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Synthesis and Characterization of Tissue-Retainable Methylsulfonyl Polychlorinated Biphenyl Isomers

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Eighty-six positional isomers of methylsulfonyl polychlorinated biphenyls (MSF-PCBs) have been synthesized by three synthetic routes: (1) the diazo coupling reaction of 3-(methylsulfonyl)chloroaniline with chlorobenzene; (2) nucleophilic substitution of PCB with methanethiolate and successive oxidation of the corresponding methyl sulfide; (3) the diazo coupling reaction of chloroaniline with chlorothioanisole and successive oxidation of the methyl sulfide. Pure isomers were characterized by their proton magnetic resonance and mass spectra and used to unambiguously identify the MSF metabolites retained in human tissues by using high-resolution capillary gas chromatography (GC). The GC analysis showed that 40 MSF derivatives were positively identified in the tissue of a patient with Yusho on the basis of comparisons of their GC retention data with those of the standard compounds.

Methylsulfonyl (MSF) derivatives of polychlorinated biphenyls (PCBs) have evoked great interest since they were found as metabolic products of PCBs in the excreta of experimental animals fed PCBs (Mio et al., 1976; Mizutani et al., 1978; Bergman et al., 1979) and wild animals (Jensen and Jansson, 1976) and human milk as well as adipose tissue (Yoshida and Nakamura, 1978; 1979). In our previous study (Haraguchi et al., 1984), several MSF-PCBs were also found to be accumulated at relatively high concentrations in the tissues of patients with Yusho, a PCB poisoning that occurred in Japan. The qualitative and quantitative analyses of these metabolites by gas chromatography (GC) are complicated by the complex composition of these MSF-PCBs and the unknown identities of the many individual components. Therefore, information concerning the precise composition of MSF-PCB mixtures is required for an understanding of the toxicity of the residual MSF-PCBs. In order to clarify the structures of these metabolites in human tissues, we synthesized various MSF-PCB congeners consisting of two to seven chlorine atoms and one or two hydrogen atoms at the lateral positions of the MSF group, because these metabolites were expected to be formed via arene oxide intermediates from PCBs that have at least two adjacent hydrogens in the phenyl ring of PCB (Preston et al., 1984). In this paper, we report the syntheses of 86 MSF-PCB isomers by three methods, their characterization by mass spectroscopy (MS) and proton magnetic resonance (^{1}H NMR) spectra, and the GC profiles of tissue-retainable MSF-PCB metabolites on three capillary columns.

EXPERIMENTAL SECTION

Chemicals. All the synthetic precursors used for this work were commercially available. 2,3-, 2,4-, 2,5-, 3,4-, and 3,5-dichloroaniline, 2,4,5-trichlorothioanisole, and methyl mercaptan sodium salt (ca. 15% in water) were purchased from Tokyo Chemical Industry Co. Ltd., Osaka, Japan. *o*-, *m*-, and *p*-dichlorobenzene, 1,2,3- and 1,2,4-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, and isoamyl nitrite were purchased from Wako Pure Chemical Industries Co. Ltd., Osaka, Japan. 2,3,4- and 2,4,5-trichloroaniline, 2,3,4,5- and 2,3,5,6-tetrachloroaniline and 1,3,5-trichlorobenzene were from Aldrich Chemical Co., Milwaukee, WI. Methanethiol (>98%) was obtained from Eastman Kodak Co., Rochester, NY. All the other chemicals employed were of reagent grade unless otherwise mentioned in the text.

