Synthesis of Possible Metabolites of 2,3,7,8-Tetrachlorodibenzofuran

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Five hydroxypolychlorodibenzofurans and the corresponding methyl ethers were synthesized and characterized. The general route of synthesis involved biphenyl formation via a diazotized aniline and a protected phenol followed by deprotection and base-catalyzed ring closure. The compounds 2-hydroxy-3,7,8-trichlorodibenzofuran, 3-hydroxy-2,7,8-trichlorodibenzofuran, 1-hydroxy-2,3,7,8-tetrachlorodibenzofuran, 3-hydroxy-2,4,7,8-tetrachlorodibenzofuran, and 4-hydroxy-2,3,7,8-tetrachlorodibenzofuran are possible biliary metabolites from rats treated with 2,3,7,8-TCDF, a highly toxic contaminant sometimes found in commercial polychlorinated biphenyls and in chlorinated phenol mixtures. The hydroxypolychlorodibenzofurans, as well as a biphenyl, 2,2′-dimethoxy-4,4′,5,5′-tetrachlorobiphenyl, can be used to establish the identity of some of the biliary metabolites and to explore possible toxicity associated with the individual metabolites.

2,3,7,8-Tetrachlorodibenzofuran (TCDF) is a highly toxic contaminant found in commercial polychlorinated biphenyls and in chlorinated phenol mixtures. In both structure and toxicity, TCDF is closely related to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Unlike TCDD, TCDF is fairly rapidly excreted via the bile and feces in rats, solely as metabolites (Birnbaum et al., 1980). A GC–MS investigation of the biliary metabolites of TCDF has been reported by Poiger et al. (1984). They treated bile with glucuronidase/sulfatase and methylated the freed phenols. Tetrachloromethoxy-, trichloromethoxy-, and trichlorodimethoxydibenzofuran isomers and a possible ring-opened product, a tetrachlorodimethoxybiphenyl, were found. The substitution patterns of the metabolites were not determined from the mass spectra. Isolation and further structural characterization, for instance by NMR spectrometry, are difficult since TCDF is very toxic and only microgram doses can be given. Indeed, it is not clear that unequivocal assignments could be made even if the mixture could be separated and ^{1}H NMR spectra obtained.

As part of a continuing effort to understand the toxicity of TCDF and related compounds, we have undertaken the synthesis of several oxygenated polychlorodibenzofurans and polychlorobiphenyls that we hope to use to establish the identity of some of the biliary metabolites of TCDF and also to investigate the toxicity of some of the hydroxylated compounds individually. Although metabolism of polyhalogenated dibenzofurans seems to result in decreased toxicity and increased elimination (Brewster and Birnbaum, 1988), an investigation of the toxicity of some of the individual metabolites would be of interest.

Several methods for the synthesis of polychlorodibenzofurans are discussed in an excellent review by Gara et al. (1981). While many of these methods produce difficult to separate mixtures of isomers, cyclization of diazotized chlorodiphenyl ethers (Gray et al., 1976) and palladium(II) acetate catalyzed cyclization of chlorinated diphenyl ethers (Norstrom et al., 1979) can produce specific isomers of chlorinated dibenzofurans. Several mono- and dichloromethoxydibenzofurans have been synthesized by photochemical cyclization of suitably substituted diphenyl ethers (Tulp and Hutzinger, 1978). A method for the synthesis of specific polychlorodibenzofurans was recently published by Safe and Safe (1984) based on the formation of biphenyls by the Cadogan method (Cadogan, 1962) and base-catalyzed ring closure. We felt that this method could be most conveniently extended and adapted for the synthesis of required oxygenated compounds.

EXPERIMENTAL SECTION

The substituted anisoles used in this work that were not commercially available were made by treating the phenol with $(CH_3)_2SO_4/K_2CO_3$; melting points of the products were in agreement with literature values. Flash chromatography was carried out as described by Still et al. (1978) with use of Baker silica gel for flash chromatography. NMR spectra were obtained at 500 MHz on a GE GN-500 NMR spectrometer. The chemical shifts are reported (ppm) relative to tetramethylsilane as the internal or external standard for $CDCl_3$ or acetone-d_6 (δ 2.04), respectively. Mass spectra were run at Triangle Laboratories, Inc., Research Triangle Park, NC, on VG 12-250 and VG 7070S mass spectrometers by direct-insertion probe in electron-impact mode at an ionizing energy of 70 eV. The most intense peaks in the molecular ion cluster are given; only the ^{35}Cl-containing ion is given for the fragments. Accurate mass measurements were obtained by peak matching versus perfluorokerosene.

