Partitioning of Mirex between Adipose Tissue and Serum

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We examined the concentration of Mirex in the serum (13 specimens) and adipose tissue (19 specimens) of residents living near a dump site in Memphis, TN, and in South Memphis, TN. Packed and capillary gas chromatography with electron capture detection as well as capillary gas chromatography with negative-ion chemical ionization (NICI) mass spectrometry was used to confirm the presence of Mirex. Regression of the adipose tissue Mirex levels (nanograms per milligram, lipid basis) on serum Mirex levels (nanograms per gram, whole weight basis; nanograms per milligram, lipid basis; nanograms per gram, albumin) resulted in the following: (1) correlation coefficients of 0.818, 0.847, and 0.838, respectively; (2) intercepts of 0.277, 0.396, and 0.285, respectively; and (3) slopes of 0.264, 1.307, and 0.0106, respectively. All correlations were significant at $p \leq 0.0006$. Although the sample size is small ($n = 13$), these data show, for the first time, paired adipose tissue and serum ratios for Mirex in humans.

Mirex is a pesticide that was used almost exclusively in the southeastern United States to control fire ants. Mounting evidence suggests that Mirex is in the food chain and is ultimately in humans. Compared with some of the other chlorinated hydrocarbons [dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs)], Mirex has not been detected as consistently in humans, even in areas where it has been used considerably. Reports in the literature vary in their estimates of how often Mirex is detected in humans.

A review of a selected number of studies in which residue levels of chlorinated hydrocarbons in milk were determined indicates varying results regarding the presence or absence of Mirex. In one of the largest single investigations—a national study involving 1436 specimens—Mirex was not positively identified in any of the samples (Savage et al., 1981). Harrod and Asquith (1980), in a study of 25 specimens, found no indication of the presence of Mirex either. In another study of 34 women living in the Mississippi Delta, a region known for high use of Mirex to control fire ants, Mirex was not found (Barnett et al., 1979). On the other hand, Mirex was detected in the milk of 26 breast-feeding women living in upstate New York (Bush et al., 1983). In a larger study of PCBs in mothers' milk, Mirex was detected in the milk of 3 of 14 samples (Mes et al., 1978). These samples had unusual gas chromatographic patterns that indicated the possible presence of Mirex. The use of gas chromatography/mass spectrometry is emphasized in both of these studies of breast milk (Bush et al., 1983; Mes et al., 1978) to ensure identification of Mirex. A review of a selected number of adipose tissue surveys indicates that, except for a few studies, Mirex is not detected more frequently in adipose tissue than in breast milk. Mirex was reported in six specimens from the Environmental Protection Agency's National Human Monitoring Program from April 1971 to April 1972 (Kutz et al., 1974). For the ensuing years through 1975, the compound was detected, but at very low frequencies (Kutz et al., 1979). In a small study (Greer et al., 1980) of persons living in northeast Louisiana, investigators found Mirex in 20 out of 22 specimens; however, electron capture gas-liquid chromatography was the only determination reported. In a larger study designed specifically to determine the prevalence and concentration of Mirex in tissue from persons living in areas specifically treated with the chemical—that is, the southeastern United States—the frequency for positive Mirex detection was estimated to be 10.2%, with a geometric mean lipid concentration of 0.286 ppm (Kutz et al., 1985). More recent data of blood analysis for pesticide residues (Murphy et al., 1983) show the frequency of Mirex detection at less than 1%.

We believe that because Mirex is rarely detected in humans, an unequivocably supported detection warrants reporting. We report the detection of Mirex in 19 adipose tissue specimens and 13 serum specimens, and we allude to its presence in 114 specimens of adipose tissue and serum. These specimens are from a larger, predominantly black cohort (297 adipose tissue; 370 serum) of persons living in close proximity to a dump site in Memphis, TN.

EXPERIMENTAL SECTION

Sample Preparation. Adipose tissue samples were obtained by needle biopsy (Daum et al., 1978). After isolating the specimen from blood and isotonic saline or demineralized water, we extracted it three times with petroleum ether, dried it with anhydrous sodium sulfate, and determined the lipid weight. Pesticides and PCBs were extracted from the lipid with 20% acetone/acetonitrile. This solution was diluted with a 2% sodium sulfate solution, and the mixture was extracted with hexane (Crist and Moseman, 1976). The hexane extract was concentrated to 1.0 mL and eluted through a 9 × 330 mm chromatographic column containing 5.4 g of 5% deactivated Woelm silica gel (70/150 mesh) sandwiched between 10-mm heights of anhydrous sodium sulfate. Two fractions were collected: hexane (35 mL) and benzene (20 mL).

The blood was obtained by venous puncture and collected in a red top vacutainer. The specimen stood at room temperature for 30 min, and the serum was isolated following centrifugation. The serum was extracted by a previously recorded method (Burse et al., 1980) in which methanol was added to the serum, vortexed, and then extracted with 1:1 hexane–ethyl ether. The organic extract was eluted through a micro Florisil column, and two fractions, 6% ethyl ether in petroleum ether and 15% ethyl ether in petroleum ether, were collected. The 6% fraction was treated with concentrated sulfuric acid (Murphy, 1972), and the organic layer was eluted through silica gel in a manner synonymous with the adipose tissue. The hexane fraction from silica gel was analyzed for hexachlorobenzene, p,p'-DDE, and PCBs (as AR 1260)