Synthesis. The 3-MSF-PCB congeners were prepared by the Cadogan coupling (Cadogan, 1962) from chlorinated 3-(methylsulfonyl)aniline and chlorinated benzene with isoamyl nitrite, as indicated in the synthetic route of 3-MSF-4,5,3′,4′-tetra-CB (Scheme I). Chlorinated 3-(methylsulfonyl)aniline was prepared by a modified procedure of the method of Mizutani et al. (1978). Dichloroaniline (4.0 g) dissolved in H_2SO_4 (20 mL) was diazotized with $NaNO_2$ (5.0 g) at 4 °C. After neutralization by sodium acetate, a mixture of $NaSCH_3$ (20 mL), copper powder (3.5 g), NaOH (2.8 g), and water (50 mL) was added to the solutions with stirring over a period of 1 h. The hexane solution of the collected solid was filtered off, washed with water, and dried over Na_2SO_4 to yield 2,3-dichlorothioanisole. The product was oxidized with an excess of hydrogen peroxide in acetic acid to give the corresponding (methylsulfonyl)chlorobenzene, which was nitrated with KNO_3 (3.0 g) in concentrated H_2SO_4 (10 mL) at 80 °C for 2 h to yield 3-(methylsulfonyl)-4,5-dichloronitrobenzene. The crude nitro product was reduced by iron powder (2.0 g) in 70% acetic acid at 80 °C for 4 h to give the corresponding 3-(methylsulfonyl)chloroaniline. The substituted aniline (0.4 g) was subsequently converted to 3-MSF-PCB by Cadogan coupling with an excess of *o*-dichlorobenzene

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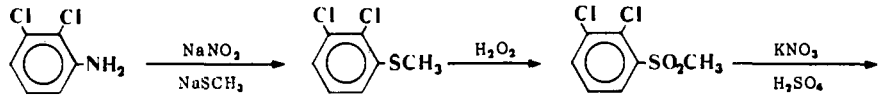
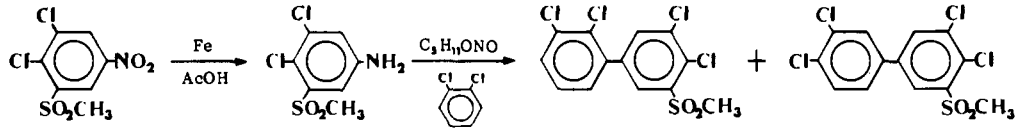
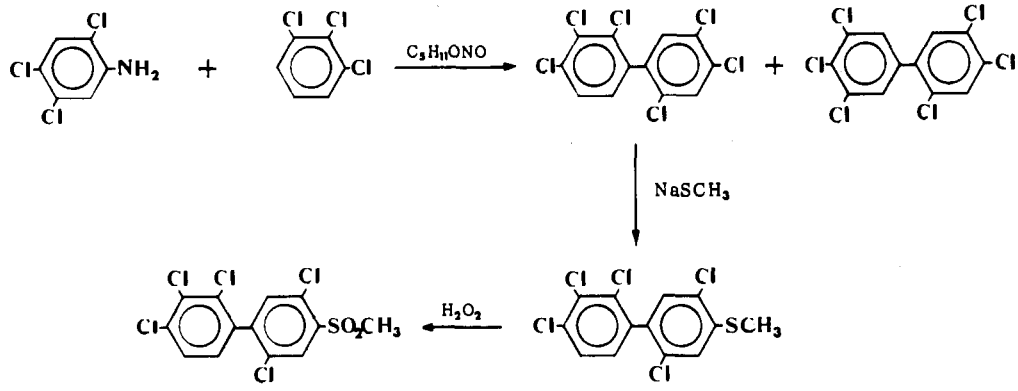
Scheme I

