Sources and Magnitude of Error Associated with PCB Measurements

Polychlorinated biphenyls (PCBs) were first produced in the United States in 1929 and quickly found use in a wide range of domestic and industrial applications. PCBs were banned by Congress in 1976. Inadvertent losses and intentional disposal of products containing PCBs ultimately led to contamination of the environment on a global scale. Because of their persistence and toxicity, PCBs remain a pollutant of concern.

There are 209 theoretically possible PCB compounds (congeners). The commercial products (synthesized in the U.S. under the trade name Aroclor) are complex mixtures containing from 50 to 80 congeners. Each mixture consists of different, but overlapping, assemblages of PCB congeners. Because several Aroclors were used in industrial and domestic applications, the PCB composition of environmental samples is complex.

Early methods of measuring PCBs relied on comparison of sample gas chromatograms with those obtained for one or more of the commercial Aroclor mixtures. The analyst was required to select the Aroclor whose chromatogram most closely resembled that of the sample. Using one or a few peaks common to the chosen Aroclor and the sample, the concentration of PCB was estimated in Aroclor equivalents. If the sample chromatogram appeared to contain peaks from more than one Aroclor, quantitation was performed for each of the Aroclors.

Chemist Marilyn Castillo packs column in preparation for sample analysis.

Selection of quantitation peaks is problematic for some Aroclors (e.g., 1254) because they contain only a few components not found in other mixtures, and these are generally in low abundance. We refer to this general approach as the "Aroclor method" recognizing that different laboratories implemented procedural variations.

While this approach appears to have the advantage of simplicity, it requires subjective decisions on the part of the analyst and is only a semi-quantitative assessment of total PCB concentration. The drawbacks of the Aroclor method became evident as technological advances in gas chromatography revealed the complexity of environmental PCB assemblages.

The most obvious problem is that PCB congeners in Aroclor mixtures have a wide range of physical properties. When Aroclors are released to the environment, their composition can be altered due to volatilization, biological metabolism, and adsorption. Moreover, there can be anthropogenic sources of non-Aroclor PCBs. Because the Aroclor quantitation method assumes that sample composition is identical to one or more of the commercial mixtures, bias is introduced whenever non-Aroclor compositions are encountered.

A second problem is the compositional similarity among the Aroclors. This leads to overlapping chromatographic patterns (coelution) which can cause over estimation of the total PCB (ΣPCB) concentration when peaks common to two or more Aroclors are selected for quantitation.

Efforts have been made over the last decade to improve the analytical chemistry of PCBs (Pellizzari *et al.* 1985). This has led to the development of "congener-specific methods" to identify and quantify individual PCB congeners. These methods can produce more accurate results for environmental samples whose PCB composition is not identical to that of the Aroclors. The methods provide information on the environmental distribution of individual compounds, which is important since PCBs differ in their toxicity. Because of their stability, individual PCBs may be useful as molecular probes of natural processes.

As increasing numbers of laboratories adopt congener-specific methods, the question will be raised about comparability of the data with the Aroclor method. This is important in monitoring programs that assess environmental change over time, particularly in response to regulatory action, and in inter-laboratory comparisons.