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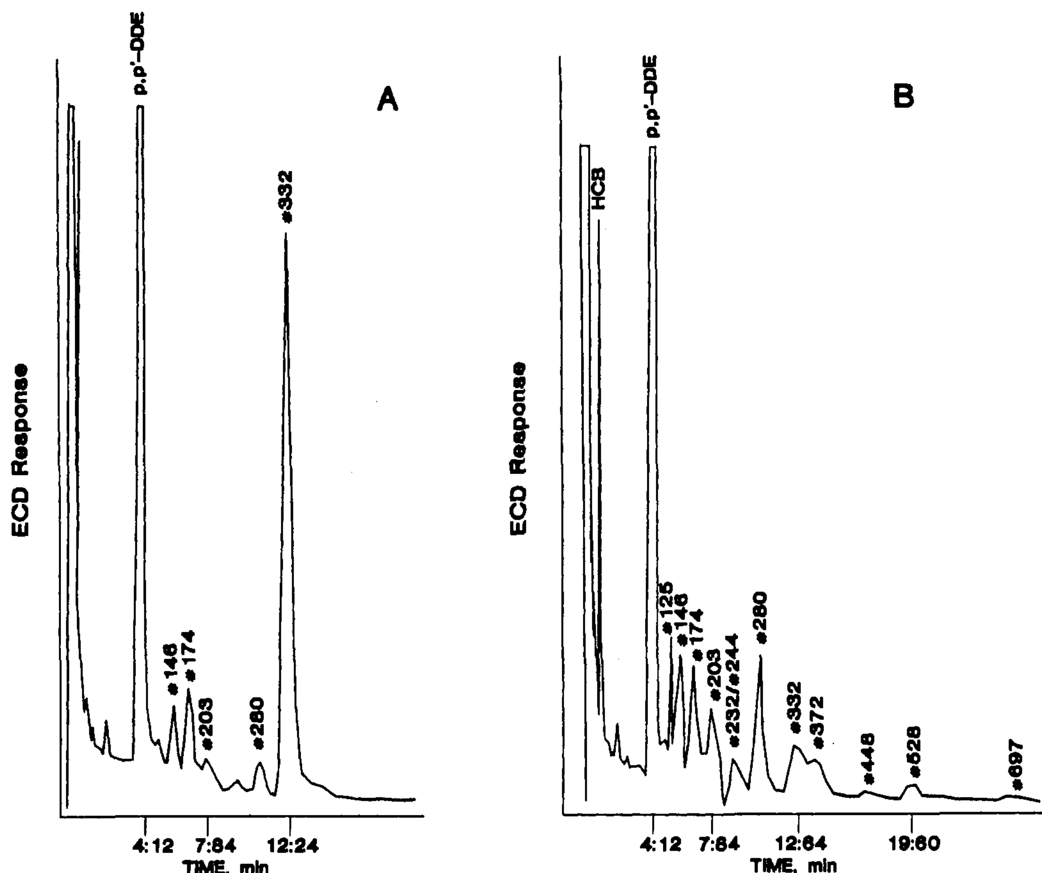

Figure 1. (A) Chromatogram of human adipose tissue sample 19 (Table III), packed column. Polychlorinated biphenyl peaks identified per Webb and McCall (1973). (B) Chromatogram of a quality control sample of lard containing in vivo PCBs (as AR 1260) and in vitro pesticides, packed column. PCB peaks identified as in Figure 1A. ECD = electron capture detection.

(adipose tissue and serum). The benzene fraction was analyzed for γ- and β-hexachlorocyclohexanes, oxychlordane, heptachlor epoxide, *trans*-nonachlor, *p,p'*-DDT (adipose tissue and serum), dieldrin, and endrin (adipose tissue only). For serum, dieldrin and endrin were determined in the 15% fraction from Florisil.

Gas Chromatography. The packed-column gas chromatography was done on a Varian Model 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector. A column, 1.83 m × 2 mm (i.d.), packed with 3% SE-30 on 80/100-mesh Gas Chrom-Q was used for the analyzing fraction I. The column was operated isothermally at 205 °C with a nitrogen carrier gas flow of 20 mL/min. The injector and detector temperatures were 250 and 330 °C, respectively.

The high-resolution capillary gas chromatography (splitless) was done on a Hewlett-Packard 5880 gas chromatograph equipped with a ^{63}Ni electron capture detector. The column was 60-m fused silica (0.32-mm i.d.) coated with DB-5 (J&W) at a 0.25-μm thickness. The oven temperature was initially held at 70 °C for 1.0 min and then programmed through three levels without delay: Level 1 was programmed at 15.00 °C/min to 160 °C, level 2 was programmed at 0.90 °C/min to 200 °C, and level 3 was programmed at 1.50 °C/min to a final temperature of 240 °C and held for 27 min. The injector and detector temperatures were 285 and 350 °C, respectively. The hydrogen carrier gas flow was 3 mL/min, and the argon–methane (95:5) makeup gas was 35 mL/min.

Gas Chromatography/Mass Spectrometry. The mass spectral analysis was done on a Finnigan 4500 GC/MS/DS operated in the negative-ion chemical ionization (NICI) mode with methane as the reagent gas. The electron multiplier setting was 1300 V with an ionizing energy of 70 eV. Scans were made from m/z 100 to 650 in 1 s. The gas chromatograph contained a 60-m fused silica column (0.25-mm i.d.) coated with DB-5 (J&W) at a 0.25-μm thickness inserted directly into the source. The oven temperature was initially held at 100 °C for 2 min and then programmed through two levels without delay: Level 1 was programmed at 20.00 °C/min to 260 °C, and level 2 was programmed at 1.00 °C/min to 300 °C and held for 20 min. The injector temperature was 300 °C, and the ionizer source was 100 °C. The carrier gas was helium at a flow rate of 0.82 mL/min.