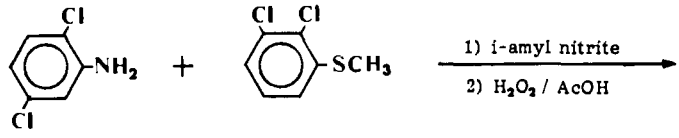


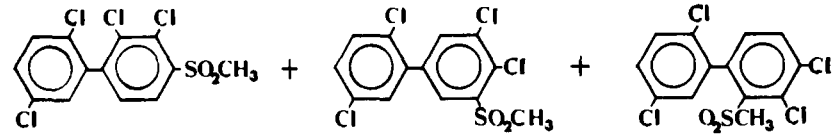


Scheme II



Scheme III





(5.0 g) in the presence of isoamyl nitrite (0.4 g). After removal of the unreacted chlorobenzene by distillation, the crude product was absorbed onto 5–10 g of neutral alumina (no. 1097, activity grade II–III, Merck, Darmstadt, Germany), put in a 10-cm length × 2-cm i.d. glass column, and eluted with diethyl ether (100 mL). The eluate contained two expected isomeric products that were separated by preparative liquid chromatography (LC) with a reversed-phase LiChroprep RP-8 column (31-cm length × 25-mm i.d., 40-63 μm, Merck, Darmstadt). The isomers were eluted with acetonitrile–water (7:3) at a flow rate of 5.6 mL/min on a KP-6H micropump (Kusano Kagakukikai Works Co., Ltd., Tokyo, Japan) and monitored at a flow cell by 254-nm ultraviolet light. The expected fraction was collected and recrystallized from methanol.

4-MSF-PCBs with at least two chlorine atoms at the 2- and 5-positions in the ring with an MSF group were prepared according to the method of Bergman and Wachtmeister (1978), showing the synthetic route of 4-MSF-2,5,2′,3′,4′-penta-CB in Scheme II for an example. 4-MSF-PCBs without a 2- or 5-chloro substituent were prepared by Cadogan coupling of the appropriate chloroaniline with chlorothioanisole in the presence of isoamyl nitrite as described above, showing the synthetic route of 4-MSF-2,3,2′,5′-tetra-CB in Scheme III. After removal of excess chlorothioanisole in vacuo, the residue was absorbed onto neutral alumina, put in a 10-cm length × 2-cm i.d. glass column, and eluted with 100 mL of hexane. The eluate was concentrated, and the residue was oxidized by excess hydrogen peroxide in acetic acid at 80 °C for 2 h to give the corresponding MSF-PCBs. The isomerized products that contained 2- and 3-MSF-PCB isomers other than the desired 4-MSF isomer were further fractionated by the preparative LC. Some of 2-MSF-PCB congeners were also obtained by the LC separation of the isomeric mixture obtained by this route.

3-MSF-4-Me-5,2′,3′,4′,5′-penta-CB was synthesized from 3-(methylsulfonyl)-4-methyl-5-chloroaniline and 1,2,3,4-tetrachlorobenzene as in Scheme I. The purities of all the separated isomers were checked by GC, and their structures were confirmed by ^{1}H NMR and MS.

Instrumental Analyses. Mass spectra were measured by a JEOL JMS-D100 double-focusing mass spectrometer with computer system (Tokyo, Japan). Samples were run at 75 eV by direct probe in the electron-impact mode.

The ^{1}H NMR spectra were measured at 90 MHz by a JEOL FX 90Q Fourier transform spectrometer (Tokyo, Japan) and were expressed in parts-per-million (ppm) with $(CH_3)_4Si$ as an internal standard in $CDCl_3$. The purities of MSF-PCB isomers were determined by a GC-6AM

Table I. Relative Retention Times (RRTs) and Response Factors (RRFs) for MSF-PCB Isomers Identified in Human Tissues