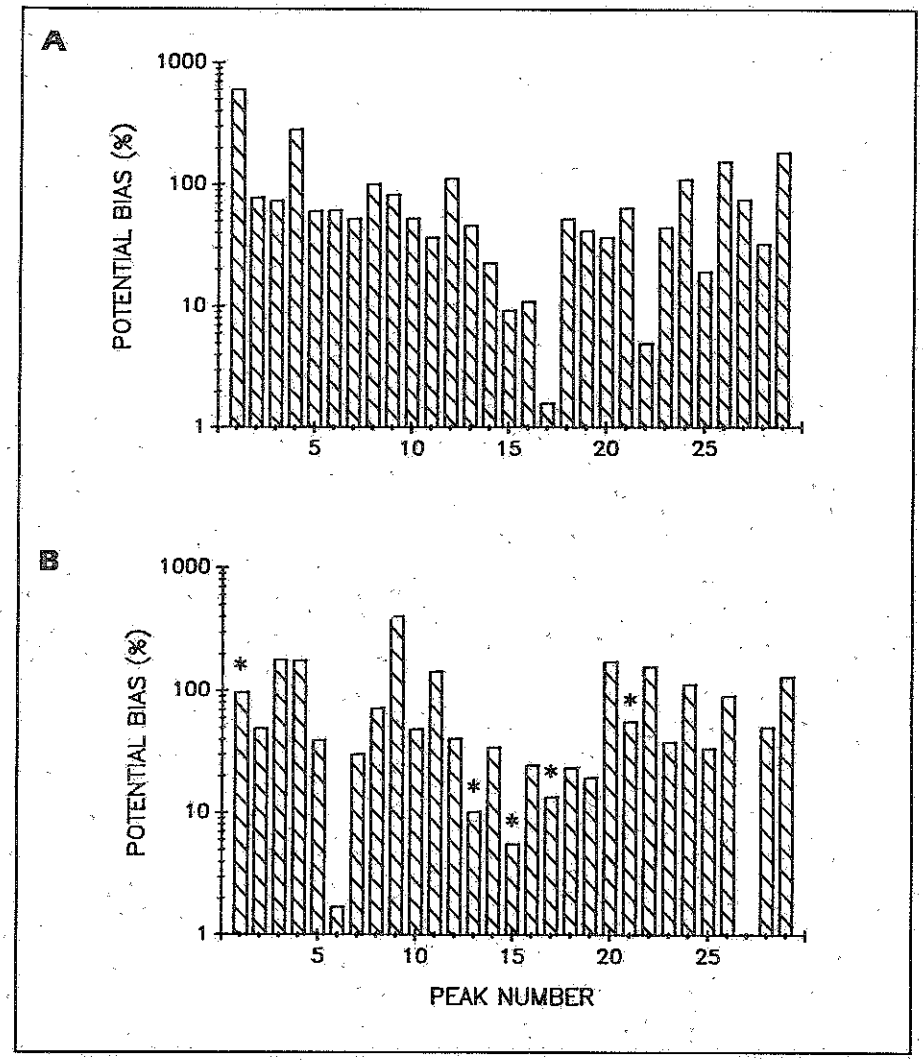
This article is about the sources and magnitude of bias during the instrumental calibration step (gas chromatography/electron capture detector: GC/ECD). No single high resolution chromatographic column has yet been demonstrated to separate (with baseline resolution) all 209 PCB congeners. Because isomeric PCBs exhibit variable ECD response, errors can arise when two or more congeners are present in a single chromatographic peak. Recent characterizations of Aroclor compositions by multidimensional GC (Shulz *et*

Table 1.
Summary of Aroclor compositions derived from Shulz et al. (1989) who analyzed the Aroclor mixtures by multidimensional GC/ECD.

	Aroclors				
	1016	1242	1254	1260	1:1:1[a]
Total number of peaks	35	53	52	54	86
Percent multicomponent peaks	40	36	29	26	36
Weight percent multicomponent peaks	76	71	63	67	67

[a]1:1:1 mixture of Aroclors 1242, 1254 and 1260

Figure 1.
Estimates of potential bias during quantitation of multicomponent peaks in Aroclors 1016, 1242, 1254, and 1260 under worst case scenario (see text for explanation). Response factor data produced on GC/ECD systems by A) Mullin et al. (1984) and B) Cooper et al. (1985).



al. 1989), and compilations of GC/ECD response factors for all 209 congeners (Mullin *et al.* 1984, Cooper *et al.* 1985), allow us to estimate the bias associated with this critical step. This will assist the bench chemist interested in using congener-specific procedures by showing how bias can be reduced. It also provides some insight into the sources and magnitude of bias associated with the Aroclor method.

Materials and Methods

Sediment samples collected from Los Angeles Harbor (see *PCBs in Los Angeles Harbor* in this volume) were used to compare the congener-specific method (Eganhouse *et al.* 1989) and the Aroclor method (Hu *et al.* 1980).

