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Problems associated with interferences in the analysis of serum for polychlorinated biphenyls

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ABSTRACT

During a recent survey to determine serum concentrations of polychlorinated biphenyls (PCBs) among people living around New Bedford, MA, U.S.A., an unidentified contaminant precluded the quantification of some early eluting Webb and McCall peaks. Loss of data is estimated to have reduced reported serum levels by 12%. Efforts to identify the contaminant by gas chromatography with an electron-capture detector, a Hall electrolytic condutivity detector, and mass spectrometer were not successful. Researchers ascertained, however, that the contaminant is not a PCB, it does not contain halogens, but it may contain phthalates. Vacutainer tubes and closures for serum storage bottles are suspected sources of contamination.

INTRODUCTION

Contaminants detected during the determination of trace amounts of environmental pollutants [*e.g.*, dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs)] in human matrices can be troublesome for the residue chemist. Historically, these contaminants have been traced to reagents [1], to glassware [2], and, in some instances, to trace components in the sample itself [3]. Contaminants can cause significant amounts of data to be lost [4] because they mask or confound the test results for the compound(s) of interest.

The Massachusetts Department of Public Health (MDPH) and the Centers for Disease Control (CDC) participated in a cooperative study to determine the

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prevalence of elevated levels (>30 ppb) of serum PCBs among residents of the greater New Bedford, MA, U.S.A. area. The area became contaminated with PCBs partly because of the high concentration of PCBs in waste waters discharged from some industries operating there [5].

During the serum analyses, we encountered a contaminant which precluded the quantification of certain PCB peaks. Here we report the steps taken to (1) identify the contaminant, (2) identify the source of the contamination, and (3) determine the amount of PCB lost because the contaminant was present.

EXPERIMENTAL[a]

Specimen collection procedure used in the field

A needle (Vacutainer brand blood collection set No. 7251, Becton Dickinson Vacutainer System, Becton Dickinson, Rutherford, NJ, U.S.A.) was used to draw venous blood into five 15-ml red-top vacutainer tubes (Vacutainer Systems, No. 6432, Becton Dickinson). The blood was allowed to stand at room temperature for 30 min to 1 h to clot. The blood was centrifuged at 2500 *g* for 10 min. For each participant, the serum yields from each vacutainer tube were pooled into one 30-ml Wheaton serum bottle [1780-H30, Thomas Scientific, Swedesboro, NJ, U.S.A.; each bottle had been previously rinsed with acetone and hexane (Nanograde quality) and allowed to air dry] by using disposable Pasteur pipets (prerinsed). Pooled serum was thoroughly mixed and, with a second Pasteur pipet, aliquots of 4–4.5 ml of serum were transferred to five 5-ml Wheaton serum bottles (1780-H05, Thomas Scientific; prerinsed as before). The five Wheaton serum bottles were capped (Silicone septa with Teflon face, 1780-K85, Thomas Scientific), sealed (Aluminum seals, 1780-L40, Thomas Scientific), crimped (seal crimper, 1780-M10, Thomas Scientific), and placed upright on dry ice packs for transport to MDPH. Three Wheaton serum bottles were shipped to CDC on dry ice, and two Wheaton serum bottles remained at MDPH stored at −30°C.

Analytical procedure

The analytical method used by MDPH/CDC to determine PCBs in serum has previously been described [6]. In this method, denatured serum is extracted with hexane and diethyl ether, and the organic extracts are eluted through deactivated silica gel with hexane. Silica gel eluates were analyzed by ^{63}Ni electron-capture detection (ECD) gas chromatography and quantified electronically by peak area with decachlorobiphenyl used as an internal standard. PCBs were quantified as Aroclor 1254. Although there is evidence that Aroclors 1016 and 1242 were used in the New Bedford area [5], the prevailing pattern observed in the serum re-

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

sembled Aroclor 1254. Webb and McCall [7] peak identification and mean weight percent factors were used.

Several samples from the New Bedford study that contained the contaminant were pooled, as were representative samples from the CDC serum bank. These samples were processed by the previously outlined method and analyzed under one or more of the following conditions listed in Table I.