no.	compound[a]	RRTs[b] A	RRTs[b] B	RRTs[b] C	RRFs[b] mean[c]	RRFs[b] RSD,[d] %
1	2-MSF-4,2′,5′-tri-CB	0.430	0.390	0.374	0.99	6.1
2	3-MSF-2,5,2′-tri-CB	0.455	0.418	0.404	0.75	2.7
3	4-MSF-2,5,2′-tri-CB	0.468	0.434	0.424	1.20	5.8
4	3-MSF-6,2′,5′-tri-CB	0.475	0.422	0.418	0.79	2.5
5	4-MSF-2,5,3′-tri-CB	0.532	0.487	0.473	0.71	7.0
6	4-MSF-2,5,4′-tri-CB	0.549	0.504	0.497	0.75	6.6
7[e]	4-MSF-3,2′,5′-tri-CB	0.550	0.505	0.509	0.71	5.6
8	3-MSF-2,5,2′,5′-tetra-CB	0.539	0.508	0.484	1.23	13.0
9	3-MSF-4,6,2′,5′-tetra-CB	0.545	0.516	0.493	1.27	7.1
10	3-MSF-4,6,2′,4′-tetra-CB	0.551	0.527	0.508	1.05	7.6
11[e]	3-MSF-4,5,6,2′-tetra-CB	0.552	0.541	0.525	0.98	7.1
12	4-MSF-2,5,2′,5′-tetra-CB	0.556	0.531	0.513	1.11	4.5
13	4-MSF-2,5,2′,4′-tetra-CB	0.567	0.541	0.527	0.73	16.4
14	3-MSF-2,5,2′,3′-tetra-CB	0.569	0.543	0.530	0.95	12.6
15	3-MSF-4,6,2′,3′-tetra-CB	0.575	0.550	0.544	0.75	11.1
16	4-MSF-2,3,6,3′-tetra-CB	0.578	0.557	0.541	1.07	6.5
17	4-MSF-2,5,2′,3′-tetra-CB	0.592	0.568	0.568	1.19	11.7
18	2-MSF-4,2′,4′,5′-tetra-CB	0.596	0.558	0.560	0.85	8.2
19	3-MSF-6,2′,3′,4′-tetra-CB	0.623	0.578	0.593	1.12	9.8
20[e]	4-MSF-2,3,2′,4′-tetra-CB	0.624	0.608	0.643	1.17	4.3
21	2-MSF-4,2′,3′,4′-tetra-CB	0.643	0.606	0.640	0.75	8.0
22	3-MSF-4,5,2′,3′-tetra-CB	0.670	0.643	0.657	0.75	5.3
23	4-MSF-2,5,3′,4′-tetra-CB	0.702	0.658	0.663	0.93	5.4
24	3-MSF-4,5,3′,4′-tetra-CB	0.804	0.759	0.780	0.59	5.1
25	3-MSF-2,5,2′,4′,5′-penta-CB	0.668	0.657	0.662	0.68	11.7
26	3-MSF-4,5,6,2′,5′-penta-CB	0.671	0.674	0.651	1.27	4.7
27	4-MSF-2,5,2′,4′,5′-penta-CB	0.698	0.689	0.669	0.63	12.7
28	3-MSF-4,5,2′,3′,6′-penta-CB	0.702	0.711	0.703	0.87	5.7
29	3-MSF-2,5,2′,3′,4′-penta-CB	0.729	0.721	0.720	1.15	10.4
30	4-MSF-2,5,2′,3′,4′-penta-CB	0.762	0.757	0.784	0.92	5.4
31	4-MSF-2,3,2′,3′,4′-penta-CB	0.815	0.819	0.918	1.19	4.2
32	3-MSF-4,5,2′,3′,4′-penta-CB	0.903	0.882	0.945	0.78	5.1
33	3-MSF-2,5,2′,3′,5′,6′-hexa-CB	0.701	0.743	0.689	1.12	11.6
34	4-MSF-2,5,2′,3′,5′,6′-hexa-CB	0.719	0.757	0.714	1.38	14.5
35	3-MSF-4,5,6,2′,3′,6′-hexa-CB	0.733	0.798	0.783	1.59	8.2
36	4-MSF-2,3,6,2′,3′,4′-hexa-CB	0.814	0.871	0.912	1.39	15.1
37	3-MSF-4,5,6,2′,4′,5′-hexa-CB	0.868	0.897	0.882	0.99	33.3
38	3-MSF-2,5,2′,3′,4′,5′-hexa-CB	0.902	0.910	0.897	0.71	18.3
39	4-MSF-2,5,2′,3′,4′,5′-hexa-CB	0.951	0.962	0.986	0.96	18.7
40	3-MSF-4,5,6,2′,3′,4′-hexa-CB	0.960	0.995	1.065	0.79	34.2

[a] Tentatively identified in human tissues by GC–ECD. [b] Relative to 3-MSF-4-Me-5,2′,3′,4′,5′-tetra-CB (retention time and response factor 1.000): A = Dexsil-410; B = OV-101; C = SP-2250. [c] Mean values measured on three columns. [d] RSD = relative standard deviation. [e] Coeluted with other isomers on any of three columns.