The congener-specific method uses a secondary calibration standard consisting of equal amounts of Aroclors 1242, 1248, 1254, and 1260. This standard contains about 130 congeners and yields 90 peaks. Sample chromatogram peaks are quantified by direct comparison with the calibration standard using the internal standard method.

Results and Discussion

Bias in the Congener-Specific Method

Individual Aroclors are characterized by 35 to 55 resolved chromatographic peaks; a mixture of Aroclors contains as many as 86 peaks (Table 1). Many of the peaks are composed of more than one PCB congener. Multicomponent peaks represent most of the total PCB by weight. Because PCB congeners exhibit highly variable GC/ECD responses, use of a single congener for calibration can lead to bias when a multicomponent peak is involved. Bias can be significant because a large proportion of the PCB peaks consist of coeluting congeners.

We estimated the bias introduced during GC/ECD calibration under several hypothetical scenarios. In the first scenario, the congeners selected for calibration of multicomponent peaks represent either the highest or lowest relative response factor (RRF). We assume that the peak is composed of one congener with the other extreme RRF value. This is a worst case scenario for computing the potential bias (PB) for individual peak and total PCB concentrations.

$$PB=\{(RRF_i-RRF_j)/RRF_j\}*100 \quad (1)$$

where RRF is the relative response factor (i.e. $\{[area]_{analyte}/[amount]_{analyte}\}/\{[area]_{IS}/[amount]_{IS}\}$ for congeners i and j

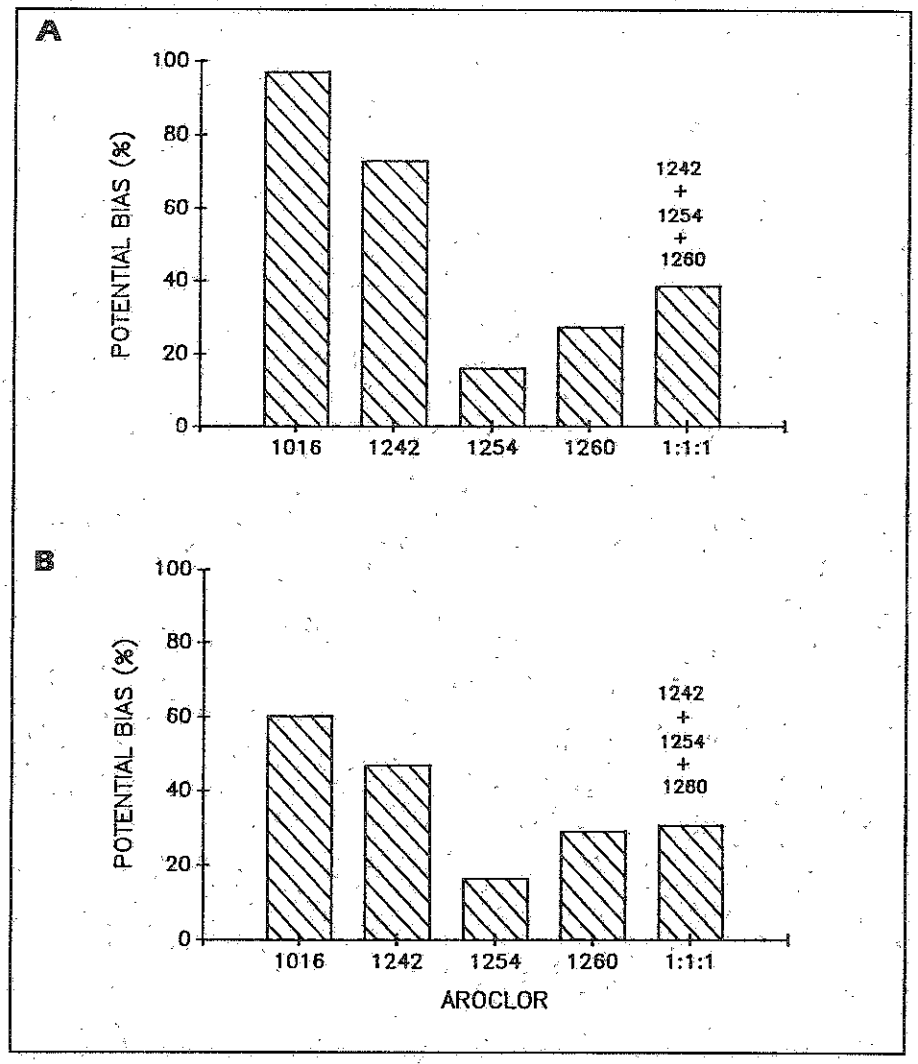
representing maximum and minimum RRF values. The internal standard (IS) was octachloronaphthalene.

