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Comparability and Precision of Serum PCB Measurements

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ABSTRACT. The 95% prediction interval for single measurements of serum "Aroclor" reported by a reputable commercial analyst was found to be approximately ± 42%. The geometric mean serum PCB levels in a population of capacitor workers who had formerly had direct exposure to the commercial PCBs—Aroclors 1016, 1242, and 1254—were found to be alternatively reportable as 1905 ppb minimum initial PCBs (as calculated from most persistent peaks present); 1093 ppb non-overlapping analytical "Aroclor" levels (as calculated by the conventional sum-of-the-peak-heights method); 303 ppb total PCBs actually present; or 19 ppb "human PCB" (as calculated by the NHMP procedure). The broad spread in reportable values was relatable to the PCB isomer distribution and clearance patterns in the occupationally exposed population.

HUMAN EXPOSURE to PCBs continues to be of popular, regulatory, and epidemiologic concern, and there have been a number of recent studies of possible correlations between health effects and PCB exposure.[1-6] In all of these studies, the extent of exposure was estimated from measurements of PCB levels in the blood serum or plasma. These measurements have been made and reported by a variety of procedures. At present, there is little information on the comparability of serum PCB meas-

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urements reported by different investigators, or on the precision of the individual measurements.

The procedures used in recent years for the measurement of low levels of PCBs in biological or environmental samples have been reviewed.[7-9] In the case of serum or plasma samples, the preferred initial step is an extraction of the PCBs from the sample using a triple solvent procedure (methanol-hexane-ethyl ether) known to be capable of denaturing lipoprotein micelles so as to favor PCB release into the solvent phase.[10,11] This is usually followed by pesticide removal over a silica gel or florisil column,[9,10] followed by solvent removal and gas chromatography using a packed column and an electron capture detector. The net result is a chromatogram showing a series of peaks representing either individual PCB isomers or clusters of several isomers having similar retention times. In addition, the chromatograms of human sera invariably show a peak for p,p′-DDE, a metabolite of DDT, which is not removed by silica gel or florisil. The p,p′-DDE peak in the tracing serves as a convenient marker for characterizing the positions (relative retention times) of the PCB peaks.[12] The heights or areas of the PCB peaks are determined not only by the quantity of PCB injected into the chromatograph, but also by the response of the electron capture detector. The output of such detectors varies as a nonlinear function of concentration, is differentially sensitive to PCB isomer structure and composition,[7-9] and requires frequent and careful calibration against PCB standards. All these extraction, purification, and chromatographic procedures, while permitting the analysis of PCBs present in biological fluids at parts-per-billion (ppb) levels, are subject to known problems and errors.[13,14]

One objective of the present study was to determine the net effect of such errors on the precision, and hence comparability, of individual measurements of serum PCB levels, as performed by a reputable commercial analyst using procedures of the type practical for epidemiological studies.

A different sort of problem in PCB data comparability is presented by the availability of several alternative procedures for reporting the same set of raw data. These alternatives arise because the commercial PCB products (e.g., Aroclors) are not pure compounds, but instead complex mixtures of isomers that are metabolized by living organisms at different rates. Hence, the distribution of isomers in a biological or environmental sample may be quite different from that in the Aroclor responsible for the exposure, or that used as an analytical reference standard. In addition, the earliest investigations of environmental PCB levels were plagued by pesticide interferences, which prevented observation of the entire distribution of PCBs present.[15-17]

The first reporting procedure evolved in response to these problems was to measure the heights of just a few of the observed PCB peaks, selected for visibility and freedom from interferences; to then reference the individual or summed peak heights to those of the corresponding peaks in an Aroclor standard; and to report the result as an "Aroclor" level. In the past, this procedure has been almost universally used in environmental PCB analysis, and quite widely also in that of serum or plasma PCBs.

A second general procedure, applicable to samples where other chlorinated hydrocarbons are either absent or removable, is to measure the height or area of every PCB peak on the chromatogram and then to reference it individually to the corresponding peak in a standard. The resulting peak PCB levels may then be reported individually, or summed over stated ranges of retention times so as to produce reportable values of, e.g., lower PCBs (LPCB), higher PCBs, (HPCB), or total PCBs (TPCB).

A third and possibly novel procedure, which will be described below, would consist of identifying in each segment of the chromatogram the individual peak that shows the least clearance relative to its neighbors, and then to calculate from the magnitudes of these persistent peaks the minimal initial concentrations (MIC's) of the commercial Aroclors required to produce them.

Four other reporting procedures, which do not permit differential determinations in the case of mixed exposures, have also been described. Zobel[18] has devised an iterative computer program that selects the Aroclor standard that best fits the observed peak distribution by a minimizing procedure, and then reports the data in terms of the "Aroclor" level. Parkinson et al.[19] constructed a mixture of PCB isomers purportedly representative of that found in human breast milk for use as a reference standard for reporting human PCB data. Berg[20] and other investigators have proposed perchlorinating the PCBs to decachlorobiphenyl, a procedure characterized by rather variable yields, so as to permit a direct determination and reporting of total PCBs (TPCB). The National Human Monitoring Program (NHMP)[21] ratios the height of a single heptachlorobiphenyl peak to that in an Aroclor 1260 standard to determine a "human PCB" value.