2,2′-Dimethoxy-4,4′,5,5′-tetrachlorobiphenyl (1a). 4,5-Dichloro-2-nitrophenol was prepared as described by Jeffcoat et al. (1977). Methylation of the phenol with $(CH_3)_2SO_4/K_2CO_3$ gave the corresponding anisole. The anisole (4.4 g) was dissolved in 80 mL of ethyl acetate, and 400 mg of platinum on sulfide carbon (Aldrich Chemical Co., Milwaukee) was added. The mixture was stirred under hydrogen (1 atm) until ca. 1800 mL of H_2 was consumed. The catalyst was removed by filtration. After evaporation of the solvent, the residue was recrystallized from ethanol/water to give 2.9 g of 4,5-dichloro-*o*-anisidine as light gray crystals, mp 94–95 °C (lit. mp 96–97 °C (Woolley and Pringle, 1952)). The anisidine (1.9 g) was dissolved in 5 mL of 3,4-dichloroanisole and heated to 120 °C; 2 mL of isopentyl nitrate was added over 30 min, and the mixture was heated at 120 °C for 6 h. Biphenyl **1a** was isolated by flash chromatography using hexane as eluent. Recrystallization from hexane/ether gave 92 mg of colorless crystals: ^{1}H NMR ($CDCl_3$) δ 3.77 (6 H, s, OCH_3), 7.04 (2 H, s, 3- and 3′-CH), 7.27 (2 H, s, 6- and 6′-CH); MS, *m/z* (relative intensity) 350 (M^+, 86), 352 (M + 2, 100), 354 (M + 4, 48), 300 (M − CH_3 − Cl, 59), 285 (300 − CH_3, 21), 257 (285 − CO, 11); accurate mass measurement for $C_{14}H_{10}O_2Cl_4$, calcd 349.9435, found 349.9441.

2,5-Dihydroxy-4,2′,4′,5′-tetrachlorobiphenyl (2b). 2,4,5-Trichloroaniline (2.5 g) was dissolved in 6.6 g of 2-chloro-1,4-dimethoxybenzene and the resultant mixture reacted with isopentyl nitrite as described above. Flash chromatography with 1:99 (v/v) ethyl acetate/hexane as eluent gave a 3:1 mixture of **2a**

and **3a**. The mixture of biphenyls was dissolved in 2 mL of methylene chloride, and 5 mL of 1 N boron tribromide in methylene chloride was added. After 6 h at room temperature, 20 mL of methylene chloride was added and the organic layer was washed with sodium bicarbonate solution, dried, and concentrated. Partial separation of dihydroxybiphenyls **2b** and **3b** was accomplished by flash chromatography using 1:9 (v/v) ethyl acetate/hexane. A fraction containing only **2b** was obtained after recrystallization from benzene/hexane: ^{1}H NMR (acetone-d_6) δ 6.83 (1 H, s, 6-CH), 6.99 (1 H, s, 3-CH), 7.56 (1 H, s, 6'-CH), 7.73 (1 H, s, 3'-CH), 8.32 (1 H, s, -OH), 8.36 (1 H, s, -OH); MS, m/z (relative intensity) 322 (M^+, 39), 324 (M + 2, 50), 326 (M + 4, 24), 287 (M - Cl, 9), 252 (M - 2 Cl, 100); accurate mass measurement for $C_{12}H_6O_2Cl_4$, calcd 321.9122, found 321.9116.

2-Hydroxy-3,7,8-trichlorodibenzofuran (8a). Biphenyl **2b** (155 mg) was dissolved in 1.5 mL of 1 N KOH in methanol and the methanol removed by a stream of nitrogen. Dimethyl sulfoxide (2 mL) was added, and the solution was heated at 140 °C for 18 h. After this time the mixture was cooled and partitioned between methylene chloride and water (50 mL each). The aqueous layer was adjusted to pH 5 and extracted 2 × 50 mL of methylene chloride. The organic layers were combined, dried, and concentrated. Flash chromatography using 1:9 (v/v) ethyl acetate/hexane and recrystallization from methylene chloride gave 38 mg of dibenzofuran **8a**: ^{1}H NMR (acetone-d_6) δ 7.688 (1 H, s, 1- or 4-CH), 7.693 (1 H, s, 4- or 1-CH), 7.84 (1 H, s, 6-CH), 8.22 (1 H, s, 9-CH), 8.91 (1 H, s, -OH); MS, m/z (relative intensity) 286 (M^+, 100), 288 (M + 2, 97), 290 (M + 4, 30), 223 (M - CO - Cl, 10), 194 (223 - CHO, 28); accurate mass measurement for $C_{12}H_5O_2Cl_3$, calcd 285.9355, found 285.9361.