The PB ranges from as much as 600% to less than 2% (Figure 1). Potential bias is greatest for peaks that contain congeners with high and low degrees of chlorination. Bias is less than 100% for the majority of multicomponent peaks. Comparison of individual peak PB values for the two data sets shows that use of the same calibration compounds on different GC/ECDs yields different results. Bars marked with an asterisk identify peaks where the sign of the bias is reversed for the two laboratories, assuming the same congeners were used for calibration. This indicates that use of the same congener-specific calibration standard on different GC/ECDs will result in variable inter-instrument bias. The sign and magnitude of the bias appears to depend on the peak in question, the compounds used for calibration, and the response of each GC/ECD. These are the only such data sets available, and we do not know whether the RRF data are representative of those from other laboratories. Consequently, it is unclear whether the PB estimates represent an upper limit.

In the second scenario, the composition of each of four Aroclors has been modified so that multicomponent peaks contain only one congener (an RRF extreme). The compositional data reported by Shulz *et al.* (1989) were combined with the bias estimates (Figure 1) to determine the potential bias in ∑PCB concentration. While this simulation is improbable—in that only multicomponent peaks were altered—it is a worst case estimate of bias in ∑PCB due to calibration with individual congeners. Aroclors dominated by either the lower or higher chlorinated congeners are subject to the greatest PB (Figure 2). The worst case is Aroclor 1016, which could be in error by about 100%. The PB estimates for Aroclors 1254, 1260, and the three-Aroclor mix are surprisingly low (<40%). Although the trends among Aroclors are the same for the two sets of data, the magnitude of bias is GC/ECD-specific. This may explain why laboratories often obtain close agreement in interlaboratory comparisons of simple PCB solutions with easily resolvable congeners, but rarely obtain agreement when actual samples are analyzed.

In the third and more realistic scenario, the congeners selected for calibration are the dominant constituent of each multicomponent peak in the original Aroclors. This situation applies to analysts implementing congener-specific procedures and using, as a basis