Obviously, if the exposure came from just a single commercial Aroclor, and if there were no selective metabolism of PCB isomers, all of these alternative procedures for reporting the raw data would result in the same numbers. An important objective of the present investigation was to determine the actual degree of divergence among the alternative differential procedures for reporting human serum PCB measurements.

MATERIALS AND METHODS

Donor population. The donor population for the study of chromatogram reading error consisted of 57 employees of a capacitor manufacturing plant that had used Aroclor 1254 during the period 1946–1954, mainly Aroclor 1242 with a little 1254 during 1954–1971, and Aroclor 1016 during the period 1971–1977. These 57 individuals had had widely varying exposures to these Aroclors during this period, and were sampled in 1981. Twelve (11 males, 1 female) of the 57 served also as the donor population for the serial dilution and peak distribution studies. These 12 had all had direct occupational exposure and were known to have high serum PCB levels. Their ages ranged from 32 to 61 yr (average 47 yr) and service times from 7 to 28 yr (average 21 yr);

none had ever presented clinical symptoms or chloracnegic disease.

Sample preparation. Venous blood samples (duplicate 10-ml tubes; plus a third if possible) were drawn from subjects in a fasting state. Each sample was allowed to clot (15 min) and centrifuged, and the serum decanted into glass vials, sealed with aluminum-lined caps, labeled, and frozen. One sample from each of the 12 directly exposed donors was used to construct a serum pool by incremental weighing. The frozen samples were shipped via air freight to Hazelton Raltech Inc., (now Hazelton Laboratories America, Inc.), Madison, WI, for analysis. That laboratory had a control human serum pool prepared from expired Wisconsin bank blood that was available for both replication studies and the serial dilutions of the capacitor workers' serum pool.

The PCB analyses undertaken included (a) duplicate measurements on the 12 directly exposed donor samples; (b) triplicate measurements on the undiluted donor pool; (c) duplicate measurements on two sets of four dilutions of the donor pool with the control pool; (d) duplicate measurements on one set of four dilutions of the donor pool with water; and (e) 45 single measurements on the other donors, to make up the total of 57 for the reading error assessment. Measurements on a third set of serum samples, obtained from 5 of the 12 directly exposed donors, agreed within ±10%, as did triplicate measurements on the undiluted serum pool. PCB concentrations in the control pool were assayed on the same day as the serial dilutions, and were used in the calculations of expected PCB levels in diluted sera.

Analytical procedures (performed by Hazleton Raltech). Using Burdick and Jackson solvents distilled in glass (P.R. grade or equivalent), 5-g serum samples were treated with methanol (2 ml, mixed on Vortex for 1 min) and then extracted 3 times with hexane : ethyl ether (1 : 1). The extracts were concentrated under a gentle stream of nitrogen and then passed over a micro-florisil column (600 mm x 6 mm i.d. glass, containing 2.2 g 60/100 mesh P.R. grade florisil, preheated to 140°C for 16–24 hr). The PCBs and p,p'-DDE were eluted with 1% methanol in petroleum ether, concentrated under nitrogen, and brought to a 2-ml final volume. PCB determinations were performed on 1.0 μl samples of the extract using a Hewlett-Packard Model 5710A gas chromatograph equipped with a 1800 mm x 4 mm i.d. column of 1.5% SP2250/1.95% SP2401 on 100/120 Supelcoport and a ^{90}Sr electron capture detector. Temperatures were as follows: injector, 250°C; column, 205°C; detector, 300°C. The carrier gas was argon-methane (95:5) at 43.5 ml/min.

All chromatotograms of both serum samples and Aroclor standards were then returned to us for further analysis, along with the analyst's own calculations of "Aroclor" levels present. Representative chromatograms are shown in Figure 1.

Chromatogram reading and reporting. The measurements of the positions and heights of the individual peaks on the chromatograms were generally straightforward except for occasional complications. Thus, for Aroclor 1242 the early peaks (e.g., peak 11) appeared along a changing elution curve at low attenuation, and were difficult to estimate. In the serum samples, there was some interference at peak 11 due to 1,2,4-trichlorobenzene. Peak 70 was a partially resolved doubie peak in both the Aroclor 1242 standard and the human sera, with the first peak (70A) smaller in the standard but higher in the sera; Aroclors 1254 and 1260 showed only a single peak in this retention time. The serum peak amplitude we recorded was that of 70A. For all our serum specimens, peaks 98 and 104 were so weak as to be obscured by the p,p'-DDE peak. The observed relative retention times for the higher congeners (peaks 203–528) differed somewhat from those reported by Webb and McCall.[12] Peaks 448 and 528 were only barely observable in human specimens at the instrument attenuation generally used.