DETERMINATION OF TOTAL LIPIDS AND ALBUMIN IN SERUM

Total cholesterol (TC) and triglycerides (TG) were determined by standard methods with the DuPont automatic chemical analyzer (ACA). Free cholesterol (FC) was determined by an enzymatic procedure (Allain et al., 1974). Phospholipids (PL) were determined by the method of Beveridge and Johnson (1949). We also determined albumin (ALB) using the ACA.

We computed the total lipids (TL) for each serum sample with confirmed Mirex using the following formula (Cheek and Wease, 1969; Akins et al., 1988):

$$TL = 1.677(TC - FC) + FC + TG + PL \quad (1)$$

These data were used to compute the Mirex concentration in serum on a lipid basis. Serum Mirex concentration was also determined on the basis of the albumin concentration.

RESULTS AND DISCUSSION

The unusual gas chromatogram was obtained in the analysis of adipose tissue for PCBs (as AR 1260) by using a packed column (Figure 1A). An adipose tissue quality control pool containing in vivo PCBs (as AR 1260) and a select number of in vitro added pesticides was also analyzed (Figure 1B). Comparison of the traces made us aware that either an unusual metabolism of PCB peak 332 (Webb and McCall, 1973) had occurred or we had encountered a contaminant. The matched serum of the adipose tissue specimen and a serum quality control pool containing in vivo PCBs (as AR 1260) and a select number of in vitro added pesticides were analyzed (Figure 2A,B). Compar-

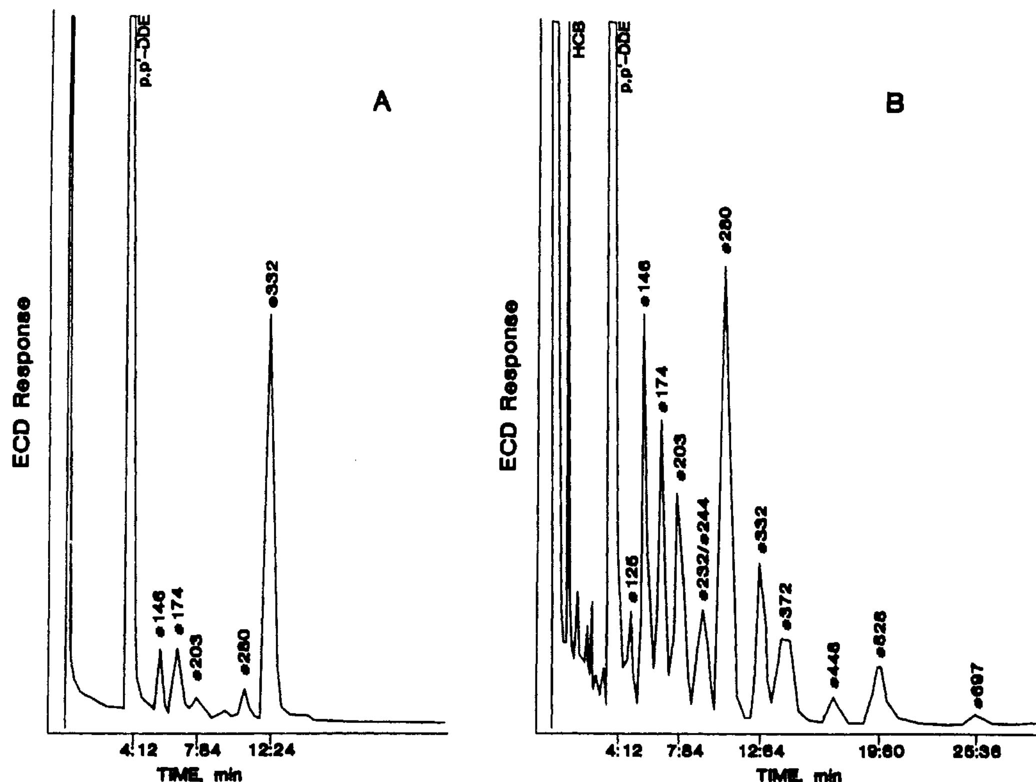

Figure 2. (A) Chromatogram of human serum sample 19 (Table III), packed column. Polychlorinated biphenyl peaks identified as in Figure 1A. (B) Chromatogram of a quality control sample of bovine serum containing in vivo PCBs (as AR 1260) and in vitro spiked pesticides, packed column. PCB peaks identified as in Figure 1A. ECD = electron capture detection.

ison of these two traces showed the same anomaly with regard to PCB peak 332 as observed with the adipose tissue. We know from elution studies within our laboratory that HCB (complete), *p,p'*-DDE (complete), *o,p'*-DDT (partial), *p,p'*-DDT (partial), and Mirex (complete) coelute with PCBs from deactivated silica gel. HCB does not interfere with any PCB peaks; however, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, and Mirex interfere with Webb-McCall peaks 98/104, 146, 174, and 332, respectively. We began to suspect the presence of Mirex in these samples. The adipose tissue and serum samples with quality control pools were then analyzed by capillary gas chromatography with electron capture detection, (Figures 3A,B and 4A,B). A peak matching the retention time of Mirex was observed in both matrices. Under these analytical conditions, Mirex is completely resolved from any of the PCB congeners characteristic of AR 1260. The adipose tissue sample was further analyzed by gas chromatography/mass spectrometry. The reconstructed ion chromatogram (RIC) of the adipose tissue sample is shown in Figure 5A. The RIC of the Mirex standard is shown in Figure 5B. Scan numbers that were later identified through their NICI spectra were 658 (HCB) and 878 (*p,p'*-DDE). HCB and *p,p'*-DDE are two pesticides commonly found in human tissue.