2-Methoxy-3,7,8-trichlorodibenzofuran (8b). Reaction of 25 mg of **8a** with CH_3I/K_2CO_3 in acetone gave 22 mg of **8b** after recrystallization from methylene chloride/methanol: ^{1}H NMR ($CDCl_3$) δ 4.02 (3 H, s, OCH_3), 7.36 (1 H, s, 1-CH), 7.61 (1 H, s, 4- or 6-CH), 7.65 (1 H, s, 6- or 4-CH), 7.97 (1 H, s, 9-CH); MS, m/z (relative intensity) 300 (M^+, 100), 302 (M + 2, 100), 304 (M + 4, 33), 285 (M - CH_3, 63), 257 (285 - CO, 43), 194 (257 - Cl - CO, 43); accurate mass measurement for $C_{13}H_7O_2Cl_3$, calcd 299.9516, found 299.9512.

2,4-Dihydroxy-5,2',4',5'-tetrachlorobiphenyl (4b). 2,4,5-Trichloroaniline (1.5 g) was added to 4.0 g of the bis(*tert*-butyldimethylsilyl) ether of 4-chlororesorcinol [prepared from 4-chlororesorcinol by adapting the procedure of Corey and Venkateswarlu (1972)]. The mixture was treated with isopentyl nitrite as described above. Biphenyl silyl ether **4a** could not be separated from the resorcinol silyl ether by flash chromatography. The silyl ethers were cleaved with tetrabutylammonium fluoride (Corey and Venkateswarlu, 1972). Flash chromatography using 1:9 (v/v) ethyl acetate/methylene chloride gave 53 mg of **4b**: ^{1}H NMR (acetone-d_6) δ 6.68 (1 H, s, 3-CH), 7.13 (1 H, s, 6-CH), 7.54 (1 H, s, 6'-CH), 7.70 (1 H, s, 3'-CH), 8.68 (1 H, s, OH), 8.87 (1 H, s, OH); MS, m/z (relative intensity) 322 (M^+, 68), 324 (M + 2, 87), 326 (M + 4, 43), 286 (M - HCl, 43), 252 (M - 2 Cl, 100); accurate mass measurement for $C_{12}H_6O_2Cl_4$, calcd 321.9122, found 321.9126.

3-Hydroxy-2,7,8-trichlorodibenzofuran (9a). Furan ring closure of the dipotassium salt from 53 mg of **4b** was carried out as described above. Flash chromatography using 1:49 (v/v) ethyl acetate/methylene chloride gave 31 mg of **9a**: ^{1}H NMR (acetone-d_6) δ 7.27 (1 H, s, 4-CH), 7.84 (1 H, s, 6-CH), 8.16 (1 H, s, 1-CH), 8.25 (1 H, s, 9-CH), 9.52 (1 H, s, OH); MS, m/z (relative intensity) 286 (M^+, 100), 288 (M + 2, 97), 290 (M + 4, 32), 252 (M - Cl + H, 8), 223 (252 - HCO, 12), 194 (223 - HCO, 30); accurate mass measurement for $C_{12}H_5O_2Cl_3$, calcd 285.9355, found 285.9361.

3-Methoxy-2,7,8-trichlorodibenzofuran (9b). Methylation of 27 mg of **9a** with CH_3I/K_2CO_3 gave 10 mg of **9b** as colorless crystals after recrystallization from methylene chloride/methanol: ^{1}H NMR ($CDCl_3$) δ 3.99 (3 H, s, OCH_3), 7.10 (1 H, s, 4-CH), 7.62 (1 H, s, 1-CH), 7.82 (1 H, s, 6-CH), 7.85 (1 H, s, 9-CH); MS, m/z (relative intensity) 300 (M^+, 100), 302 (M + 2, 97), 304 (M + 4, 31), 285 (M - CH_3, 75), 257 (M - $COCH_3$, 27), 194 (257 - Cl - CO, 20); accurate mass measurement for $C_{13}H_7O_2C_{13}$, calcd 299.9512, found 299.9513.

2,2'-Dimethoxy-3,4,6,4',5'-pentachlorobiphenyl (5a). 4,5-Dichloro-*o*-anisidine (1 g) and 3.4 g of 2,3,5-trichloroanisole were treated with isopentyl nitrite as described above. Flash chromatography using 1:99 (v/v) ethyl acetate/hexane as eluent gave biphenyl **5a** (178 mg) as an oil: ^{1}H NMR ($CDCl_3$) δ 3.55 (3 H, s, OCH_3), 3.78 (3 H, s, OCH_3), 7.08 (1 H, s, 3'-CH), 7.21 (1 H, s, 5-CH), 7.40 (1 H, s, 6'-CH); MS, m/z (relative intensity) 384 (M^+, 65), 386 (M + 2, 100), 388 (M + 4, 66), 390 (M + 6, 22), 334 (M - CH_3 - Cl, 29), 319 (334 - CH_3, 14), 314 (M - 2 Cl, 11), 291 (334 - $COCH_3$, 7); accurate mass measurement for $C_{14}H_9O_2Cl_5$, calcd 383.9045, found 383.9057.