The heights of the individual peaks (H_p) in the Aroclor standards did not vary linearly with concentration, but did give straight lines on log-log plots. Taking the weight fractions of the Aroclor standards represented by such individual peaks, C_p, from Webb and McCall[12] for Aroclors 1242, 1254, and 1260, and from Sawyer[22] for Aroclor 1016, calibration plots were constructed as the best straight lines through each set of log (H_p) vs. log Aroclor (C_p) data points, as measured under fixed conditions of injection volume and attenuation. These calibration plots were then used to determine the concentrations of the PCB isomer(s) responsible for each individual peak in the chromatograms of the serum samples.

Similarly, the summed heights of the peaks selected by the analyst for analytical "Aroclor" determinations, ΣH_s, were also found to give straight lines against Aroclor or log-log plots, permitting the construction of similar calibration plots for determining "Aroclor" levels. Examples of such calibration plots are shown in Figure 2. This procedure for constructing calibration plots differed slightly from that used by the analyst, which was to draw smooth curves between successive data points on a simple plot of ΣH_s vs. Aroclor.

In order to calculate the minimal initial concentrations (MIC's) of the Aroclors present in the serum samples, the heights of all the peaks relative to those in the Aroclor standards were examined to identify the most persistent peaks in each region of the chromatogram, and then to select those that were also present at reasonably different levels in the different Aroclors. The peaks selected were for Aroclor 1260, peak 448/528; for Aroclor 1254, peak 186; for 1242, peak 84; and for 1016, peak 70. Taking the observed levels of PCB isomer(s) responsible for these four peaks as a, b, c, and d, respectively, the MIC's for the four Aroclors were calculated from these calculations:

$$MIC_{1260} = \frac{1}{0.021}\,a$$

$$MIC_{1254} = \frac{1}{0.084}\,(b - 0.124MIC_{1260})$$

$$MIC_{1242} = \frac{1}{0.027}\,(c - 0.173MIC_{1254} - 0.047MIC_{1260})$$

$$MIC_{1016} = \frac{1}{0.034}\,(d - 0.103MIC_{1242} - 0.132MIC_{1254} - 0.0027MIC_{1260})$$