Shimadzu gas chromatograph (Kyoto, Japan) fitted with a 2-m length × 3-mm i.d. glass column packed with 1.5% OV-17 on Chromosorb W AW DMCS (100–120 mesh) and were generally greater than 99%. The temperatures of the inlet, column, and detector were maintained at 280, 250, and 280 °C, respectively. The carrier gas used was pure nitrogen at a flow rate of 40 mL/min.

Capillary GC separation for MSF-PCB isomers was performed on a Shimadzu GC-7AG gas chromatograph equipped with a ^{63}Ni electron-capture detector (ECD) and a Chromatopac C-R3A data system. Three fused silica capillary columns were employed, and their temperature programs were as follows: (1) a 50-m length × 0.25-mm i.d. OV-101 column (Shimadzu Co.), 180 °C (isothermal for 2 min) to 270 °C at 6 °C/min; (2) a 30-m length × 0.2-mm i.d. Dexsil-410 column (Shimadzu), 200 °C (isothermal for 2 min) to 265 °C at 4 °C/min; (3) a 30-m length × 0.25-mm i.d. SP-2250 column (Supelco Inc., Bellefonte, PA), 190 °C (isothermal for 2 min) to 260 °C at 4 °C/min. The temperatures of the inlet and detector were maintained at 290 °C. Pure nitrogen was used as carrier and makeup gas at flow rates of 0.7 and 40 mL/min, respectively. The carrier gas was split at a ratio of 1:10 at the injection port. A sample solution in hexane was introduced into the gas chromatograph via a moving precolumn SPL-7 solventless injector (Shimadzu). GC retention times and response factors of MSF-PCB isomers were expressed relative to those of 3-MSF-4-Me-5,2′,3′,4′,5′-penta-CB by using integrated peak areas.

RESULTS AND DISCUSSION

Methylsulfonyl metabolites of PCBs are presumably formed from the PCBs that possess two adjacent unsubstituted carbon atoms, via arene oxide intermediate (Bakke et al, 1982) and glutathione conjugate (Preston et al., 1984). The structures of MSF-PCBs that were present in the human tissue, therefore, would be expected as follows: (1) the 3- or 4-MSF substituents in the 2,5-dichlorophenyl nucleus; (2) the 2- or 3-MSF substituents in the 4-chlorophenyl nucleus; (3) the 3- or 4-MSF substituents nonchlorinated in the lateral position of PCB. On the basis of this assumption, pure MSF-PCB isomers were prepared by three synthetic routes: (a) the Cadogan coupling reaction of 3-(methylsulfonyl)chloroaniline with chlorobenzene (Scheme I); (b) the nucleophilic aromatic substitution of the 4-position in 2,4,5-trichloro-substituted biphenyl with methanethiolate and subsequent oxidation of the methyl sulfide (Scheme II); (c) the Cadogan coupling reaction of chloroaniline with chlorothioanisole and subsequent oxidation of the methyl sulfide (Scheme III). The routes outlined in Schemes I and II, in many cases, made it possible to prepare a single MSF-PCB isomer. In Scheme II, nucleophilic substitution in 2,4,5-trichloro-substituted biphenyl occurs at the 4-position involving the

p-quinonoid intermediate (Binns and Suschitzky, 1971) to give the corresponding 4-methylthio derivatives. Unfortunately, analogous reaction to obtain the corresponding isomers nonchlorinated at the 2- or 5-positions were unsuccessful. These isomers had to be prepared from the mixtures that were generated by the coupling reaction as in Scheme III. The LC separation of the resulting compounds, however, proved to be difficult since the mixtures often contained some 2-, 3-, and 4-MSF derivatives that behaved similarly on the chromatographic column.