The spectra of scan number 1234 in the sample and 1233 in the Mirex standard are shown in Figure 6A,B. The spectra do not show a molecular ion. They do, however, indicate the presence of fragment ions at m/z 435, 400, 366, 332, and 235, with isotopic ratios indicative of the presence of Cl_9, Cl_8, Cl_7, Cl_6, and Cl_5. These represent the successive loss of three, four, five, six, and seven Cl^-, respectively, from the parent molecule. These spectra are quite similar to those previously reported (Stemmler and Hites, 1985). Table I displays the comparison of the significant ions observed in the Mirex standard with those in the adipose tissue sample. The ion cluster at m/z 400 contains the base peak (m/z 404) in both spectra. In Table II the chlorine isotopic intensities for a Mirex standard and the adipose tissue sample are compared with the theoretical

Table I. Comparison of Significant Frequent Ions in Mirex Standard and Human Adipose Tissue

	rel intens	
major ions	Mirex std	adipose tissue
435	14.32	13.19
400	29.68	31.59
366	16.28	13.46
332	15.85	12.09

Table II. Comparison of Mirex Standard and Adipose Tissue Extract with Theoretical Chlorine Isotope Intensities (%)

	no. of Cl atoms				
theor	5	6	7	8	9
m	61.5	51.2	43.9	33.8	26.3
$m + 2$	100.0	100.0	100.0	87.9	76.9
$m + 4$	65.0	81.2	97.5	100.0	100.0
$m + 6$	21.2	35.2	52.8	65.0	75.8
$m + 8$	3.4	8.5	17.1	26.4	36.9
$m + 10$		1.1	3.3	6.8	12.0
$m + 12$				1.1	2.6
	molecular ions, m/z				
theor	235	332	366	400	435
	Mirex Standard				
m	52.6	62.4	40.9	29.7	24.0
$m + 2$	100.0	100.0	100.0	81.0	75.9
$m + 4$	62.6	61.2	86.5	100.0	100.0
$m + 6$	20.4	25.5	46.2	69.7	80.9
$m + 8$				30.8	38.1
	Adipose Tissue Extract				
m	50.3	51.8	37.4	31.6	21.3
$m + 2$	100.0	100.0	100.0	88.7	77.3
$m + 4$	58.0	70.6	95.4	100.0	100.0
$m + 6$	14.5	15.3	50.4	75.0	80.0
$m + 8$				22.5	37.8