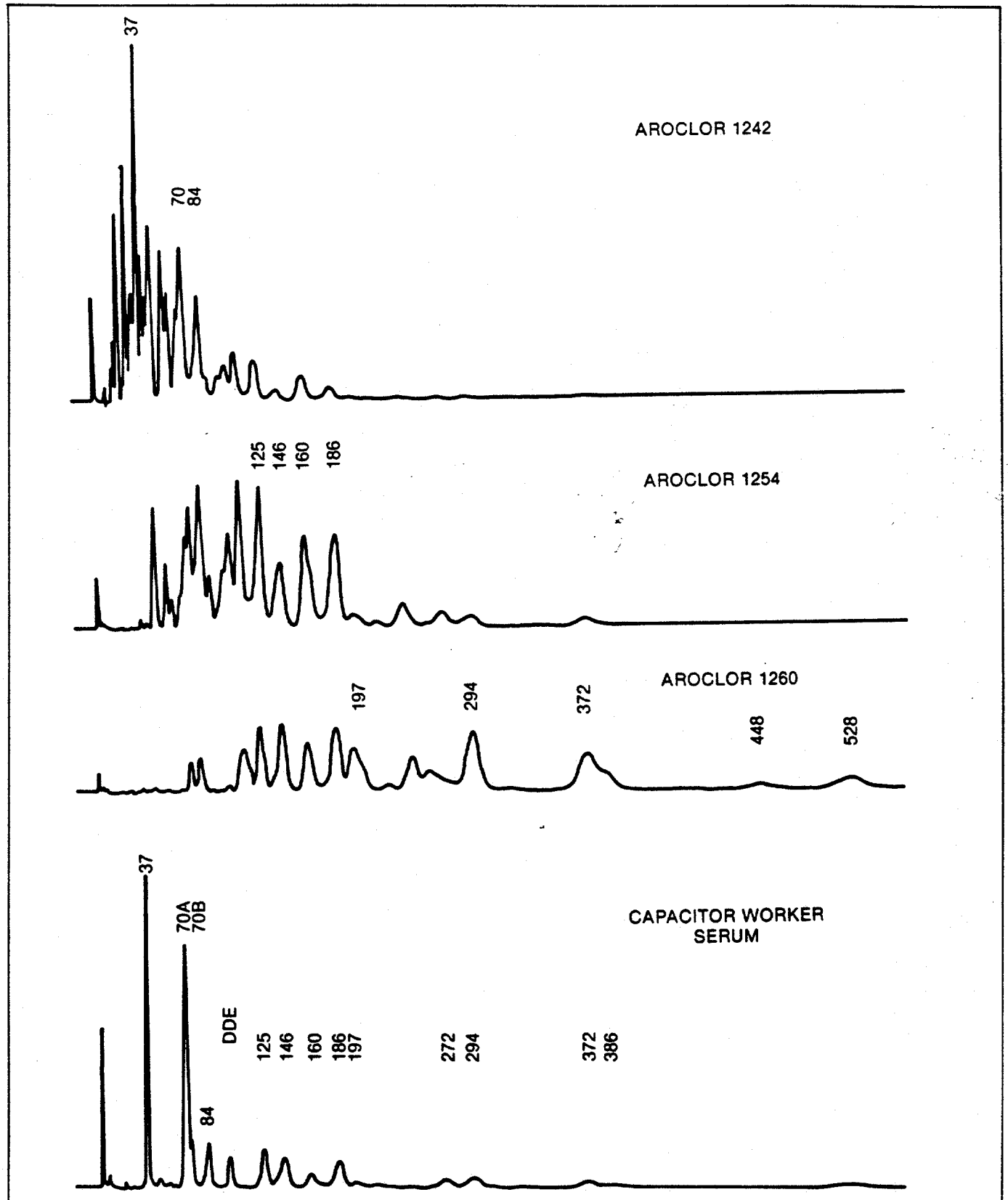

Fig. 1. Packed column gas chromatograms of PCB standards (Aroclor 1242, 1254, and 1260) and the pooled serum of 12 capacitor workers. Peak numbers indicate retention times relative to p,p'-DDE = 100. Peaks used for measurement of "Aroclors" by the sum-of-selected-peak-heights method were, for Aroclor 1242, 37, 70, and 84; for Aroclor 1254, 125, 146, 160, and 186; for Aroclor 1260, peaks of Aroclor 1254 plus 197, 294, and 372.

where the numerical coefficients represent the C_p values taken from Webb and McCall[12] for Aroclors 1260, 1254, and 1242, or from Sawyer[22] for Aroclor 1016.

Statistical analyses. Statistical analyses were performed on a Honeywell 600/6000 computer with time-sharing applications programs[23] for curve fitting (CURFIT) and linear regression with confidence limits (COLINR). The assumptions of normality of distribution were examined by the *W*-test.[24] Library guide examples[23] or problems in textbooks were employed for routine checks on program operation.

RESULTS

Alternative representations of the data. Table 1 lists the geometric mean observed levels of gas chromatographically resolvable PCBs in the sera of the directly exposed study population, along with the calculated levels of minimum initial concentration and minimum clearance. The relative quantities of retained and cleared PCBs are also displayed graphically in Figure 3 in such a way as to facilitate comparison with the raw data of Table 1. It is evident from Table 1 and Figure 3 that most of the di-, tri-, and tetrachlorobiphenyls had undergone extensive clearance in the population studied, but that there had been little selective clearance of hexa-, hepta-, and octachlorobiphenyls, i.e., those isomers giving peaks after 125. The sum of the minimum clearance values for peaks 146 through 448/528 (i.e., those characteristics of most of the Aroclor 1260 range) was indistinguishable from zero.

The peaks used for calculating analytical "Aroclor" levels by the sum-of-selected-peak-heights method are denoted by asterisks (*) on Table 1. The data in the last column show that while these were generally among the more persistent peaks, some of them were being cleared in the population examined. The rate of clearance of the persistent peaks used in calculating minimum initial concentrations was only briefly examined. The major component of peak 70, which was identified by capillary gas chromatography and mass spectrometry as 2,4,4′,5-tetrachlorobiphenyl, in widely scattered data on eight subjects, exhibited a mean decline of 56% over the 20-month period preceding the sampling giving the data shown in Table 1.

Table 2 summarizes the alternative ways of reporting the Table 1 and "Aroclor" data, using various ways of grouping the PCB peaks into reportable aggregates and

Table 1.—Observed Mean Levels of (Packed Column) Gas Chromatographically Resolvable PCBs in the Sera of 12 Heavily Exposed Capacitor Workers, Designated Indicator Peaks*†, and Calculated Levels of Minimum Initial Concentrations and Minimum Clearance