RESULTS AND DISCUSSION

The gas chromatogram in Fig. 1 is characterized by the presence of peaks with retention times (t_R) of 0.85, 1.02–2.17, 2.47, and 3.00–8.17 min. These t_R values indicate the presence of hexachlorobenzene (HCB) (0.85 min), 1,1-dichloro-2,2-di-(*p*-chlorophenyl)ethylene (p,p'-DDE) (2.48 min), and PCBs (3.00 to 8.17 min). The t_R 1.03–2.18 min characterizes the contaminant peak. HCB and DDE will be used as markers to bracket the gas chromatographic elution of the contaminant peak in subsequent studies. The contaminant response in Fig. 1 is an extreme example, because none of the PCB peaks eluting before p,p'-DDE (Webb and McCall peak 100) [7] could be quantified. In our experience, during the New Bedford project, PCB peaks eluting before Webb and McCall peak 70 usually were not quantifiable when the contaminant peak was present. A composite specimen from the CDC serum bank was also analyzed, and its gas chromatogram is shown in Fig. 2. Note the presence of peaks with t_R values of 0.85, 2.45, and 2.98–7.92 min matching the t_R values of HCB, DDE, and PCBs, respectively. Note also the absence of the contaminant peak between t_R 1.02 and 2.18 min.

We used the Hall electrolytic conductivity detector (HECD) [8], which is very selective for halogenated compounds, to analyze (semiquantitatively) the New Bedford composite sample and the composite specimen from the CDC serum bank. The contaminant peak was not detected by the HECD; however, this does not imply unequivocally that the peak does not contain chlorine, because differences in the sensitivity of the electron-capture detector and the HECD are significant.

Standards analyzed by the HECD were ~20 to 100 times more concentrated than the electron-capture detector standards, so we could obtain a significant response. The sample sizes used for the HECD were also ~100 to 200 times more concentrated than those used for ECD. If we assume that the contaminant peak is chlorinated and that its responses by ECD and HECD are somewhat analogous to the response of DDE, then the contaminant peak should be detected under these chromatographic conditions, but it was not.

The New Bedford composite was also analyzed by capillary gas chromatography using an electron-capture detector. The capillary approach was not as informative as the packed column approach in defining the contaminant and consequently was not pursued further.

We further analyzed the New Bedford composite sample by gas chromatogra-

TABLE I

INSTRUMENTATION USED IN ATTEMPTS TO IDENTIFY SERUM CONTAMINANT

Instrument	Detector	GC column	Essential parameters	Figures
Varian Vista 6000 GC	ECD (^{63}Ni)	1.83 m × 2 mm I.D. packed with 3% SE-30 on 80–100 Gas Chrom Q	Temperatures (°C): injector, 250; column, 205; ECD, 330; N_2 carrier gas at 20 ml/min	1–2
Varian Vista 6000 GC	HECD	1.83 m × 2 mm I.D. packed with 1.5% SP-2250–1.95% SP-2401 (mixed phase) on 100–120 Supelcoport	Temperatures (°C): injector, 225; column, 200; reactor tube; 900; reactor base, 250; He carrier gas at 30 ml/min; H_2 reaction gas at 60 ml/min; solvent type and flow, *n*-propanol at 0.35 ml/min; vent time, 0.3 min	
Hewlett-Packard 5880 GC	ECD (^{63}Ni)	60 m × 0.23 mm I.D. fused silica coated with DB-5 (0.25 μm thickness)	Temperatures (°C): injector, 260 (splitless); ECD, 350; column, initially held at 90 for 2 min and then temperature-programmed through three levels without delay: level 1, programmed at 15.00 °C min^{-1} to 160 °C; level 2, programmed at 0.90 °C min^{-1} to 200 °C; level 3, programmed at 150 °C min^{-1} to a final temperature of 240 °C, which is held for 27.00 min. H_2 carrier gas at 1.5 ml/min; Ar–CH_4 (95:5) make-up gas at 35 ml/min	
Finnigan TSQ-46		1.83 m × 2 mm I.D. packed with 3% SE-30 on 80–100 Gas Chrom Q	Temperatures (°C): injector, 240; column, 200; He carrier gas at 25 ml/min; MS: Single MS mode scanning Q1; MS reagent gas, CH_4 at 0.5 Torr; ionizer temperature, 100; scan range, −CI, +CI, EI: 30 u to 600 u at 1.95 s/scan; ionizing voltage, 70 eV; electron multiplier, 1200 V	3–4
VG 7070E-HF		1.83 m × 2 mm I.D. packed with 3% SE-30 on 80–100 Gas Chrom Q	Temperatures (°C): injector 240; column, 200; He carrier gas at 25 ml/min; MS reagent gas, CH_4 at $5 \cdot 10^{-5}$ Torr indicated source pressure to give CI ratio of 17/16 = 50:1; source temperature, 200; scan range, CIMS 100–600 at 1 s/decade; reference, NCI PFK; resolution (10% valley definition), low = 1000, high = 6500	