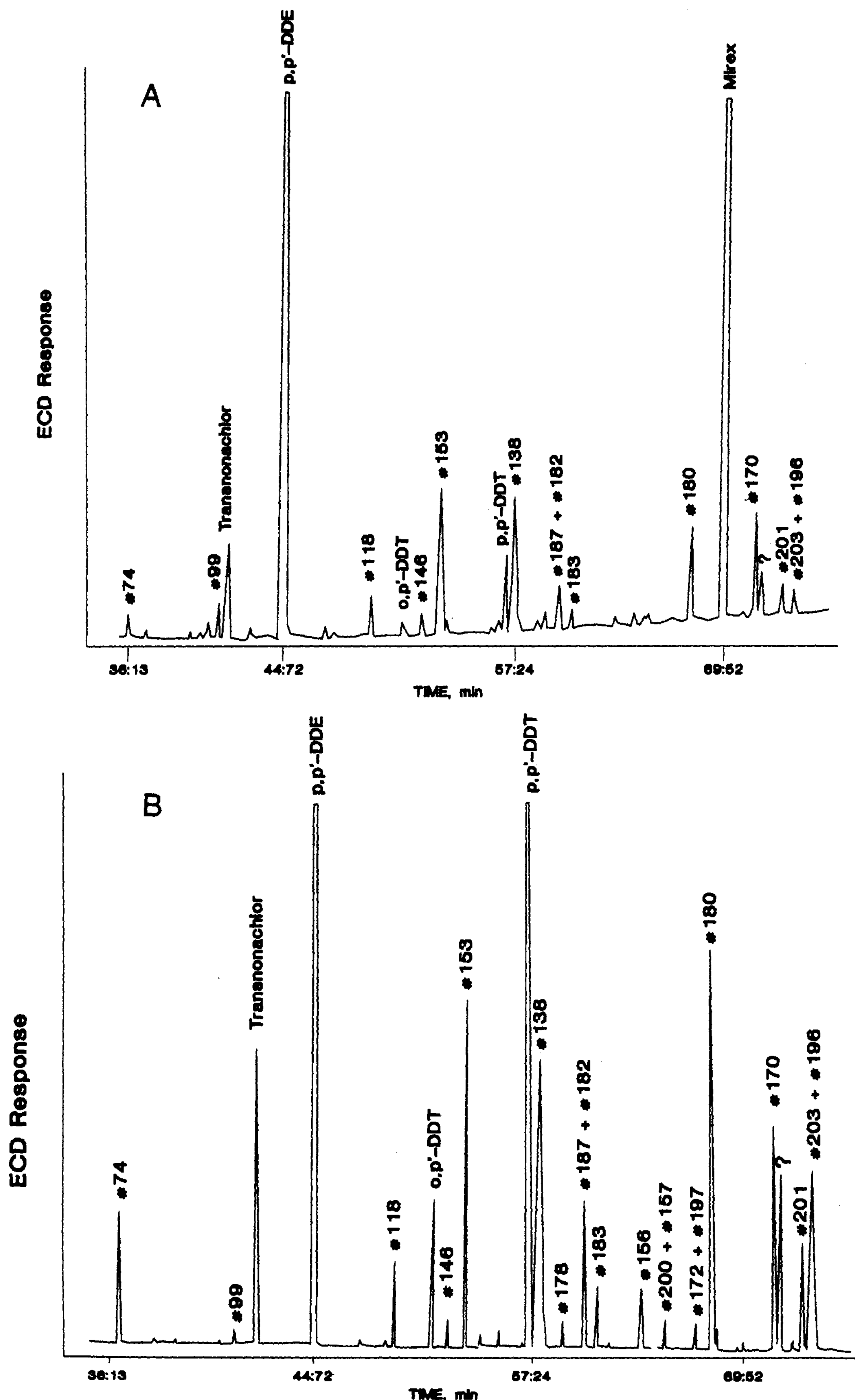

Figure 3. (A) Chromatogram of human adipose tissue sample 19, capillary column. Polychlorinated biphenyl congeners identified per Ballschmiter et al. (1980) and Mullins (1985). ECD = electron capture detection. (B) Chromatogram of a lard quality control sample (Figure 1B), capillary column. Polychlorinated biphenyl congeners identified as in Figure 3A. ECD = electron capture detection.

intensities. Relatively good agreement is obtained for isotope intensities between the Mirex standard and the adipose tissue sample.

After confirming Mirex in the sample with the unusual gas chromatographic traces, we reviewed the traces of the remaining adipose tissue samples in the study for indica-

Table III. Concentration of Mirex and Polychlorinated Biphenyls in Samples for Which Adipose Tissue Exhibited an Unusual Ratio of Webb-McCall Peaks (332 to 372)

		adipose tissue			serum			
specimen	age, sex	AR 1260,[b] ppm	332:372[c]	Mirex, ppm	AR 1260, ppb	332:372[d]	Mirex, ppb	concn ratio:[a] adipose tissue to serum
1[e,f]	39, F	2.40	3.94	1.44	12.9	3.68	5.87	245
2[e,f]	29, F	2.24	2.76	0.431	10.8	2.68	1.56	276
3[e,f]	40, F	4.43	2.56	0.828	22.1	2.41	2.75	301
4[e-g]	19, M	3.73	2.92	2.01	12.1	2.60	6.64	303
5[e,f]	58, F	3.84	2.22	0.107	25.2	3.52	5.65	19
6[e,f]	47, F	2.03	2.45	0.598	9.23	2.08	1.90	315
7[e,f]	75, F	1.40	2.56	1.11	8.33	2.43	2.04	544
8[e,f]	45, F	1.26	3.10	0.408	6.62	1.64	2.62	156
9[e]	24, M	1.82	2.45	1.78	6.68	1.18	2.17	820
10[e,f]	41, F	2.78	2.14	0.866	14.1	1.91	1.56	555
11[e]	18, F	0.662	2.80	0.086	3.74	1.32	<0.33	RND[h]
12[e,f]	31, M	1.13	5.69	1.76	4.36	4.66	4.66	378
13[e]	21, F	0.480	2.44	0.064	3.56	15.0	<0.28	RND
14[e]	26, F	0.951	3.10	0.201	5.01	1.50	<0.66	RND
15[e]	20, F	1.48	2.83	0.160	5.23	1.36	<0.33	RND
16[e-g]	35, M	2.94	4.28	3.72	7.28	4.20	6.78	549
17[e]	29, F	0.690	2.54	0.030	2.62	2.36	<0.33	RND
18[e]	21, M	0.652	2.76	0.140	1.56	2.92	<0.33	RND
19[e-g]	33, F	1.42	47.0	4.68	8.35	32.9	16.8	279

[a] Mean concentration ratio for Mirex in adipose tissue (lipid basis) to Mirex in serum (whole weight basis) is 364, with an SE of 57. [b] Concentration based on lipid weight. [c] Ratio of 332 to 372 in AR 1260 adipose tissue quality control pool was 1.55 + 0.148, (mean + 1SD, n = 20). [d] Ratio of 332 to 372 in serum of goat given a single dose of AR 1260 (100 mg/kg) and allowed to recover for 30 days was determined to be 1.31 + 0.116 (mean + 1SD, n = 14). [e] All specimens quantified by using capillary chromatography with electron capture. [f] Confirmed the presence of Mirex in adipose tissue by using capillary gas chromatography with negative-ion chemical ionization mass spectrometry. [g] Confirmed the presence of Mirex in serum by using capillary gas chromatography with negative-ion chemical ionization mass spectrometry. [h] RND, ratio not determined because Mirex was not detected in serum.

tions of Mirex. The review consisted of determining the ratio of the concentration of Webb-McCall PCB peaks 332 to 372 in an adipose tissue quality control pool analyzed in a series of analytical runs. The pool chosen had a characterized AR 1260 concentration of 2.01 ppm ± 0.494 (mean ± 1 SD, n = 20). The mean 332 to 372 ratio was 1.55 ± 0.148 (mean ± 1 SD). The quality control pool was prepared from adipose tissue taken from a goat that had been given a single dose of AR 1260 (100 mg/kg) and allowed to recover for 30 days. Any adipose tissue sample having a ratio more than 3 standard deviations (+1.994) from the mean was suspect for containing Mirex. Eighteen adipose tissue samples met the criteria and were reanalyzed by using capillary gas chromatography with electron capture detection, as were their matching sera. The 19 persons whose samples were analyzed included 5 males and 14 females, with an age range of 18–75 years. Other adipose tissue samples (114) were borderline (e.g., 332:372 < 1.994) but will be investigated later. Table III lists Mirex and PCB concentrations as well as 332 to 372 ratios for suspect samples. Although adipose tissue ratios were used as the primary indicator of the presence of Mirex, serum ratios of suspect specimens were also computed. In six instances, the ratio criteria for serum were met but Mirex was not detected by capillary gas chromatography. We found the limit of detection for Mirex in serum to be 0.5 ppb with a signal to noise ratio of 2.5:1. This discrepancy is probably attributable to a large partitioning ratio for Mirex between adipose tissue and serum, allowing for increased detectability in adipose tissue.