Obs. G. C. Peak‡	No. Cl per PCB§	PCBs in Peak (ppb) Standard Used§ A1242	A-1254	A1260	Mean	Minimum Initial Concentration (ppb) A-1016§	A-1242§	A1254§	A-1260§	Total	Min. Clearance (ppb)
11//	1	(6.1)			(6.1)	(2.6)	(5.0)			(7.6)	(1.5)
16	2	0.3			0.3	50.0	13.2			63.2	62.9
21	2	0.5			0.5	122.4	51.5			173.9	173.4
28	2,3	2.1			2.1	221.1	50.1			271.2	269.1
32	3	0.6			0.6	100.0	27.8			127.8	127.2
37	3	76.7*			76.7	243.4	52.4			259.8	219.1
40	3	0.6			0.6	192.1	50.6			242.7	242.1
47	4	3.8	2.2		3.0	152.6	40.1	7.9		200.6	197.6
54	3,4	1.3	0.9		1.1	101.3	31.0	3.7		136.0	134.9
58	4	1.1	1.1		1.1	84.2	25.5	1.8		111.5	110.4
70	4,5	112.4*	123.5	90.8	108.9	44.7*	46.9	16.7	0.5	108.8	0†
78	4	7.2			7.2		16.4	–	–	16.4	9.2
84	5	52.4*	28.6	24.3	35.1		12.3†	21.9	0.9	35.1	0†
98	5						6.8	9.5	0.4	16.7	16.7
104	5						10.5	17.2	0.3	28.0	28.0
117	6			0.2	0.2		–	–	0.6	0.6	0.4
125	5,6	14.2	18.5*	21.8*	18.2		7.3	19.0	2.3	28.6	10.4
146	5,6	29.4	21.0*	15.7*	22.0		4.6	13.2	2.9	20.7	-1.3
160	6,7		1.1*	4.0*	2.6			1.7	0.9	2.6	0
186	6		12.0*	14.0*	13.0			10.6†	2.4	13.0	0†
197	6,7		3.7	1.0*	2.4			2.3	1.8	4.1	1.7
222,244	6,7		2.3	1.3	1.8			1.3	1.9	3.2	1.4
272,294	7			3.7*	3.7				2.1	2.1	-1.6
332	7								0.8	0.8	0.8
372,386	8			1.5*	1.5				0.8	0.8	-0.7
448,528	8			~0.4	~0.4				0.4†	0.4	0†
Total	–	–	–	–	303	1312	447	127	19	1905	1602

* Peaks used for calculating indicated "Aroclor" level by sum-of-selected-peak-heights method.
† Peaks used for calculating minimum initial concentrations by method described in test; for such peaks the minimum clearance is zero by definition.
‡ Peaks indicated by retention times relative to p,p′-DDE = 100.
§ Peak compositions from Webb and McCall[12] for Aroclors 1242, 1254, and 1260; from Sawyer[22] for Aroclor 1016.
// Peak 11 in this population believed to represent 1,2,4-trichlorobenzene rather than monochlorobiphenyls; not included in totals.

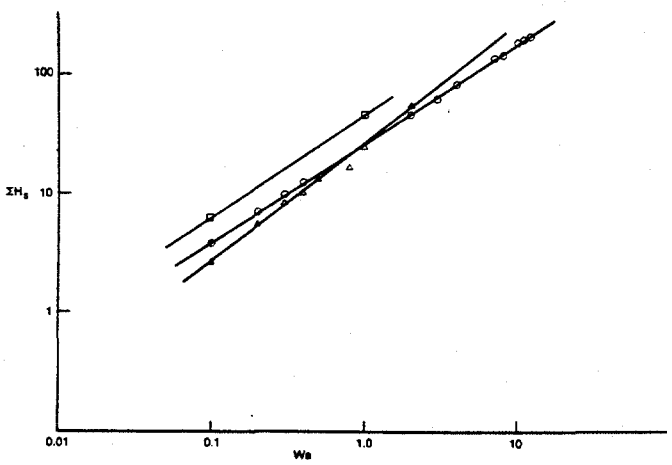

Fig. 2. Representative calibration plots showing the sum of the selected peak heights (ΣH_s) in scale division vs. the weight of the Aroclor standard (W_s, in micrograms) injected into the gas chromatograph. Data for Aroclor 1242, ○; for Aroclor 1254, △; for Aroclor 1260, □. Aroclor 1254 outlier not included in fitting calibration line.

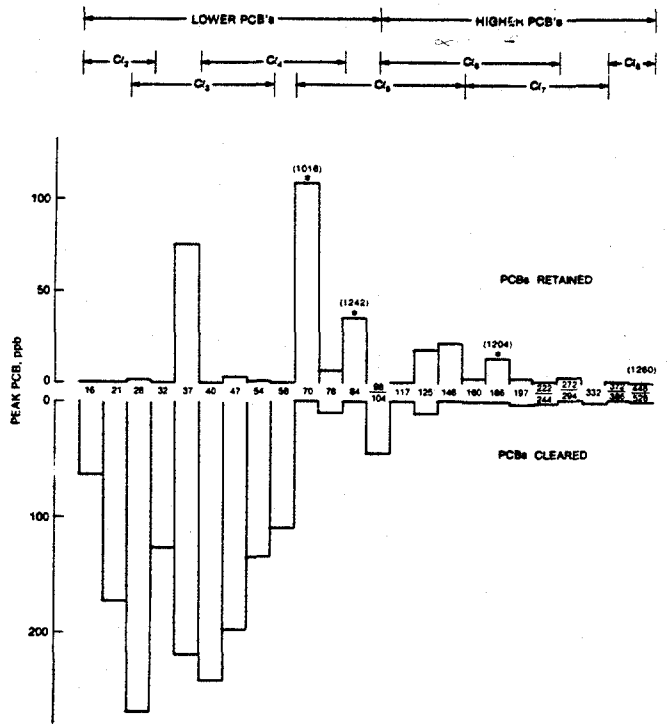

Fig. 3. Observed mean levels of retained PCBs in the sera of 12 heavily exposed capacitor workers, compared with calculated levels of minimum clearance, listed according to the relative retention times of the observable gas chromatographic peaks. The asterisks denote those peaks used for the calculations of minimum initial concentration (MIC) and minimum clearance.

various reportable descriptors of the aggregated PCB levels. The total analytical "Aroclor" is reported as the sum of only the "Aroclor 1242" and "Aroclor 1260" levels because there is only a 5% overlap in composition between those two Aroclors,[12] whereas the 1242-1254 and 1254-1260 overlaps are so extensive that the sum of all three "Aroclor" values would present a double reporting of the Aroclor 1254 data.