Structures of the purified 86 isomers consist of 2 dichlorinated, 10 trichlorinated, 40 tetrachlorinated, 20 pentachlorinated, 13 hexachlorinated and 1 heptachlorinated (methylsulfonyl)biphenyls, which are characterized by melting points, molecular ions of MS, and ^{1}H NMR spectra.

Electron-impact MS data for MSF-PCBs were characterized as studied earlier (Mizutani et al., 1978; Bergman et al., 1980). In general, most of the isomers gave a relatively intense molecular ion (M^+) and a fragment ion peak (M – 114) due to loss of SO_2CH_3Cl. As noted previously (Bergman et al., 1980), 2-MSF-PCBs containing an *o*-chlorine in the second ring gave a weak molecular ion and characteristic fragment ions of M – 35 and M – 98 due to loss of Cl and $SOCH_3Cl$, respectively. In the case of a non *o*-chloro substituent, however, their mass spectra showed intense M^+ but no $[M - Cl]^+$ ions. On the other hand, 3- and 4-MSF-PCBs gave rise to M – 79 due to loss of SO_2CH_3 and M – 91 due to successive loss of $SOCH_3$ and CO after rearrangement to the methyl sulfinate ester, which were rather weak or absent for 2-MSF isomers. 4-MSF-PCBs showed a prominant $[M - CH_3SO]^+$, which were explained by the formation of a quinoide-type structure as observed for methoxy PCBs (Jansson and Sundstrom, 1974; Tulp et al., 1977), whereas the 3-MSF-PCB isomers showed a more abundant $[M - CH_2SO]^+$ ion rather than $[M - CH_3SO]^+$ ion in many cases. It, however, is difficult to distinguish between 3- and 4-MSF-PCBs on the basis of their fragmentation patterns.

The ^{1}H NMR spectral data of MSF-PCBs were in accordance with the proposed structure. The chemical shifts for the aromatic protons on the MSF-substituted ring were more downfield than those on the second ring, due to the electron-withdrawing effect of methylsulfonyl. The shift of the aromatic proton adjacent to the MSF group was influenced by the chloro substitution. In the case of 3- and 4-MSF-PCBs, when one of the lateral positions of the MSF group was chlorinated, chemical shifts for the proton adjacent to MSF group were more downfield, as much as 0.06–0.41 ppm for 3-MSF isomers and 0.08–0.44 ppm for 4-MSF isomers, compared to those of non-chlorine substituents. Similarly, the shift for the methyl protons of 3- and 4-MSF isomers also showed downfield displacements of 0.18–0.27 and 0.14–0.21 ppm, respectively. In the case of 2-MSF isomers, the signal of 3-aromatic and methyl protons showed at 8.10–8.30 and 2.84–2.89 ppm, respectively, which were distinguished from those of 3- or 4-MSF-PCBs.

Since a large number of MSF-PCBs have been detected in the lung, liver, and adipose tissue of Yusho patients (Haraguchi et al., 1986), these standards were used for isomer-specific determination of MSF-PCBs in human tissues by high-resolution GC using the three capillary columns coated with the OV-101, Dexsil-410, and SP-2250. GC-ECD analyses showed that several GC peaks observed in human tissue extracts were in accordance with those of the 40 authentic isomers on the retention times. The components are listed in Table I. The GC retention times were calculated relative to 3-MSF-4-Me-5,2′,3′,4′,5′-penta-CB, which was assigned a value of 1.000. Due to the differences in the respective liquid phases, the coeluting isomers are resolvable on the alternate column, except for three isomers (7, 11, 20). These columns, when used together, serve as useful tools for MSF-PCB analysis. The effects of the structure on the capillary GC retention times were relatively consistent throughout the MSF-PCB congeners in the elution order of 2-, 3-, and 4-MSF isomers without change in the position and total number of chlorine atoms, but the effects of the relative degrees of phenyl ring chlorination were not readily assessed. The relative response factors (RRFs) for the MSF-PCBs are also shown in Table I. The extreme RRF values determined with ECD varied over 2.7-fold, but there were no apparent structural features that correlated with relative electron-capture response sensitivities. The relative standard deviation for the RRFs obtained from the three columns ranged from 2.5 to 34%.