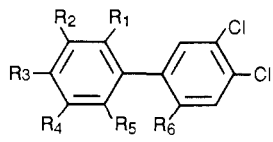
2,2'-Dihydroxy-3,4,6,4',5'-pentachlorobiphenyl (5b). Boron tribromide treatment of 178 mg of **5a** gave 90 mg of **5b** after flash chromatography using 1:9 (v/v) ethyl acetate/hexane as eluent: ^{1}H NMR ($CDCl_3$) δ 5.30 (1 H, br s, OH), 5.96 (1 H, br s, OH), 7.14 (1 H, s, 3'-CH), 7.24 (1 H, s, 5- or 6'-CH), 7.29 (1 H, s, 6'- or 5-CH); MS, m/z (relative intensity) 356 (M^+, 51), 358 (M + 2, 68), 360 (M + 4, 50), 362 (M + 6, 13), 321 (M - Cl, 13), 286 (321 - Cl, 48), 257 (286 - CO, 31), 223 (286 - Cl - CO, 22), 194 (223 - HCO, 29), 43 (CH_3CO, 100); accurate mass measurement for $C_{12}H_5O_2Cl_5$, calcd 355.8732, found 355.8734.

1-Hydroxy-2,3,7,8-tetrachlorodibenzofuran (10a). The dipotassium salt from 74 mg of **5b** was heated in 1 mL of DMSO at 160 °C for 4 h. Flash chromatography with 1:9 (v/v) ethyl acetate/hexane gave 35 mg of **10a**: ^{1}H NMR (acetone-d_6) δ 7.50 (1 H, s, 4-CH), 7.93 (1 H, s, 6-CH), 8.17 (1 H, s, 9-CH); MS, m/z (relative intensity) 320 (M^+, 79), 322 (M + 2, 100), 324 (M + 4, 51), 326 (M + 6, 10), 285 (M - Cl, 13), 257 (M - Cl - CO, 33), 228 (257 - HCO, 9), 221 (257 - HCl, 10), 194 (257 - CO - Cl, 17); accurate mass measurement for $C_{12}H_4Cl_4O_2$, calcd 319.8965, found 319.8963.

1-Methoxy-2,3,7,8-tetrachlorodibenzofuran (10b). Methylation of 7 mg of **10a** using CH_3I/K_2CO_3 gave **10b** as a solid that was recrystallized from CH_2Cl_2/methanol: ^{1}H NMR ($CDCl_3$) δ 4.10 (3 H, s, OCH_3), 7.52 (1 H, s, 4-CH), 7.68 (1 H, s, 6-CH), 8.11 (1 H, s, 9-CH); MS, m/z (relative intensity) 334 (M^+, 81), 336 (M + 2, 100), 338 (M + 4, 49), 340 (M + 6, 10), 319 (M - CH_3, 40), 291 (M - CH_3CO, 46), 228 (291 - CO - Cl, 24); accurate mass measurement for $C_{13}H_6O_2Cl_4$, calcd 333.9122, found 333.9119.

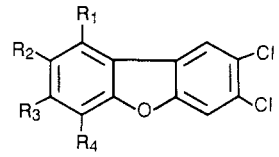
3-Hydroxy-2,4,7,8-tetrachlorodibenzofuran (11a) and 4-Hydroxy-2,3,7,8-tetrachlorodibenzofuran (12a). 4,5-Dichloro-*o*-anisidine (2.1 g) and 2,3,6-trichloroanisole (10 g) were treated with isopentyl nitrite at 120 °C. Flash chromatography using 1:19 (v/v) ethyl acetate/hexane as eluent gave 440 mg of a 1:1 mixture of dimethoxybiphenyls **6a** and **7a**, which we could not separate upon further chromatography: ^{1}H NMR ($CDCl_3$) δ 3.91 (3 H), 3.92 (3 H), 7.02 (1 H), 7.03 (1 H), 7.16 (1 H), 7.18 (1 H), 7.20 (1 H), 7.24 (1 H), all singlets. The mixture (400 mg) was demethylated with boron tribromide to give 275 mg of a 1:1 mixture of dihydroxybiphenyls **6b** and **7b**: ^{1}H NMR ($CDCl_3$) δ 7.07 (1 H), 7.10 (2 H), 7.22 (1 H), 7.23 (1 H), 7.27 (1 H), all singlets. Dihydroxybiphenyls **6b** and **7b** (275 mg) and 220 mg of potassium carbonate were heated in 3 mL of DMSO at 155–160 °C for 8 h. Flash chromatography using 1:1 (v/v) hexane/methylene chloride as eluent gave 34 mg of the major product, 16 mg of a minor product, and 70 mg of recovered dihydroxybiphenyls. The major product was assigned as 3-hydroxy-2,4,7,8-tetrachlorodibenzofuran (**11a**): ^{1}H NMR (acetone-d_6) δ 7.80 (1 H, s, 6-CH), 8.00 (1 H, s, 1-CH), 8.11 (1 H, s, 9-CH); MS (EI), (relative intensity) 320 (M^+, 75), 322 (M + 2, 100), 324 (M + 4, 49), 326 (M + 6, 10), 285 (M - Cl, 2), 257 (285 - CO, 3), 228 (257 - HCO, 17), 221 (257 - HCl, 11), 194 (257 - CO - Cl); accurate mass measurement for $C_{12}H_4O_2Cl_4$, calcd 319.8965, found 319.8963. The minor product was assigned as 4-hydroxy-2,3,7,8-tetrachlorodibenzofuran (**12a**): ^{1}H NMR (acetone-d_6) δ 7.82 (1 H, s, 1- or 6-CH), 7.86 (1 H, s, 6- or 1-CH), 8.28 (1 H, s, 9-CH); MS, m/e (relative intensity) 320 (M^+, 69), 322 (M + 2, 100), 324 (M + 4, 46), 326 (M + 6, 16), 285 (M - Cl, 1), 257 (285 - CO, 3), 228 (257 - HCO), 194 (257 - CO - Cl, 16); accurate mass measurement for $C_{12}H_4O_2Cl_4$, calcd 319.8965, found 319.8963. The recovered biphenyls were still a 1:1 mixture according to NMR.