Partitioning data of lipid soluble xenobiotics between adipose tissue and serum have been reported by two methods: (1) In the first method, the mean (or the median) concentration ratio between the two matrices in a given cohort is determined (Anderson, 1985). (2) In the second method, the regression coefficient slope—referred to here as the partitioning ratio that results from a linear regression plot of the concentration of the compound in the adipose tissue versus its concentration in serum in a given cohort—is determined (Patterson et al., 1988). This slope,

Table IV. Albumin and Lipid Concentrations in Serum with Confirmed Mirex[a]

specimen	ALB, g/dL	FC, mg/dL	PL, mg/dL	TC, mg/dL	TG, mg/dL
1	4.40	86	273	249	63
2	4.43	80	248	233	46
3	3.73	63	215	200	99
4	3.94	53	179	175	38
5	4.46	80	227	215	212
6	4.08	64	216	196	94
7	4.34	82	265	221	122
8	4.09	69	219	207	104
9	4.54	54	177	169	68
10	4.32	62	183	193	34
12	4.82	67	188	213	66
16	3.74	61	221	164	113
19	4.08	71	229	140	81

[a] Key: ALB, albumin; FC, free cholesterol; PL, phospholipids; TC, total cholesterol; TG, triglycerides.

however, actually represents the change of adipose tissue level per unit change of serum level. Therefore, this slope is indicative of partitioning only if the intercept is not statistically different from zero.

In this study, we compared the Mirex levels in adipose tissue (lipid basis) with Mirex levels in serum on a whole weight basis and Mirex levels in serum on a lipid basis (lipid determined as in eq 1) by both methods. We also compared Mirex levels in adipose tissue (lipid basis) with Mirex levels in serum on an albumin basis, using the linear regression technique only.

The mean concentration ratio for Mirex in the 13 specimens with detectable Mirex serum concentration was 364 with a standard error (SE) of 57 (Table III). Table IV shows the albumin and lipid concentrations in the 13 serum specimens with confirmed Mirex. Table V shows the serum Mirex concentration corrected for these analytes. Linear regression of the adipose tissue Mirex levels (nanograms per milligram, lipid basis) on serum levels (nanograms per gram, whole weight basis) produced a slope of 0.264. The correlation coefficient (r) was 0.82 (Table

Table V. Serum Mirex Concentrations Corrected for Total Lipids and Albumin

specimen	total lipids, mg/dL	Mirex concn lipid, ng/mg	concn ratio:[a] adipose tissue (lipid basis) to serum (lipid basis)	Mirex concn albumin, ng/g
1	695	0.866	1.66	136.9
2	631	0.254	1.70	36.1
3	607	0.465	1.78	75.6
4	475	1.435	1.40	172.9
5	745	0.778	0.14	130.0
6	595	0.327	1.83	47.8
7	702	0.298	3.72	48.2
8	623	0.431	0.95	65.7
9	492	0.453	3.93	49.0
10	499	0.321	2.70	37.0
12	566	0.845	2.08	99.2
16	568	1.225	3.04	186.0
19	497	3.470	1.35	422.5

[a] Adipose tissue values are from Table III. Serum values from Table III have been corrected for total lipids. Mean concentration ratio is 2.02, with an SE of 0.30.

VI). A similar regression plot was performed on these concentration levels except for specimen 19, which was omitted because of the extremely high Mirex levels. The slope changed to 0.275, and the r changed to 0.58. In both cases, the intercept was not significantly different ($p > 0.05$) from zero. Therefore, when partitioning data are calculated in this manner, the partitioning ratio of Mirex between adipose tissue and serum is 264 (275 with specimen 19 omitted).

Brown and Lawton (1984) showed that human serum PCB concentrations were equivalent to adipose tissue PCB levels when both were reported on a lipid basis. Similarly, Patterson et al. (1988) showed that the mean concentration ratio between lipid-corrected adipose tissue and lipid-corrected serum 2,3,7,8-TCDD levels was 1.09. Mirex is likewise a lipophilic molecule that tends to concentrate in the adipose tissue. Therefore, using the lipid data shown in Table V, we calculated the concentration ratios and the partitioning ratio for Mirex between adipose tissue and serum, both on a lipid basis. The mean concentration ratio was 2.02 (SE 0.30) (Table V). The partitioning ratios that we found for Mirex were 1.307 ± 0.494 (slope ± 2SE) and 1.733 ± 1.174 (slope ± 2SE), depending on whether specimen 19 was included in the analysis (Table VI).

Chlorinated hydrocarbons similar to those we studied can attach to albumin or to lipoproteins in plasma (Maliwal and Guthrie, 1982). The correction of serum Mirex concentrations for albumin did not greatly change the correlation between adipose tissue and serum Mirex levels (Table VI). This may be attributed to any one or a combination of the following: (1) Compounds similar to Mirex are known to attach to proteins, and albumin accounts for 53%–65% of the total serum protein in adults (Mitruka and Rawnsley, 1981). (2) Albumin concentrations within these samples exhibit little variation, with a range of 3.74–4.82 g/dL.