For the group studied here, the observed mean ratios between the reportable quantities LPCB and "Aroclor 1242," and between HPCB and "Aroclor 1260," were 0.237 and 0.71, respectively (Table 2). However, the peaks used in calculating "Aroclor 1242" were found to represent 93% of the total counted as LPCB, and those used for "Aroclor 1260" 96% of those counted as HPCB (Table 1), so that these conversion factors should also be approximately applicable to other similarly exposed populations as well. Likewise, the procedure for MIC_{1260} calculation and the observed persistence of relative peak heights in the heptachlorobiphenyl region (Table 1; Fig. 3) indicate that the parameter reported in Table 2 as "Aroclor 1260 minimum initial concentration" may be taken as substantially equivalent to that reported by NHMP as "human PCB."

Data precision. Table 3 presents the results of 24 replicate determinations on the control serum pool made on 6 successive days, assuming a normal distribution of values. This serum contained only about 15 ppb total PCBs, in accord with U.S. background levels reported by others.[24] The distribution assumption for each "Aroclor" was evaluated using the *W*-test for all measurements, for two individual days (there were unequal numbers of measurements on different days), and for the daily means. For this limited set of data we obtained mixed results, as a consequence of significant day-to-day variations and the effects of outlying values, but found no clear grounds for rejecting the normal distribution as a model of the analytical error at constant concentration. For the normal distribution the coefficients of variation (CVs) were 13.7% for DDE and 17–27% for the "Aroclors." For the three "Aroclors" the 96% prediction interval for a single measurement ranged from ±34 to ±53%.

In additional studies on the control serum pool we omitted the use of methanol in the extraction step, and observed PCB and DDE values only 45% and 49% of the Table 3 averages. These values were not increased by repeated (4X) hexane extraction.

The duplicate serial dilutions of the capacitor workers' serum pool with control serum produced the data shown as a log-log plot in Figure 4, which indicates homogeneous variance at different concentration levels. Assuming the analytical errors to be normally distributed at each concentration level (constant CV as in Table 3), then $CV_x \cong$ standard deviation of $\ln_x$ = 21.2%. The 95% prediction interval, which includes a single future measurement with 95% confidence, was ±42%, in general agreement with the data of Table 3. In experiments where the serial dilutions were made with water rather than control serum, the results were more erratic, and the 95% prediction interval was approximately ±80%.

To evaluate the portion of the total variance attributable to reading and calibration errors, we examined the original gas chromatograms obtained during the analysis of 57 different capacitor workers' sera, measured the sum of the heights of the selected peaks 37, 70, and 84 (ΣH_x), normalized for sample weight and injection volume, and used our own calibration plots (e.g., Fig. 2) to determine "Aroclor 1242" values. Com-

Table 2.—Alternative Ways of Reporting Same Analytical Data on PCB Levels in Capacitor Workers' Sera

Reportable PCB Aggregate		Reportable Parameter (ppb)		
Decription	Peak Numbers	Actual PCBs Present	Analytical "Aroclor"	Minimum Initial Conc.
~2-4 Cl-PB	16-70	195	-	1732
~5-6 Cl-PB	78-197	101	-	166
~7-8 Cl-PB	222-528	7	-	7
Total	16-528	303	-	1905
LPCBs	16-98	237	-	1800
HPCBs	104-528	66	-	105
Total	16-528	303	-	1905
Aroclor 1016	16-70	-	-	1312
Aroclor 1242	16-146	-	1000	447
Aroclor 1254	47-244	-	150	127
Aroclor 1260	70-528	-	93	19
Total	16-528	303	1093*	1905

* Sum of values for "Aroclor 1242" and "Aroclor 1260."

Table 3.—Results of Replicate Measurements of PCB and DDE in Control Serum Pool

Measurement	N	Mean (ppb)	Standard Deviation	CV (%)	95% Prediction Interval (%)
"Aroclor 1242"	24	7.93	2.13	26.8	52.5
"Aroclor 1254"	24	13.45	2.38	17.3	33.9
"Aroclor 1260"	12	7.37	1.52	20.6	40.4
p,p'-DDE	24	10.34	1.42	13.7	26.9

parison of our values to those reported by the analyst (Fig. 5) indicated the mean deviation from the line of identity to be −20% at 10 ppb and +20% at 4000 ppb, and the 95% prediction interval for a single reading and calibration to be approximately ±40%. Generally similar behavior was noted for the reading of the "Aroclor 1254" and "Aroclor 1260" peaks.