3-Methoxy-2,4,7,8-tetrachlorodibenzofuran (11b). Methylation of **11a** with CH_3I/K_2CO_3 and recrystallization from methylene chloride gave **11b**: ^{1}H NMR ($CDCl_3$) δ 4.01 (3 H, s, OCH_3), 7.75 (1 H, s, 6-CH), 7.82 (1 H, s, 1-CH), 7.94 (1 H, s, 9-CH); MS, m/z (relative intensity) 334 (M^+, 76), 336 (M + 2,



1a	R_1=R_4=H,	R_2=R_3=Cl,	R_5=R_6=OCH_3
2a	R_1=R_4=H,	R_3=R_6=Cl,	R_2=R_5=OCH_3
2b	R_1=R_4=H,	R_3=R_6=Cl,	R_2=R_5=OH
3a	R_1=R_3=H,	R_4=R_6=Cl,	R_2=R_5=OCH_3
3b	R_1=R_3=H,	R_4=R_6=Cl,	R_2=R_5=OH
4a	R_1=R_4=H,	R_2=R_6=Cl,	R_3=R_5=OTBMS
4b	R_1=R_4=H,	R_2=R_6=Cl	R_3=R_5=OH
5a	R_2=H,	R_1=R_3=R_4=Cl,	R_5=R_6=OCH_3
5b	R_2=H,	R_1=R_3=R_4=Cl,	R_5=R_6=OH
6a	R_1=H,	R_2=R_3=R_5=Cl,	R_4=R_6=OCH_3
6b	R_1=H,	R_2=R_3=R_5=Cl,	R_4=R_6=OH
7a	R_1=H,	R_2=R_4=R_5=Cl,	R_3=R_6=OCH_3
7b	R_1=H,	R_2=R_4=R_5=Cl,	R_3=R_6=OH

Figure 1. Structures of biphenyls synthesized.



8a	R_1=R_4=H,	R_3=Cl,	R_2=OH
8b	R_1=R_4=H,	R_3=Cl,	R_2=OCH_3
9a	R_1=R_4=H,	R_2=Cl,	R_3=OH
9b	R_1=R_4=H,	R_2=Cl,	R_3=OCH_3
10a	R_4=H,	R_2=R_3=Cl,	R_1=OH
10b	R_4=H,	R_2=R_3=Cl,	R_1=OCH3
11a	R_1=H,	R_2=R_4=Cl,	R_3=OH
11b	R_1=H,	R_2=R_4=Cl,	R_3=OCH_3
12a	R_1=H,	R_2=R_3=Cl,	R_4=OH
12b	R_1=H,	R_2=R_3=Cl,	R_4=OCH_3

Figure 2. Structures of dibenzofurans synthesized.

100), 338 (M + 4, 45), 340 (M + 6, 9), 321 (M + 2 - CH_3, 13), 319 (M - CH_3, 10), 291 (319 - CO, 43), 228 (291 - CO - Cl, 33); accurate mass measurement for $C_{13}H_6O_2Cl_4$, calcd 333.9122, found 333.9122.