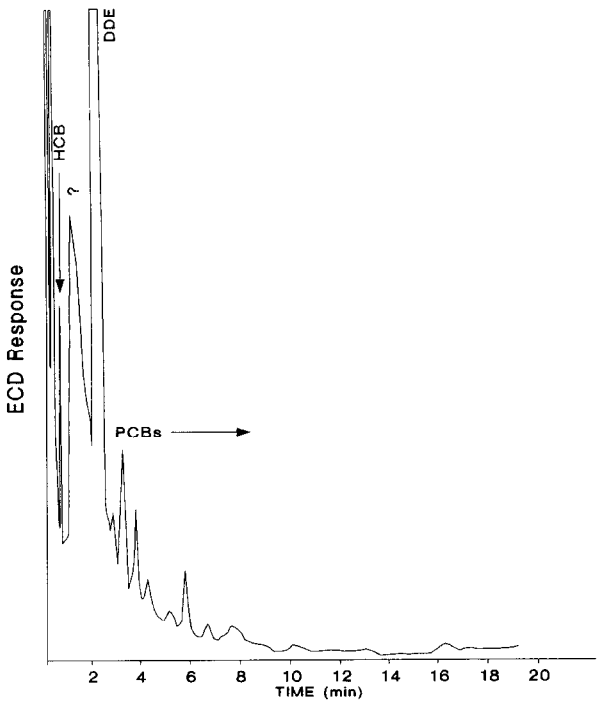

Fig. 1. ECD gas chromatogram of the New Bedford composite analyzed per protocol: equivalent of 2.4 mg of serum injected. See Table I for GC conditions.

phy–mass spectrometry. A packed gas chromatographic column interfaced to a Finnigan TSQ-46 GC/MS/MS/DS was used. Electron-capture negative chemical ionization (EC-NCI) was chosen as the initial experimental mode because this mode is most likely to detect compounds observed by the electron-capture detector.

Two marker compounds, HCB and p,p'-DDE, were used to establish the time frame of interest within the chromatogram. The compound of interest elutes between these two marker compounds under the conditions of the GC–ECD analysis. Three different compounds were detected in the NCI mass spectrometric analysis (Fig. 3). These compounds were not observed in serum from the CDC composite prepared by the same methodology. The first and third compounds yield only one ion in this mode, m/z 148, which is characteristic of the ubiquitous phthalate esters [9].

The second and largest peak observed in the time frame of interest yielded an NCI mass spectrum with ions at m/z 286 (assumed to be the parent ion), 272 (the base peak), 256, 241, and 148 (Fig. 4). The observation of individual ion chromatograms indicates that this is a single component peak and not a mixed mass spectrum. The mass spectrum is not that of a PCB, as indicated by the lack of the characteristic fragmentation of these compounds which we observed in NCI, that

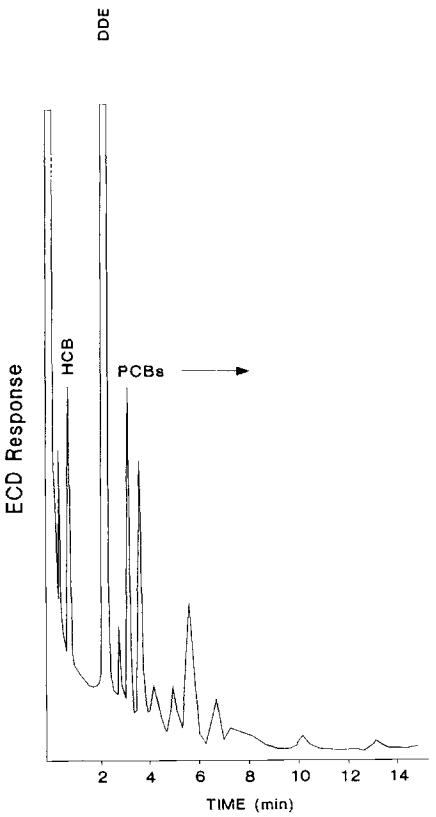

Fig. 2. ECD gas chromatogram of the CDC serum bank pool. Equivalent of 12 mg of serum injected. See Table I for GC conditions.

is M–35. In addition, the mass spectrum does not indicate that the contaminant contains chlorine, since chlorine-containing compounds exhibit a characteristic chlorine cluster in the mass spectrum that varies, depending upon the number of chlorine atoms in the molecule.