DISCUSSION

The most striking finding of the present investigation was the wide divergence among the numbers generated by alternative modes of reporting the same serum PCB data. Thus, the numbers for NHMP "human PCBs," total PCBs actually present (TPCB), total non-overlapping "Aroclors," and total minimum initial concentrations (MIC) in our study population were 19, 303, 1093, and 1905 ppb, respectively.

The divergence of the first of these figures arises because the NHMP records only one peak near the far end of the distribution, and then proceeds with a calculation like the one presented here for an Aroclor 1260 MIC. The other three values all describe total PCBs present, but at different points in time. Thus, the TPCB value sums up the levels of all PCB peaks present at the time of sampling. The total MIC indicates the minimum past Aroclor levels needed to account for the present levels of the most persistent peaks. The conventionally reported analytical "Aroclor" levels turn out to be intermediate between the TPCB and total MIC values, but closer to the latter, since they are calculated from fairly persistent peaks.

The divergence between the actual PCB and MIC values reflects the extent of selective PCB clearance, which varies with both the PCB and the exposed population involved. The present results showed that all peaks listed as significant constituents of Aroclor 1016[22] were >98% cleared by asymptommatic occupationally exposed capacitor workers within 4 yr after exposure except for peaks 37 and 70, and that both of these peaks were also being cleared, but more slowly. Thus, it would appear that Aroclor 1016, which was originally developed as an Aroclor 1242 replacement that would be totally biodegradable by environmental microorganisms, is also entirely metabolizable in the asymptommatic human, albeit somewhat unevenly.

On the other hand, it is also apparent from the Table 1 data that our occupationally exposed population was clearing the most slowly metabolized pentachlorobiphenyl isomers, those which are mainly responsible for peaks 84, 125, and 146,[26] at a rate at least 10-fold lower than has been reported[26] for Yusho (rice oil) poisoning victims, who ingested PCBs in admixture with the highly chloracnegenic polychlorinated dibenzofurans (PCDFs). In severe cases, such individuals eliminated almost all of their lower PCBs within 4 yr, and pre-

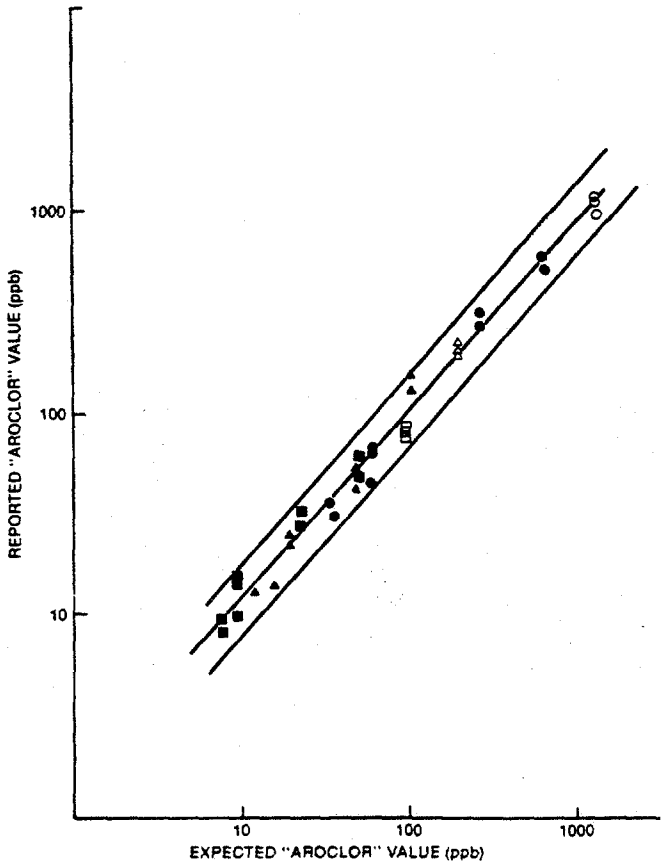

Fig. 4. Reported "Aroclor" values for serum samples prepared by serially diluting a capacitor workers' serum pool (*open symbols*) with a control serum pool, plotted against values calculated from the relative weights of the pools used. Line for regression equation ($y = 1.376x^{0.939}$; $N = 33$; $r^2 = 0.98$) and associated 95% prediction interval for individual measurements shown. ● = "Aroclor 1242;" ▲ = "Aroclor 1254;" and ■ = "Aroclor 1260."

sented chromatograms dominated by the peaks of the more refractory hexa- and heptachlorobiphenyls.[26,27] Thus, the PCB chromatograms shown in Figure 1, and the clearance patterns presented in Table 1 and Figure 3, may not be representative of those exhibited in cases where concurrent exposure to a cytochrome-inducing toxicant has occurred.