4-Methoxy-2,3,7,8-tetrachlorodibenzofuran (12b). Methylation of **12a** with CH_3I/K_2CO_3 and recrystallization from acetone/hexane gave **12b**: ^1H NMR ($CDCl_3$) δ 4.28 (3 H, s, OCH_3), 7.69 (1 H, s, 1- or 6-CH), 7.72 (1 H, s, 6- or 1-CH), 7.96 (1 H, s, 9-CH); MS, *m/z* (relative intensity) 334 (M^+, 57), 336 (M + 2, 68), 338 (M + 4, 30), 340 (M + 6, 7), 321 (M + 2 - CH_3, 100), 319 (M - CH_3, 70), 291 (M - OCH_3, 21), 228 (291 - CO - Cl, 34); accurate mass measurement for $C_{13}H_6O_2Cl_4$, calcd 333.9122, found 333.9122.

RESULTS AND DISCUSSION

The general procedure for synthesis of the hydroxypolychlorodibenzofurans involved an initial coupling between properly substituted anilines and anisoles (or in the case of **4a**, a silylated phenol) to give biphenyls **1a–7a** (Figure 1). The ether bonds were cleaved to produce dihydroxybiphenyls **2b–7b**, which were then cyclized with base in DMSO at ca. 160 °C to yield hydroxypolychlorodibenzofurans **8a–12a** (Figure 2). Since the compounds were ultimately to be used for GC–MS, methyl ethers **8b–12b** were subsequently prepared and characterized.

A symmetrical biphenyl, 2,2′-dimethoxy-4,4′,5,5′-tetrachlorobiphenyl (**1a**), was the first compound synthesized. This compound is not suitably substituted for cyclization; however, we were interested in determining whether the dimethoxytetrachlorobiphenyl reported by Poiger et al. (1984) might be this compound. Biphenyl **1a** could arise from metabolism of TCDF by oxygenation of the C4–C4a bond followed by furan ring-opening and reduction. The biphenyl was synthesized in a straightforward manner from 4,5-dichloro-*o*-anisidine and 3,4-dichloroanisole.

Two hydroxylated trichlorodibenzofurans (**8a** and **9a**) were synthesized. These compounds would result from replacement of chlorine by a hydroxyl group in the metabolism of TCDF. Compounds of this class accounted for about 10% of the biliary metabolites reported by Poiger et al. (1984). Coupling of 2,4,5-trichloroaniline and the dimethyl ether of chlorohydroquinone gave a 3:1 mixture of biphenyls **2a** and **3a**, the assignment of **3a** as the minor product was based on the observation of meta coupling in the NMR spectrum. Biphenyls **2a** and **3a** were not separable by flash chromatography; however, the dihydroxybiphenyls **2b** and **3b** were separated by chromatography and crystallization. Dihydroxybiphenyl **2b** was cyclized to dibenzofuran **8a**. Coupling of 2,4,5-trichloroaniline to 1,3-dimethoxy-4-chlorobenzene gave almost exclusively the biphenyl resulting from coupling at the carbon between the two methoxy groups (assignment based on the observation of ortho coupling in the NMR spectrum of the product). Substituting the bis(*tert*-butyldimethylsilyl) (TBMS) ether of 4-chlororesorcinol for the dimethyl ether effectively blocked reaction at C2 to give biphenyl **4a**, but the added steric hindrance also decreases reactivity at the desired site. Dihydroxybiphenyl **4b** was obtained by cleaving the silyl ethers with fluoride ion. Base-catalyzed cyclization of **4b** gave dibenzofuran **9a**.

Three hydroxylated tetrachlorodibenzofurans were prepared. These compounds would arise from hydroxylation without dechlorination during the metabolism of TCDF. Compounds of this class accounted for about 40% of the reported metabolites (Poiger et al., 1984). Coupling of 4,5-dichloro-*o*-anisidine and 2,3,5-trichloroanisole gave biphenyl **5a**. Methyl ether cleavage and base-catalyzed cyclization resulted in formation of dibenzofuran **10a**. Coupling of 4,5-dichloro-*o*-anisidine with 2,3,6-trichloroanisole resulted in a 1:1 mixture of biphenyls **6a** and **7a**. These compounds were not separable by flash chromatography; neither were the dihydroxybiphenyls **6b** and **7b**. The mixture of **6b** and **7b** was subjected to the cyclization reaction to give dibenzofurans **11a** and **12a** in a 2:1 mixture, which were easily separated. Apparently **12a** is not stable to the cyclization conditions. The ratio of **11a** to **12a** was 2:1, but the ratio of **6a** to **7a** in the recovered biphenyl from the cyclization reaction was the same as before the reaction, i.e., 1:1.

