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Note

Characterization of interferences in the analysis of serum for DDT and its metabolites

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The appearance of peaks due to contamination and interfering substances is common during the course of trace organic analysis by chromatographic procedures. Additives and plasticizers are often extracted from laboratory equipment during the course of sample preparation[1–3] and appear systematically in the analysis. Interferences have been observed previously from sample containers[4], laboratory environment[5], solvents[6], added reagents[7,8] and trace components in the sample itself[9,10]. The cause of an interference can be quite unusual, such as static electricity which caused a change in observed counts in a radioimmunoassay procedure[11]. For analytes at ultra-low levels, control of interferences from all sources may be the most difficult part of methodology development[12,13].

In the course of developing a gas chromatographic (GC) method for DDT and its metabolites in human serum[14], a series of contaminants was encountered, one of which eluted between *p,p'*-DDD and *p,p'*-DDT. Appearance of these contaminants or interferences at the same retention indicies (however, at irregular times and at varying concentrations during the course of a day) not only interfered in the observation of these two analytes, but also produced a significant change in the response of the electron-capture detector (ECD) to all the DDT-type compounds. GC–mass spectrometry (GC–MS) analysis indicated the interfering compounds were a series of low-molecular-weight polymeric materials extracted by hexane from a Kel-F® valve in the solvent dispensing system.

EXPERIMENTAL*

The GC method with an (ECD) has been described[14].

Analyses of a series of samples and combined extracts were conducted by using a Finnigan 4023 GC–MS–data system fitted with the PPINICI accessory. The glass chromatographic column was 6 ft. × 2 mm I.D. packed with 1.5% OV-17+1.95% QF-1 on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, PA, U.S.A.) and maintained at 200°C isothermally. The helium flow-rate was 20 ml/min

* Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

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with an electron multiplier setting of 1200 V and an ionizing energy of 70 eV. Positive and negative chemical ionization (CI) were run by using methane and isobutane (Matheson, East Rutherford, NJ, U.S.A.) as reagent gases.

RESULTS AND DISCUSSION

Several serum extracts were combined and concentrated to obtain a sample suitable for mass spectral analysis. Several of the compounds observed in the sample (Table I) were also observed in an extract from a broken cap liner, but the origin of the other compounds was not investigated. The three intermittent peaks of primary interest which interfered in the quantitation of DDT and its metabolites were located at 0.26, 0.60 and 1.63 relative to *p,p'*-DDE (Table I).

TABLE I

COMPOUNDS OBSERVED IN SERUM EXTRACT ANALYSES

*Compound**	*Retention index***	*Major ions m/z (rel. intensity)*
Diethyl phthalate	0.17	99(57), 105(19), 149(100), 177(22), 222(M^+, 1.7)
Chlorofluorocarbon	0.26	See Fig. 1.
Methyl hexadecanoate	0.28	74(100), 87(70), 143(15), 227(6), 270(M^+, 5)
Butyl isobutyl phthalate	0.34	104(5.9), 135(3.6), 149(100), 205(2), 223(6), 278(M^+, 0.2)
Methyl esters of saturated and unsaturated C_{18} acids	0.52	
Chlorofluorocarbon	0.60	See Fig. 1.
Long-chain hydrocarbon	0.70	57(100), 71(94), 85(58), 97(36), 111(17)
Butyl octadecanoate	1.26	56(100), 73(37), 129(28), 285(21), 340(M^+, 6)
Chlorofluorocarbon	1.63	See Fig. 1.
Long-chain hydrocarbon	1.50 and 2.11	57(100), 71(75), 85(54), 99(18), 113(10)

* Identification is on the basis of comparison (when available) with reference spectra, as well as interpretation of both chemical-ionization and electron-impact mass-spectral fragmentation patterns.

** Retention times are relative to *p,p'*-DDE, a reference compound for environmental analysis[13].

The mass spectrum for the peak at 0.60 is shown in Fig. 1 along with the rationalization for the major fragment ions. The use of the library search routine on the data system with a background-subtracted spectrum indicated a good match (FIT = 890) for tetrachlorohexafluorobutane. The library search routine also indicated chlorofluorocarbons as possible compounds for the peaks at 0.26 and 1.63 relative retention time. These types of compounds do not exhibit molecular ions in electron impact[15] and attempts to observe molecular ions with methane or isobutane positive-ion chemical ionization were unsuccessful. The negative-ion CI spectrum showed only an intense [Cl_2^-] fragment ion.

Based in part on these results, an examination of equipment used in the method showed a Kel-F valve in a solvent dispensing system. This polymer is composed of trifluorochloroethylene units[16]. Studies show that this system leaches these fluorochlorocarbon contaminants in the process of dispensing hexane. The fact that this solvent was used only to calibrate glassware for volume and was not used in an

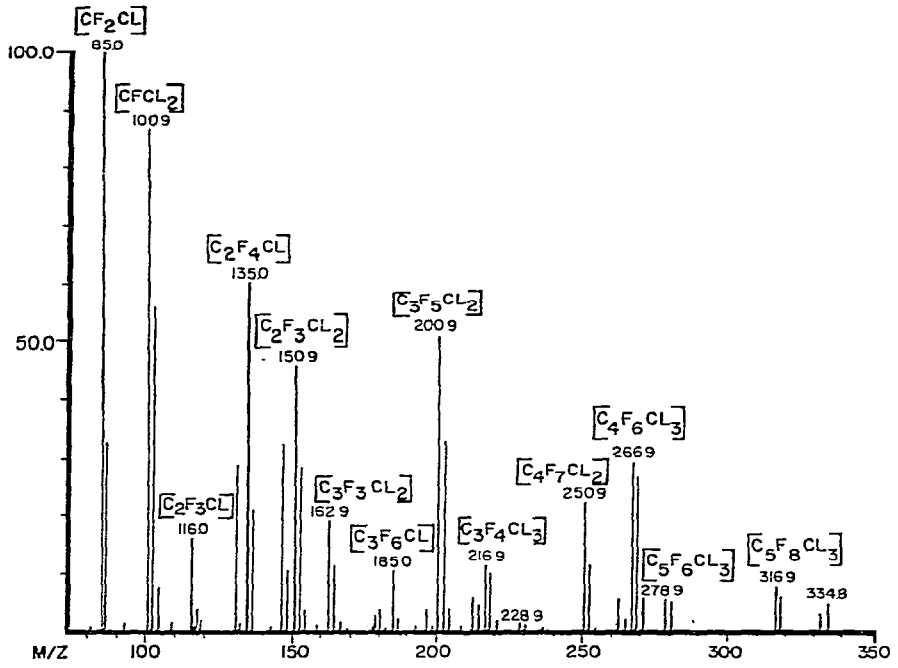

Fig. 1. The mass spectrum of the second eluting contaminant (0.6 relative retention time) from the hexane solvent system.

extraction procedure caused the appearance of these components to be very sporadic, and, hence, difficult to explain.

The similarity in mass spectra of these components and the increased retention times suggested a series of lower-molecular-weight homologues. The small ion intensities at $m/z = 279,281$; $m/z = 317,319$; and $m/z = 333,335$ in Fig. 1 and in the mass spectrum of the component at 0.26 indicate that these compounds are at least trimers. The observed sensitivity changes in the ECD can be due to the formation of the Cl_2^- species in the unit or unobserved traces of high-boiling, higher-molecular-weight components eluting from the column system. Although the interferences were too dilute to be observed directly in the reconstructed total-ion chromatogram from a single serum extract, their presence was confirmed by examining the selected-ion chromatogram for ions characteristic of these compounds.

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