The calculations of MIC values for individual PCB peaks, and that of the total MIC, should both be as reliable as the reported C_n values[12,22] and the peak height measurements. However, the allocation of the total MIC among the individual Aroclors involved in a mixed exposure must be considerable less reliable because of the overlapping and variable PCB distributions present in the Aroclors. Nevertheless, for the population studied the calculated Aroclor MIC values (Tables 1 and 2), in contrast to the analytical "Aroclor" values, were in reasonable accord with the known occupational exposures to Aroclor 1026, 1242, and 1254, and a background-only exposure to a composition equivalent to Aroclor 1260.

In addition to the apparent interlaboratory variations arising from the use of alternative data reporting procedures, there are undoubtedly some real variations in serum PCB measurements resulting from the choice of analytical method. Fat extraction and clean-up procedures have varied widely in the past. Ouw et al.,[17] in a widely cited paper, used a method that did not remove pesticides and calculated PCB values from single peaks identified by retention times relative to Aldrin. The survey of human PCB levels conducted by Karppanen and Kolho[28] employed the single solvent extraction method described by Helminen et al.[29] Our observations, in general agreement with earlier reports,[10,11] indicated that this extraction method recovers only half the PCB present.

The results of the replicate analyses on serum samples of known composition, i.e., the serum-diluted pooled serum samples, indicated the 95% prediction interval for an individual measurement to be approximately ±42%. The corresponding measures of variance were similar for the replicate measurements on the control serum pool, but somewhat larger for those on the water-diluted serum samples.

The serum "Aroclor" measurements involved in these multiply replicated determinations incorporated manual procedures for reading the chromatograms of both test samples and Aroclor standards, and also for preparing and reading the calibration curves. Our check on the reproducibility of such procedures (Fig. 5) indicated the 95% prediction interval for an individual measurement to be approximately ±40%. Thus, these manual reading and calibration procedures, which have been used in much of the epidemiological literature, may have accounted for part of the interlaboratory variance in serum "Aroclor" levels.

More recently, computer techniques have been developed for the automated reading of specified peak areas

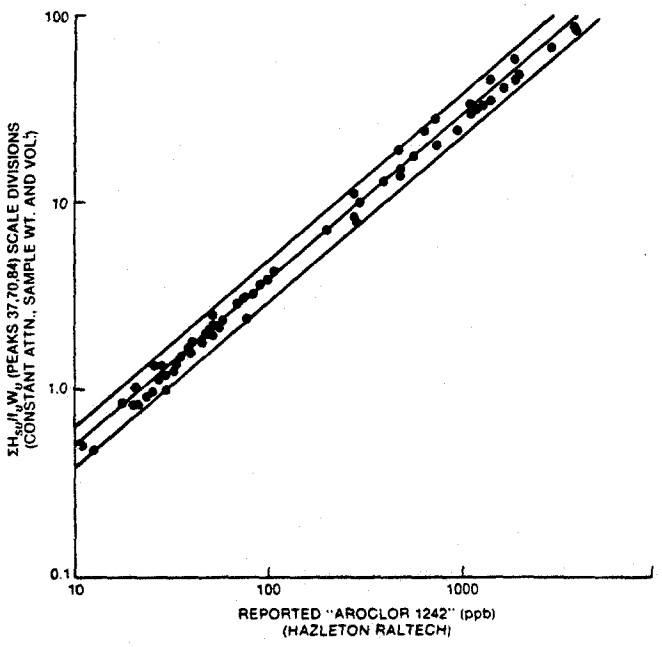

Fig. 5. "Aroclor 1242" levels in the sera of 57 capacitor workers: comparison between values reported by a commercial analyst and those recalculated from the original chromatograms of the Aroclor standards and the sera. Line for regression equation ($y = 0.657x^{1.075}$; $N = 57$; $r^2 = 0.99$) with its associated 95% prediction interval for individual recalculations, and the line of identity (————————) are shown.

and comparison with standards. Using such techniques, ESE[30] reported CVs of 7.3–14% for "Aroclor 1242" and "Aroclor 1254" in two pooled serum samples (N = 26). In another set of two pooled serum samples (N = 66), however, the CV rose to 12–20%, which was attributed to interfering peaks. In a study by Wolff et al.,[31] in which peak areas before and after p,p'-DDE were summed, 27 replicates gave the following CVs: p,p'-DDE, 9.8%; peaks before DDE, 11.96%; peaks after DDE, 23.1%. Both studies suggest an improvement in precision with automated peak reading techniques, but with considerable variance still remaining.

In summary, this investigation shows that random errors, interlaboratory variations in procedure, and methods of data reporting can all have considerable impacts on apparent human PCB levels. Such effects should not interfere with the use of serum PCB data obtained from a single laboratory for assessing environmental exposures of populations, or for statistical correlations with clinical parameters in epidemiological studies. However, they do merit attention when comparing exposure estimates or health effect studies reported by different investigators, or when considering the use of a specific serum PCB tolerance limit[17] as a basis for administrative action.

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