Assignment of structure **12a** as the minor product is based on comparison of the NMR spectra of the compounds to those of **8a–10a**. The NMR resonance for the methoxy group in **12b** at δ 4.28 is considerably downfield from the resonances for **8b–11b** (δ 3.99–4.10). This difference is attributed to deshielding of the methoxy group by the furan oxygen. The chemical shift of the 1-CH (δ 8.00) is also in agreement with the assignment of **11a** as the major product since it agrees well with the δ 8.16 chemical shift for compound **9a** which also has a hydroxy group meta to 1-CH. Difference NOE experiments were attempted to determine whether enhanced relaxation of the C6 proton by the methyl protons could be observed; however, no NOE was seen with either **11b** or **12b**.

Table I. Summary of NMR Spectra of Oxygenated Dibenzofurans

compd[a]	chemical shift (δ) 1-CH	4-CH	6-CH	9-CH	OCH_3
8a	7.688[b]	7.693[b]	7.84	8.22	
9a	8.18	7.27	7.84	8.25	
10a		7.50	7.93	8.17	
11a	8.00		7.82	8.11	
12a	7.82[b]		7.86[b]	8.28	
8b	7.36	7.61[b]	7.65[b]	7.97	4.02
9b	7.62	7.10	7.82	7.85	3.99
10b		7.52	7.68	8.11	4.10
11b	7.82		7.75	7.94	4.01
12b	7.69[b]		7.72[b]	7.96	4.28

[a] NMR spectra of **8a–12a** were recorded in acetone-d_6; spectra of **8b–12b** were recorded in $CDCl_3$. [b] Assignments may be reversed.

While a specific synthesis of **12a** would have been preferred, and several were attempted, **11a** is also of interest as a possible metabolite of TCDF. It could result from arene oxide formation at the C3–C4 bond followed by NIH shift of the chlorine. An NIH shift of a chlorine during the metabolism of TCDD in the dog has been reported (Poiger et al., 1982). Oxidation of the C3–C4 or C4–C4a bond is predicted to be a preferred metabolic pathway since substitution of a chlorine at C4 (as in 2,3,4,7,8-pentachlorodibenzofuran) greatly decreases rates of metabolism and elimination compared to the 1,2,3,7,8-pentachloro isomer or TCDF (Brewster and Birnbaum, 1987, 1988). This observation is rationalized by the supposition that one of the preferred sites of metabolism is blocked when C4 is chlorinated.

NMR chemical shift assignments of dibenzofurans **8–12** are given in Table I. The assignments are based on comparison of spectra from the various isomers. Since all the signals are singlets, there is no coupling information to aid in the assignments. While the assignments are internally consistent, it is not clear that the NMR spectra of an individual isolated metabolite could be assigned without the assistance of the data in Table I. Spectra for **8a–12a** were all determined in acetone-d_6 because of solubility; those of **8b–12b** were determined in deuteriochloroform. There is a considerable chemical shift difference between the two solvents, for example in deuteriochloroform the NMR signals for **11a** (δ 7.59, 7.73, 7.96) and **12a** (δ 7.71, 7.77, 7.89) are at about 0.2 ppm higher field than the values given in Table I. Similar solvent shifts are reported for TCDF in the literature; for example, in $CDCl_3$ TCDF has resonances at ca. δ 7.7 and 7.9 (Gray et al., 1976), and in THF-d_8, they are at δ 7.9 and 8.2 (Norstrom et al., 1979).

Mass spectra for the dibenzofurans are given in the Experimental Section. Loss of chlorines and carbon monoxide account for most of the fragmentation seen in the hydroxy compounds; loss of chlorines, methyl, and CH_3CO account for most of the fragmentation seen in the methyl ethers. Relative magnitudes change from isomer to isomer, but there is no fragment unique to any one isomer. In their analysis of the mass fragmentation patterns of mono- and dichloromethoxydibenzofurans, Tulp and Hutzinger (1978) also conclude that substitution patterns of these compounds cannot be deduced from mass spectra.

It is possible to make comparisons between the data reported by Poiger et al. (1984) to that obtained from the synthetic compounds reported here. The supposed ring-opened product, a dimethoxytetrachlorobiphenyl, reported by Poiger et al. (1984) has the same major fragments as we find with compound **1a**; however, since relative intensity data were not given for the bile metabolite, other isomers cannot be ruled out. The mass spectrum of one of the major trichloromethoxydibenzofurans was illustrated in Poiger et al. (1984); the relative intensities of the parent ion and the three major fragments are intermediate between those we find for **8b** and **9b** so no assignments can be made. One of the major metabolites isolated by Poiger et al. (1984), accounting for about 40% of the total metabolites isolated from bile, is a tetrachloromethoxydibenzofuran. The mass spectrum of this compound compares very closely that of **12b**. Data from Poiger et al. (1984): *m/e* 334, 62%; 321, 100; 291, 15; 228, 8. Data from this report: *m/e* 334, 57%; 321, 100; 291, 21, 228, 35). Only **12b** of the three compounds, **10b**, **11b**, and **12b**, has the M – CH_3 (*m/e* 321) fragment as the base peak. The difference in intensities of the *m/e* 228 fragment makes the assignment somewhat equivocal at this point, and verification must await GC–MS analysis of bile from TCDF-treated rats.