Additional efforts to identify this compound by using positive chemical ionization and electron-impact mass spectrometry were not successful. The mass spectra obtained in these modes were not characteristic of the compounds being investigated. Thus, the compound is probably at a concentration below the detection limit for these ionization modes.

We tried to obtain accurate mass NCI on the contaminant peak in the New Bedford composite. A packed gas chromatographic column was interfaced to a VG 7070E-HF high-resolution GC/MS/DS. The elution region of interest was again bracketed with standards of HCB and DDE; however, efforts to detect the unknown peak under low- or high-resolution NCI conditions were not successful. The sensitivity of the instrument for the marker compounds was more than adequate for the unknown to be detected. A chromatographic problem either with

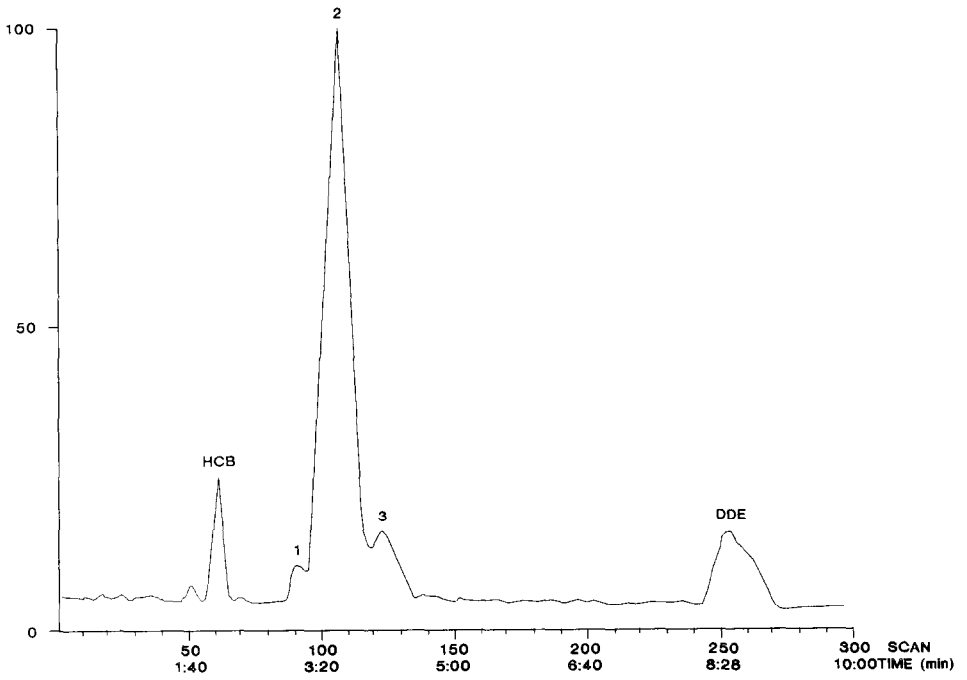

Fig. 3. Reconstructed ion chromatogram obtained in EC-NCI analysis of New Bedford serum composite. See Table I for analytical conditions.