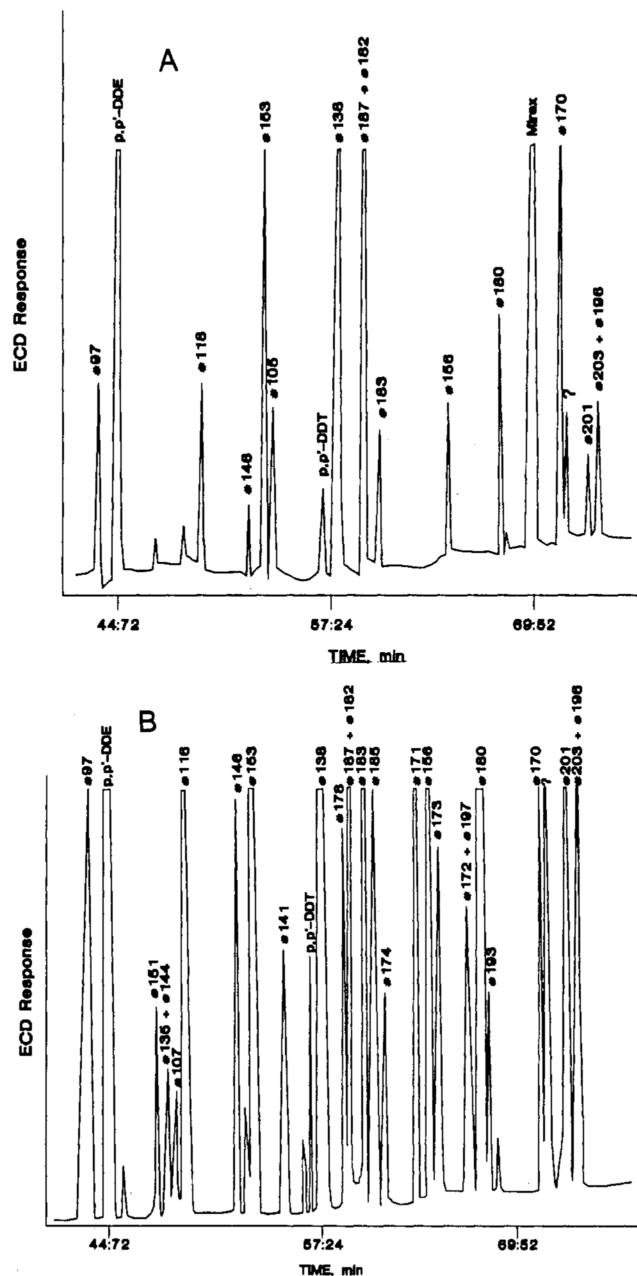

Figure 4. (A) Chromatogram of human serum sample 19, capillary column. Polychlorinated biphenyl congeners identified as in Figure 3A. ECD = electron capture detection. (B) Chromatogram of a serum quality control sample (Figure 2B), capillary column. Polychlorinated biphenyl congeners identified as in Figure 3A. ECD = electron capture detection.

Mirex was not included in the quality control material for adipose tissue or serum, since it was not an analyte in the original protocol. We did analyze a fat pool (adipose no. 129), however, provided by the U.S. Environmental Protection Agency Environmental Monitoring Systems

Table VI. Statistics Associated with Regression Analysis for Mirex in Adipose and Serum

independent variable	correln coeff	P value[a]	estd intercept	std error	P value[b]	estd slope	std error	P value[c]
serum Mirex, whole weight	0.818	0.0006	0.277	0.345	0.44	0.264	0.056	0.0006
	(0.578)[d]	(0.0488)	(0.239)	(0.514)	(0.65)	(0.275)	(0.123)	(0.0488)
serum Mirex, lipid	0.847	0.0003	0.396	0.296	0.21	1.307	0.247	0.0003
	(0.682)	(0.014)	(0.142)	(0.435)	(0.75)	(1.733)	(0.587)	(0.014)
serum Mirex, albumin	0.838	0.0003	0.284	0.322	0.40	0.0106	0.0021	0.0003
	(0.636)	(0.03)	(0.197)	(0.466)	(0.68)	(0.0117)	(0.0045)	(0.03)

[a] P value is from t-test testing H_0: Pearson correlation coefficient $R = 0$. [b] P value is from t-test, H_0: intercept 0. [c] P value is from t-test, H_0: slope 0. [d] The values in parentheses are for 12 paired data points excluding specimen 19, which had very high adipose and serum Mirex concentrations.

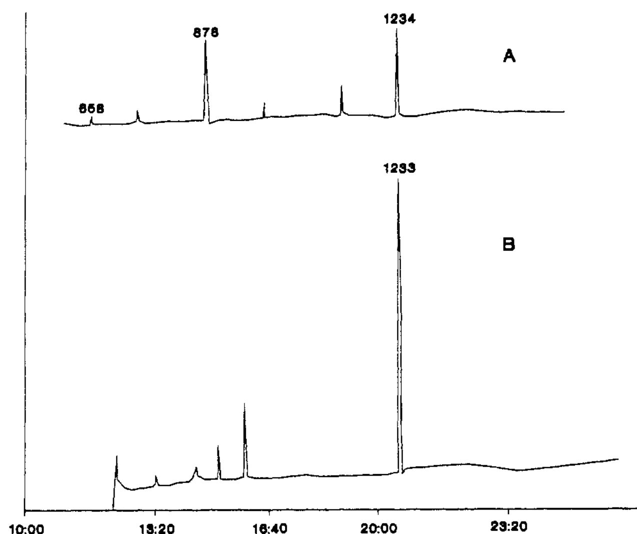

Figure 5. (A) Reconstructed ion chromatogram (RIC) of adipose tissue sample 19. (B) RIC of a Mirex standard at 10 ng/μL. Operational parameters given in text.

