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Detection Limits of Organochlorine Pesticides and Related Compounds in Blood Serum

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Determinations of organochlorine pesticides and similar chemical residues in blood serum have often reported detection limits of 1 ng/mL (ppb; Murphy and Harvey 1985). When a study group has incurred body burdens lower than this, underestimates and misclassifications of exposure may occur because persons with pesticide residue concentration below the limit of detection are usually treated as "zeros." Thus in order to more accurately assess exposures in such populations, analysis of adipose tissue has been done (Kahn *et al.* 1988, Wolff *et al.* 1982). Recently, with TCDD, use of a sufficient volume of serum, as much as 0.5 L, in conjunction with appropriate analytical techniques has been shown to achieve detection limits necessary for epidemiological assessment, *i.e.*, comparable to analysis of adipose tissue (parts per trillion; Patterson *et al.* 1988). This practical application followed a number of studies during the past decade in which the correlation between serum levels and adipose concentration was established (Anderson 1985).

In a population-based study involving children in which we were involved, it was not feasible to obtain specimens of either adipose or a large volume of serum (Baker *et al.* 1991). There was no compelling health motivation for such measures, nor did we wish to impair participation rates. Therefore, we chose to optimize the existing serum analysis, in order to achieve a detection limit low enough to assess reasonably the anticipated exposures.

MATERIALS AND METHODS

Blood (5 mL) was drawn in 7-mL red-top vacutainers. Wax or marble top tubes were avoided because they can introduce chemicals which interfere with the analysis. The serum was stored frozen (-20°C) in precleaned (acetone/ hexane) 5-mL teflon-lined capped, glass vials. Specimens were shipped on dry ice to the laboratory via overnight express in batches of 50 samples.

The sample preparation method was equivalent to that developed by the Centers for Disease Control (CDC; Burse *et al.* 1980) and used previously by us (Wolff *et al.* 1982). Serum (2 mL) was treated with methanol (1 mL) and extracted three times with 2.5 mL of 1:1 diethyl ether-hexane. The combined extracts were reduced to 0.5 mL under nitrogen and were chromatographed on a 1.8-g Florisil column in a 5-mL disposable pipet topped with 0.5 cm of anhydrous Na_2SO_4. The use of Florisil

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column chromatography follows the Environmental Protection Agency (EPA) method for separation of pesticide residues (EPA 1980). Two fractions were eluted, the first (13-mL hexane) containing nonpolar residues (PCBs, *p,p'*-DDE, *trans*-nonachlor); the second (13-mL 6% ether-hexane) containing oxychlordane, heptachlor epoxide, *trans*-nonachlor, and *p,p'*-DDT. Each fraction was evaporated under reduced pressure using a rotary evaporator to 0.50 mL for gas chromatographic analysis.

Both fractions were analyzed by gas chromatography (Perkin-Elmer Sigma 1) with electron-capture detection (^{63}Ni) and autosampler injection, using external standards for quantitation. Gas chromatographic conditions were: glass column (6 ft x 1/8 inch id) packed with 6%SE30/4%OV210 on 100/120 mesh Supelcoport; 50 cc/min argon/5% methane; column temperature 180°C, inlet 260°C, detector 300°C. Compound identity was confirmed on a 1.5% SP-2250/1.95% SP-2401 column under the same conditions for a few samples. Identification was based on retention time relative to *p,p'*-DDE, within $\pm$0.01 of the authentic standards for peaks earlier than *p,p'*-DDE or $\pm$0.05 for later peaks.

Reference standards (PCBs, *p,p'*-DDE, *trans*-nonachlor, oxychlordane, heptachlor epoxide, *p,p'*-DDT) were obtained from the EPA reference library, and were prepared as solutions in isooctane. These residues were chosen because PCBs and DDE are practically ubiquitous in human tissues (Murphy and Harvey 1985) and because the chlordane residues were targeted in the epidemiologic study. External standard quantitation was done using integrated peak areas with standard concentrations from 0.2 to 100 ng/mL. Peak heights were used for heptachlor epoxide and oxychlordane. PCBs were calculated as Aroclor 1260 using the method of Webb and McCall (1973) to quantitate those peaks with retention time longer than *p,p'*-DDE (P117-P372). The instrumental limit of sensitivity was approximately 1 pg injected (20% full scale deflection of γ-HCH).

For quality assurance, two serum specimens from a pool were included in each laboratory batch of 12 unknown samples. These pools were prepared using dated blood bank plasma fortified with standards in dimethylsulfoxide solution. Solvent recoveries and blanks (hexane) were also included in each laboratory batch. Surrogate sera from the same pools were also inserted as coded (blind) field samples into the specimens obtained in the field.