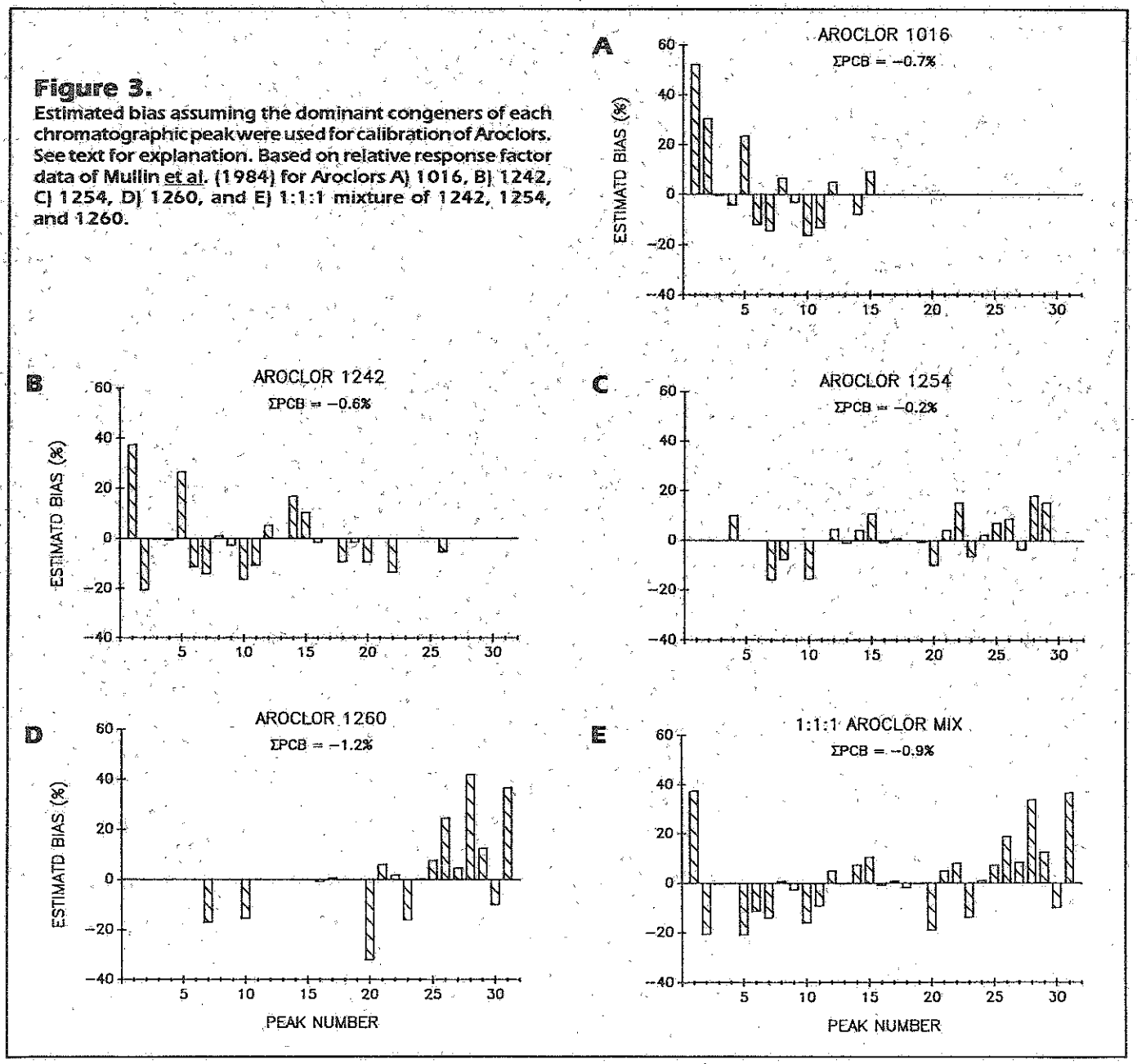
Figure 2.
Potential bias in total PCB concentration due to calibration with individual congeners. See text for explanation. Response factor data produced on GC/ECD systems by A) Mullin et al. (1984) and B) Cooper et al. (1985).



for compound selection, the data provided on Aroclor compositions by Shulz *et al.* (1989). Using the RRF results obtained by Mullin *et al.* (1984), we computed the bias that would result if the dominant congeners (of each chromatographic peak) were used for calibration during quantitation of the Aroclors (Figure 3).

The greatest bias involves the lower and higher chlorinated congeners. However, the magnitude of the bias is substantially reduced over the PB. Bias for individual peaks never exceeds 55%. Tetra-, penta- and hexachlorobiphenyls have much lower variation in ECD response; peaks containing these congeners have less bias. Not surprisingly, the average bias associated with multicomponent peaks in Aroclor 1254 is lower than those of Aroclors containing greater amounts of the higher and lower chlorinated congeners (e.g., 1016 and 1260). Because the sign varies, calibration bias for individual peaks is effectively cancelled and total PCB concentrations deviate from actual concentrations by less than 1.2%.

Figure 3.
Estimated bias assuming the dominant congeners of each chromatographic peak were used for calibration of Aroclors. See text for explanation. Based on relative response factor data of Mullin et al. (1984) for Aroclors A) 1016, B) 1242, C) 1254, D) 1260, and E) 1:1:1 mixture of 1242, 1254, and 1260.