Current research on the unambiguous syntheses of other potential MSF-PCB isomers is in progress, and it is anticipated that these components will facilitate identification of MSF-PCBs in human tissues.

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Registry No. 1, 66640-69-5; **2**, 104085-98-5; **3**, 104085-99-6; **4**, 104086-00-2; **5**, 66640-53-7; **6**, 66640-67-3; **7**, 106352-68-5; **8**, 60640-54-2; **9**, 104086-01-3; **10**, 66640-55-9; **11**, 106352-69-6; **12**, 60640-55-3; **13**, 69797-52-0; **14**, 104086-02-4; **15**, 104086-03-5; **16**, 104086-04-6; **17**, 104086-05-7; **18**, 104086-06-8; **19**, 104086-07-9; **20**, 106352-70-9; **21**, 104086-08-0; **22**, 104085-96-3; **23**, 69797-51-9; **24**, 104104-34-9; **25**, 66640-60-6; **26**, 104086-09-1; **27**, 66640-61-7; **28**, 104086-10-4; **29**, 66640-58-2; **30**, 66640-59-3; **31**, 104086-11-5; **32**, 104086-12-6; **33**, 104086-13-7; **34**, 104086-14-8; **35**, 104086-15-9; **36**, 104086-16-0; **37**, 104086-17-1; **38**, 104086-18-2; **39**, 104086-19-3; **40**, 104086-20-6; 3-MSF-4-Me-5,2′,3′,4′,5′-penta-CB, 106419-00-5; 4-MSF-2,3,2′,5′-tetra-CB, 106418-96-6; 3-MSF-4,5,2′,5′-tetra-CB, 106418-97-7; 2-MSF-3,4,2′,5′-tetra-CB, 106418-98-8; 2,4,5,2′,3′,4′-hexa-CB, 35065-28-2; 2,4,5,3′,4′,5′-hexa-CB, 52663-72-6; 4-(methylthio)-2,5,2′,3′,4′-penta-CB, 66640-49-1; *o*-$Cl_2C_6H_4$, 95-50-1; 2,6-dichloroaniline, 95-82-9; 2,3-dichloroaniline, 608-27-5; 2,3-dichlorothioanisole, 17733-25-4; (methylsulfonyl)-2,3-dichlorobenzene, 106418-92-2; 3-(methylsulfonyl)-4,5-dichloronitrobenzene, 106418-93-3; 3-(methylsulfonyl)-4,5-dichloroaniline, 106418-94-4; 2,3-dichlorobenzenediazonium sulfate, 106418-95-5; 2,4,5-trichloroaniline, 636-30-6; 1,2,3-trichlorobenzene, 87-61-6; 3-(methylsulfonyl)-4-methyl-5-chloroaniline, 106418-99-9; 1,2,3,4-tetrachlorobenzene, 634-66-2.

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Transmission of [^{14}C]Deoxynivalenol to Eggs following Oral Administration to Laying Hens[1]

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Following a single oral dose of [^{14}C]deoxynivalenol (2.2 mg of DON, 2.4 μCi/bird) low levels of residues were transmitted to eggs. Maximum radioactivity, which occurred in the first eggs laid after dosing (within 24 h), amounted to 1.9 μg DON-equivalents/60-g egg (0.087% of dose); levels dropped rapidly in ensuing eggs. During daily consumption of DON, administered in spiked feed over a 12-day period (2.2 mg of DON/bird per day for 6 days followed by 2.2 mg of [^{14}C]DON, 1.5 μCi/bird per day for 6 days), radioactivity levels increased with each subsequent egg laid up until the last exposure to the toxin; maximum levels accounted for 4.2 μg DON-equivalents/60-g egg. Residues quickly declined once the birds were switched to clean feed. Results indicate that although residues appear to accumulate in eggs, levels do not persist once the contaminated source is withdrawn. Preliminary analysis of egg material showed only about 10% of radioactivity present could be identified as the parent toxin, DON.