RESULTS AND DISCUSSION

The limit of detection has been designated by the International Union of Pure and Applied Chemistry (IUPAC) as 3 times the standard deviation of the blank (Long and Winefordner 1983). For practical purposes, the standard deviation of the lowest level known concentrations is used. The instrumental limits of detection, as shown by external standards used for gas chromatography (~0.2 ng/mL), were approximately 0.1 ng/mL for the pesticides and 1 ng/mL for Aroclor 1260, assuming 2 mL of serum. The limits of detection demonstrated by the lowest surrogate plasma specimens (quality assurance specimens) were 0.15 to 0.3 ng/mL for the pesticides. The results of analyses of the quality control specimens are presented in Table 1. The Shewhart

Table 1. Results of Analyses of Fortified Plasma as Quality Control Pools for Pesticide Residue Determination

pool	added, ng/mL	mean	n	sd	cv (%)	(pool[+])
			PCB			
c	3.02	4.86	21	0.970	20.0	4.23
a	10.2	11.3	44	1.67	14.8	1.54
a*	10.2	11.9	18	1.96	16.5	
			p,p'-DDE			
c	0.150	4.54	21	0.0391	8.6	3.67
a	0.482	9.54	24	0.830	8.7	7.39
a*	0.482	9.68	10	1.12	11.6	
a	1.45	9.82	19	0.881	9.0	
a*	1.45	10.4	8	0.749	7.2	
			Oxychlordane			
c	0.195	0.307	21	0.0404	13.2	0.161
a	0.628	0.830	24	0.0831	10.0	0.202
a*	0.628	0.747	10	0.0992	13.3	
a	1.88	1.97	19	0.193	9.8	
a*	1.88	1.83	8	0.170	9.3	
			Heptachlor Epoxide			
c	0.201	0.570	6	0.122	21.4	ND
a	0.646	0.752	24	0.0943	12.5	0.307
a*	0.646	0.671	9	0.0615	9.2	
a	1.94	2.00	19	0.169	8.4	
a*	1.94	1.85	8	0.187	10.1	
			trans-Nonachlor			
c	0.159	0.257	21	0.0928	36.1	0.0822
a	0.512	0.760	24	0.198	26.1	0.0980
a*	0.512	0.626	10	0.109	17.4	
a	1.54	1.58	19	0.200	12.7	
a*	1.54	1.59	8	0.0993	6.2	

[+] Results of two determinations of blank plasma. ND = not detected. Pool C determinations were complicated by an interfering peak, which separated from heptachlor epoxide in six analyses.

* Same pool, incorporated as blind field specimens.

plot for oxychlordane is shown in Figure 1 (Westgard *et al.* 1981). This analytical procedure follows those that have been developed by CDC for analysis of PBB and PCBs. We reported a detection limit for PBBs using this method of approximately

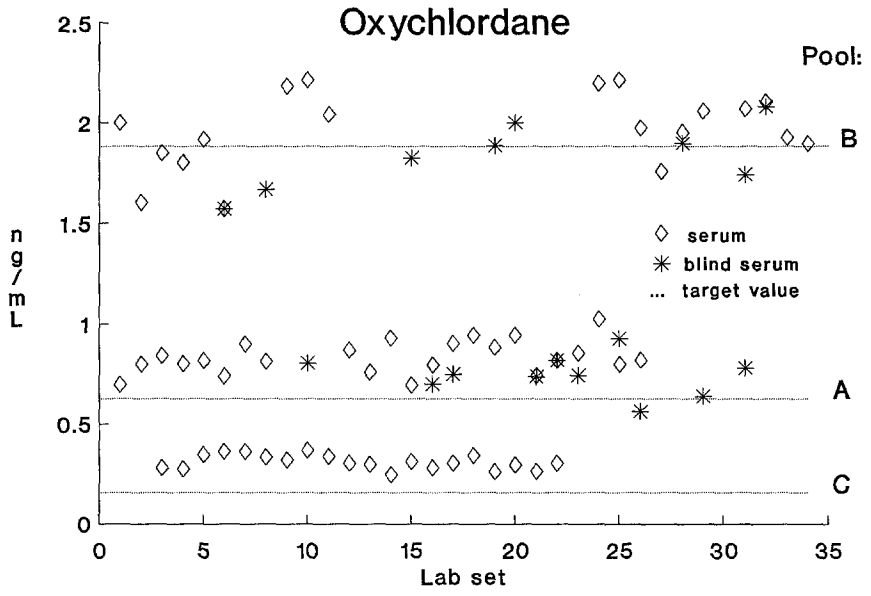

Figure 1. Shewhart plots of three quality control pools for oxychlordane (not corrected for native oxychlordane)

0.2 ng/mL (ppb; Wolff *et al.* 1982). More recently, other techniques have been developed for analyses such as these by varying the column chromatography cleanup step. The data from one such study suggest detection limits that appear comparable to those we report here, although the procedures are more laborious (Burse *et al.* 1990). Various components of current analytical protocols contribute to better detection of pesticide residues in blood serum. Polar extraction gives properly quantitative recoveries. Thus the quality control results are better, and since more material is retrieved, instrumental response is greater. Adequate sample cleanup with column chromatography provides better instrumental performance and longer detector life. In addition, the chromatographic cleanup step furnishes evidence of component identity by its presence in the proper fraction. Automated data processing and sample injection within the gas chromatography step reduce operator and other systematic errors.

In conclusion, the current methods of serum analysis for organochlorine pesticides can be optimized to achieve lower detection limits which are essential to epidemiologic studies involving low-level exposures.

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