In summary we have synthesized and characterized six possible TCDF metabolites. One of these, **12a** (or more probably its glucuronide or sulfate conjugate), appears to be one of the major biliary metabolites of TCDF. Verification of the presence of **12a** as well as the other possible TCDF metabolites in bile from rats treated with TCDF is in progress.

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Hydroxyatrazine and Atrazine Determination in Soil and Water by Enzyme-Linked Immunosorbent Assay Using Specific Monoclonal Antibodies

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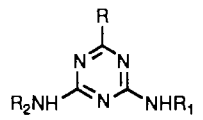
Monoclonal antibodies (MAbs) were obtained against the herbicide atrazine and its metabolite hydroxyatrazine by immunizing mice with protein conjugates of both compounds. By competitive ELISA, we observed that the anti-hydroxyatrazine MAbs cross-reacted predominantly with hydroxypropazine. The anti-atrazine MAbs cross-reacted with propazine and, to a much lower extent, with a few other *s*-triazines and hydroxy-*s*-triazines. Atrazine could be detected in water samples with a sensitivity of 0.05 ng/mL. Average recoveries from soil samples fortified with atrazine or hydroxyatrazine, measured by ELISA, were comparable to those measured by GLC or HPLC. Soil samples of unknown atrazine and hydroxyatrazine content were analyzed by GLC, HPLC, and ELISA. Interference during UV monitoring of hydroxyatrazine by HPLC analysis was observed. Despite limited specificity due to cross-reacting substances, the results demonstrate that the ELISA immunoassay represents a valuable method for detecting trace amounts of atrazine and hydroxyatrazine in the soil.

Derivatives of *s*-triazines (atrazine, propazine, simazine) have been extensively used as herbicides during the past 25 years. Atrazine undergoes degradation in the soil, an important pathway being its conversion to the nonphytotoxic hydroxyatrazine (Jordan et al., 1970). The growing number of soil samples to be analyzed has encouraged the development of simple and inexpensive assays, able to monitor not only the concentration of the active substances but also major metabolites remaining in the soil. The determination of hydroxyatrazine was done previously by TLC (Koudela, 1970; Cee and Gasparic, 1971) or UV spectrophotometry (Hurter, 1966; Sirons et al., 1973) and is currently done by HPLC (Ramsteiner and Hörmann, 1979). HPLC determination of hydroxyatrazine in the soil requires a cumbersome cleanup procedure. The latter could be avoided by using an immunoassay as an alternative approach to residue analysis.



atrazine: R = Cl, $R_1 = CH_2CH_3$, $R_2 = CH(CH_3)_2$
hydroxyatrazine: R = OH, $R_1 = CH_2CH_3$, $R_2 = CH(CH_3)_2$

Such immunochemical determination based on competitive binding of residues to an antibody has been recently developed for the determination of several herbicides such as 2,4-dichlorophenoxyacetic acid (Fleeker, 1987) and chlorosulfuron (Kelley et al., 1985) or pesticides such as diflubenzuron (Wie and Hammock, 1982), metalaxyl (Newsome, 1985), and parathion (Ercegovich et al., 1981). [For a review, see Hammock and Mumma (1980).] For the determination of *s*-triazines in water, a kit is available commercially (ImmunoSystems Inc., Biddeford, ME) and several immunoassays for atrazine have been described (Dunbar et al., 1985; Huber, 1985; Bushway et al., 1988).

To develop our present immunoassay for atrazine and hydroxyatrazine, we took advantage of hybridoma technology to obtain MAbs, which allowed better definition of the specificity of the assay, as well as an unlimited supply of reagents.

EXPERIMENTAL SECTION

Materials. Atrazine and its analogues were synthesized in our laboratories. Bovine serum albumin (BSA) was purchased from Fluka (Buchs, Switzerland) and keyhole limpet hemocyanin (KLH) from Calbiochem (Lucerne, Switzerland). Freund's adjuvant was obtained from Difco (Detroit, MI), 2,6,10,14-tetramethylpentadecane (pristan oil) from Aldrich Chemical Co. (Steinheim, FRG), poly(ethylene glycol) 4000 (PEG) from Merck (Zurich, Switzerland), and Tween 20 from Serva (Heidelberg, FRG). All other reagents were of the highest purity grade. RPMI 1640 medium from Seromed (Biochrom, Berlin, FRG) was supplemented with 15% fetal calf serum (Gibco), 0.01% gentamy-

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