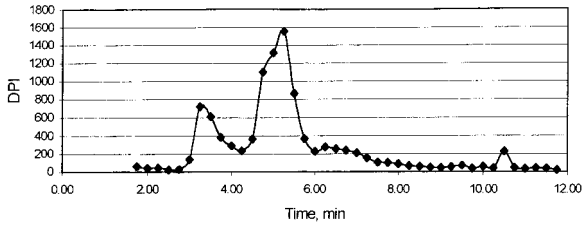


FIGURE 3. HPLC radiochromatogram from photolysis of 1,6-[^{3}H]-2,3,7,8-TCDD in hexane at 300 nm: mobile phase, 100% MeOH flow rate, 1 mL/min.

labeled) TCDD and its photolysis products for a fixed aliquot of Ah receptor. In the absence of unlabeled TCDD, the radioligand occupied all the receptor binding sites, and maximum protein-bound radioactivity was observed (100% specific binding). In the unphotolyzed sample, the unlabeled TCDD was in large excess over the radioligand, and specific binding was low. Specific binding increased with the percent photoconversion of TCDD, eventually reaching 100% at 100% conversion, when no unlabeled TCDD remained to compete with the radioligand (Figure 4). Because the reaction products at 100% conversion have no Ah receptor affinity, they will not exhibit dioxin-like toxicity.

The increase of specific binding lagged behind the percent conversion at intermediate stages of conversion (Figure 4). The binding affinities of **1** and **2** relative to unlabeled 2,3,7,8-TCDD were 3.4×10^{-4} and 5.7×10^{-5}, respectively; hence, **1** could contribute to the Ah receptor binding at intermediate stages of photolysis. The relative binding affinities of a group of chlorinated diphenyl ethers were in the same range as **1**: 2,3′,4′,6-tetrachloro-, 1.2×10^{-4}; 3,3′,4,4′-tetrachloro-, 1.0×10^{-2}; 3,3′,4,4′,5-pentachloro-, 1.4×10^{-2}; 2,3′,4,4′,5-pentachloro, 7.7×10^{-3}. The 3,3′,4,4′-chlorines, corresponding

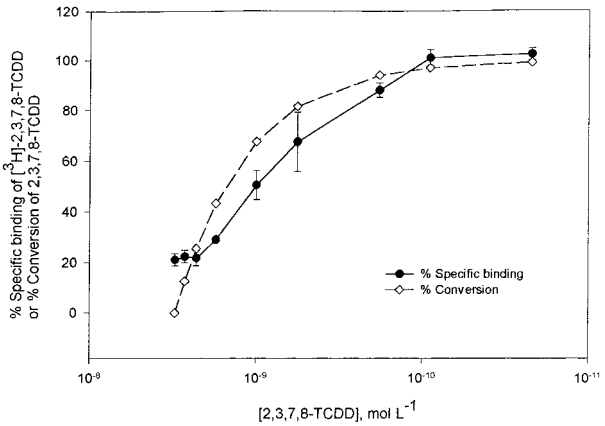


FIGURE 4. Relationship between percent specific binding of 1,6-[^{3}H]-2,3,7,8-TCDD to rat hepatic Ah receptor and the photolytic conversion of 2,3,7,8-TCDD.

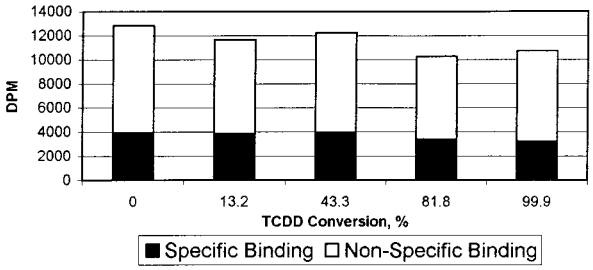


FIGURE 5. Specific and nonspecific binding of [^{3}H]estradiol for the estrogen receptor when competed against photolysis products 2,3,7,8-TCDD.

to the 2, 3, 7, and 8 chlorines of PCDDs, impart moderately strong Ah receptor binding activity, which is somewhat diminished by the presence of the polar 2-OH group of **1**.

Knowing that the major primary products of PCDDs are hydroxylated, the second bioassay was a test of estrogenic activity, which has been documented for numerous phenolic compounds (*36*, *37*), including hydroxylated PCBs (*38*−*40*). The photolysis products from 2,3,7,8-TCDD were competed against [^{3}H]estradiol for occupation of the estrogen receptor from female Long-Evans rat liver. Gel filtration chromatography (*22*) allowed the direct determination of percent specific binding vs extent of photoreaction. Samples taken at 0, 13, 43, 88, and 99% conversion of 2,3,7,8-TCDD showed no significant decrease in the area of specific binding with increasing conversion (Figure 5). Thus, the photolysis products possess neither dioxin-like nor estrogenic activity, indicating that dioxin wastes that have been photolyzed to nondetect levels can be treated as regular organic waste.

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