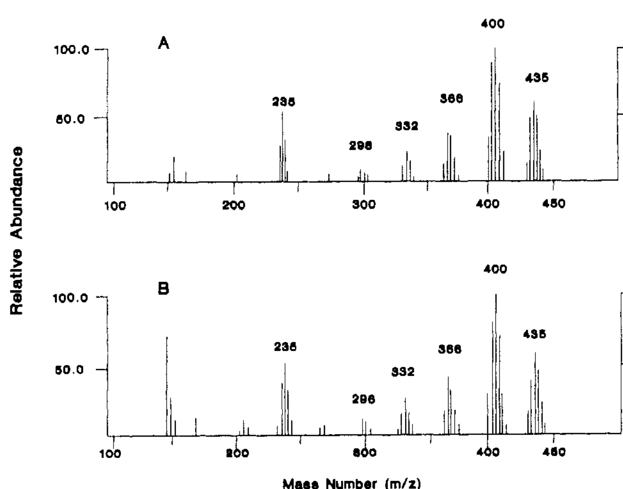

Figure 6. (A) Negative-ion chemical ionization (NICI) spectra of scan number 1234 of adipose tissue sample 19 (B) NICI spectra of scan number 1233 of Mirex standard at 10 ng/μL. Operational parameters given in text.

Laboratory (Las Vegas, NV) that contained 65 ppb of Mirex. In duplicate analyses using the analytical procedure we have outlined, we obtained values of 40 and 41 ppb indicative of recoveries of 61.5% and 63.0%, respectively.

To evaluate the serum procedure used to determine Mirex, we prepared base bovine serum in vitro spiked with Mirex in acetone at 3.50 ppb. In duplicate analyses we obtained values of 3.44 and 3.23 ppb indicative of recoveries of 98.2% and 92.2%, respectively. No correction has been made in reported residues based on recovery experiments.

This study, although limited by sample size, to our knowledge, provides for the first time, paired Mirex data for serum and adipose tissue in humans and corresponding partitioning coefficients. Results indicate that lipid correction may increase the adipose tissue–serum concentration correlation somewhat and suggest that Mirex may partition more into the adipose tissue than in serum on a lipid basis.

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Secondary Metabolites of *Fusarium* Species: Apotrichothecene Derivatives[1,2]

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Two epimers (3α-OH, 3β-OH) of 3,13-dihydroxy-11-epiapotrichothec-9-ene have been isolated from liquid cultures of *Fusarium* species. These epimers are common minor metabolites of *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, and *Fusarium sporotrichioides*, the ratio of the epimers being species dependent. The absolute configuration of the 3α-OH epimer was determined by X-ray crystallography and shown to have a trans A/B ring system with H-11 in the β-configuration as opposed to the cis A/B ring system of the trichothecenes. Both epimers readily undergo oxidation. The 3α-OH epimer also is involved in a unique rearrangement to form a ketal, 3,11-epoxy-13-hydroxyapotrichothec-9-ene, a compound also detected in the crude extracts of *F. culmorum* and *F. sporotrichioides* fermentations. The mass and NMR spectral data of these compounds and their epoxy derivatives are discussed. Speculations are made on the biosynthesis of the apotrichothecenes and sambucinol, another minor metabolite ubiquitous to the *Fusarium* species.

The impact of *Fusarium* mycotoxins on animal and human health and on the economy has resulted in an increase of interest in the trichothecenes (Ueno, 1983; Foster et al., 1986), which are the major toxic metabolites. The trichothecenes are tricyclic sesquiterpenes having in common a 9,10 double bond and a 12,13-epoxide moiety but varying in the degree of oxidation and acylation in rings A and C. Oxidation of the trichothecene ring appears to be species dependent. The stereochemistry of the oxygen moieties at any specific carbon atom is well-defined. This together with the degree of substitution appears to be related to the relative toxicity, with T-2 toxin being the most toxic.

In addition to the trichothecenes, all the *Fusarium* species studied by us to date, i.e. *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, and *Fusarium sporotrichioides*, also produce a variety of minor metabolites in liquid culture. Some of these compounds are common to all four species, e.g. sambucinol (IX), first isolated by Mohr et al. (1984), and 3,13-dihydroxy-11-epiapotrichothec-9-ene (I) (Greenhalgh et al., 1986; Zamir et al., 1987). This latter compound possesses two asymmetric methine centers (C-11 and C-3), but to date only isomers at C-3 have been reported (Lauren et al., 1987). Other compounds including sambucoin (X) (Mohr et al., 1984) and its 8-hydroxy analogues (Corley et al., 1987b) have been reported to be formed by specific *Fusarium* species, the latter on a solid medium. These minor metabolites differ from the trichothecenes in the size and stereochemistry of the ring system.

Although apotrichothecenes appear to be noncytotoxic to animal cells (Grove and Mortimer, 1969), they are phytotoxic (Wang and Miller, 1988). In addition, the biosynthetic origins of these compounds are of interest since they appear to arise from trichodiene, in common with the trichothecenes but by a different cyclization mechanism. This paper describes the resoluton of the two epimers of I, the absolute configuration of the 3α-hydroxy isomer IA, and the characterization of some rearranged products.

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EXPERIMENTAL SECTION

Apparatus. Gas chromatography/mass spectrometry (GC/MS) analysis was performed on a Finnigan Model 4500 system using a DB-5 capillary column (15 m × 0.32 mm (i.d.), 0.25-μm film), which was temperature programmed from 140 to 260 °C at 15 °C/min. The helium carrier gas was set at 10 psi. Some MS data were generated also by a Finnigan ion trap detector (IT). High-performance liquid chromatography (HPLC) was carried out with a Varian Model 5500 system equipped with a UV-200 variable-wavelength detector set at 205 nm. Separations were