Deoxynivalenol (DON, vomitoxin), a *Fusarium*-produced mycotoxin, has been the subject of intensive testing since its identification in eastern Canada and the midwestern United States as an important contaminant of various field crops infected with *Fusarium* fungi (Trenholm et al., 1983; Cotê et al., 1984). Since exposure to DON has been associated with a number of toxic effects in farm and laboratory animals (i.e., feed refusal, emesis, anorexia), this has resulted in concern about potentially toxic residues in food products intended for humans, not only contaminated grains but also tainted animal products (meat, eggs, milk) obtained from livestock or poultry previously exposed to DON-contaminated feeds.

Several investigators have studied the transmission of DON from contaminated feed to tissues of livestock and poultry, the toxin being administered either as the pure compound or as naturally or intentionally infected corn or wheat. At detection limits of 10 ng of DON/g of tissue, neither El-Banna et al. (1983) nor Kubena et al. (1985) were able to detect DON in either eggs and/or tissues of poultry fed, respectively, a 4–5 mg of DON/kg diet for periods of between 28 and 190 days or a 9–18 mg of DON/kg diet for up to 35 days. Prelusky et al. (1984) found only trace levels of DON in milk (<4 ng/mL) following a single oral administration of the toxin (2 mg/kg of body weight) to lactating dairy cows. However, in these previous studies the milk and poultry products were analyzed for the presence of DON, but not the presence of possible metabolites. Recent studies though have demonstrated extensive metabolism of DON can indeed occur; between 50 and 75% of the administered dose given to swine (Coppock et al., 1985) or sheep (Prelusky et al., 1986a) appears to be metabolized prior to its elimination. Therefore, it is important from a human health viewpoint to determine whether or not DON, either as the unchanged toxin or as potentially toxic metabolites, can be introduced into the human food chain through edible products obtained from farm animals exposed to DON. A subsequent study (Prelusky et al., 1986b), involving the oral administration of radiolabeled DON to chickens (2.2-mg single dose or 2.2 mg/day for 6 days), demonstrated that measurable levels of residues could be found in tissues: <40 ng DON-equivalents/g for most tissues, except liver, kidney, and gastrointestinal (GI) tract, which were marginally higher. These results suggest that although the parent toxin DON itself may not be transmitted to animal products, metabolites may account for the low levels of residues detected.

The present study to measure the transmission of radioactivity to eggs was part of a larger experiment (Prelusky et al., 1986b) designed to determine the fate of DON in laying hens. DON was administered as the ^{14}C-labeled compound either as a single oral dose or in spiked ration fed to the birds over an extended period.

MATERIALS AND METHODS

Chemicals. Both nonradioactive and ^{14}C-labeled DON were produced biosynthetically and purified as previously described by Miller and Arnison (1986). Purity of each compound (>95%) was established by reversed-phase high-performance liquid chromatographic analysis with a Hewlett-Packard Model 1090 liquid chromatograph (Hewlett-Packard Ltd., Palo Alto, CA) equipped in series with a diode array detector and Berthold Model LB 504 radioactivity monitor. Operating conditions: RP-18 column, 5-μm OD-5A Spheri-5, 25 cm × 4.6 mm (Brownlee Labs Inc., Santa Clara, CA); mobile phase, acetonitrile–water (1:9), flow, 0.8 mL/min; wavelengths monitored, 220, 234, and 254 nm; oven temperature, 40 °C. The original

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