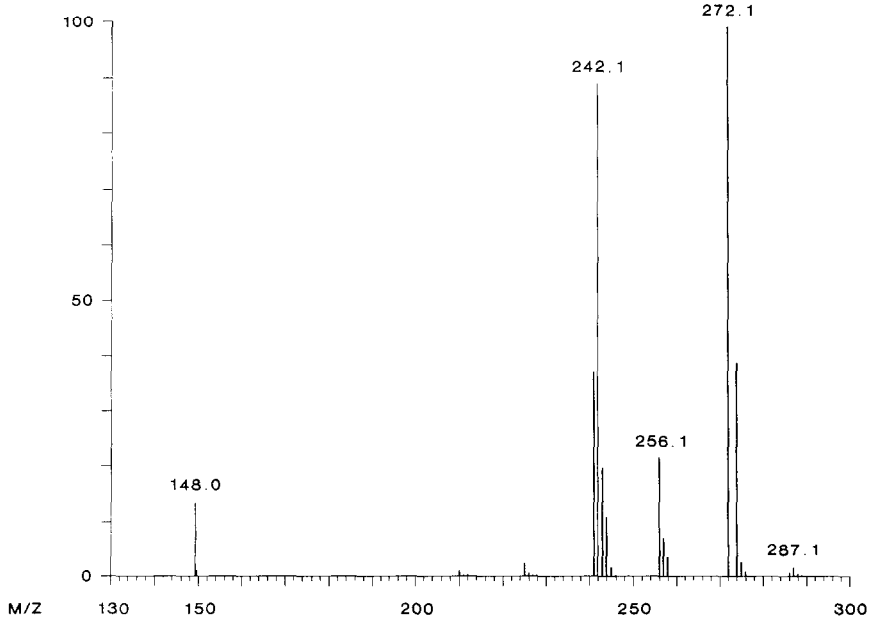

Fig. 4. EC-NCI mass spectrum for peak 2 (see Fig. 3) from New Bedford composite serum. See Table I for analytical conditions.

the GC column or the GC–MS interface (*e.g.* adsorption due to active sites) or both precluded the detection of the unknown. Efforts to rectify the problem (*e.g.* silanization) were not successful.

Data losses due to contamination

Determining the extent of PCB concentration loss on a sample-per-sample basis would be difficult, since those PCB peaks interfered with could not be quantified. For those samples that did not contain the contaminant, it would be naive to extrapolate the concentration of these peaks to other samples.

A more practical approach to obtaining an estimate of the extent of loss in PCB concentration would be through the analysis of serum quality control pools that contain *in vivo* PCBs as AR 1254. The peak distribution found in the quality control pool that contains *in vivo* AR 1254 is shown in Table II.

Those peaks that contribute to the total concentration of *in vivo* AR 1254 and that would be interfered with by the contaminant peak are Webb and McCall peaks 47, 54, and 58, which account for 12% of the quantifiable concentration. A 12% loss in quantifiable PCBs residues would not significantly change the reported prevalence of PCBs among the New Bedford residents [10].

The interferents were detected in about 70% of the New Bedford study serum samples, with varying intensities among samples. Results of an analysis of specimen-container bottles (MDPH/CDC), reagent blanks (MDPH/CDC), quality control pools (MDPH/CDC), and miscellaneous serum samples (CDC) did not

TABLE II

CONCENTRATION AND PERCENTAGE DISTRIBUTION OF WEBB AND McCALL PEAKS. *IN VIVO* AROCLOR 1254

Peak identification	Concentration (mean ± S.D., n = 20) (ppb)	Contribution to the total (%)
47	0.62 ± 0.154	6.6
54	0.36 ± 0.060	3.8
58	0.14 ± 0.025	1.6
70	0.65 ± 0.058	7.0
84	1.12 ± 0.102	11.9
98/104[a]	–	
125	1.90 ± 0.113	20.2
146	1.94 ± 0.104	20.7
174	1.86 ± 0.047	19.8
203/232	0.80 ± 0.114	8.5
Total PCB (as AR 1254)	9.38 ± 0.584	100.1

[a] Presence of in vitro spiked DDE precludes quantitation of these Webb and McCall peaks.

indicate the presence of the contaminants. Data resulting from the GC–ECD analysis of hexane extracts of a few vacutainers (remaining from the same lot used during the study) by MDPH did show the presence of several extraneous peaks, one of which had a t_R that matched the t_R of the contaminant peak. The remaining peaks, however, had ECD responses equal to or greater than the peak that matched the contaminant peak and had t_R values closer to the t_R for DDE. Elution of vacutainer extracts through silica gel appreciably reduced the response of the peak with a t_R matching that of the contaminant peak, but had no effect on the response of the remaining peaks. The presence of these additional peaks was not characteristic of the interferent observed during the study. CDC could not conduct an analysis because the lot had been depleted.

Although neither the contaminant nor its origin were ever identified, the analytical data do not support its being a PCB, PCB metabolite, a chlorine-containing compound, or a halogen-containing compound. Apparatus used to take blood (*e.g.* the needle or the vacutainer tube, or both) or store blood (*e.g.* the non-Teflon-coated surface of the silicone septa) is the most logical suspected source of the contaminant.

This problem, encountered by MDPH and CDC, does emphasize the need for testing a large enough number of lots of all materials and reagents when ultra low concentrations of analytes are to be measured in large populations [11].

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