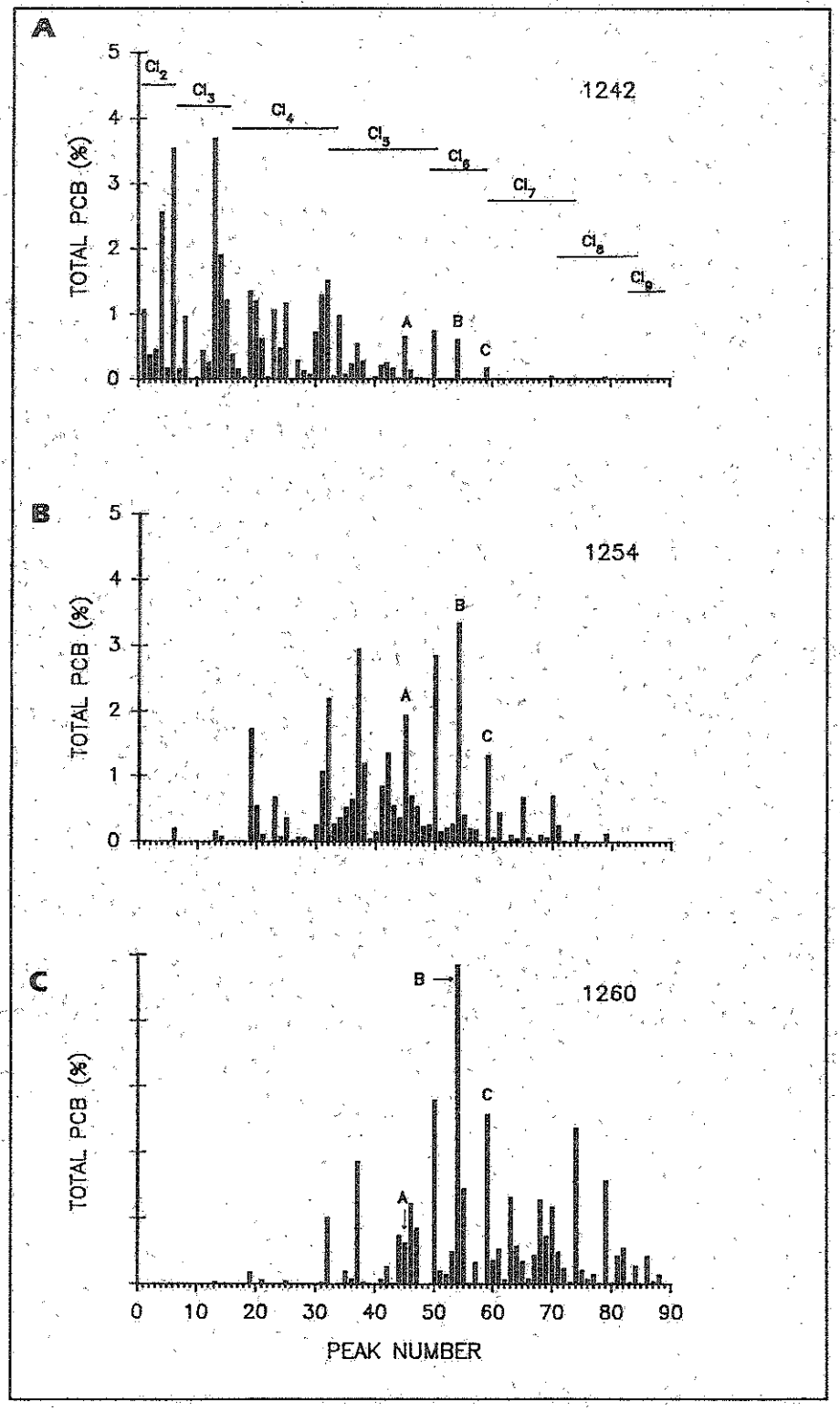
If the total PCB concentration is of interest, using the dominant constituent of each multi-component peak (in Aroclor mixtures) for calibration leads to little bias. On the other hand, if the PCB concentration of individual multi-component peaks is of interest, bias will vary depending on the peak under consideration. This simulation examined the bias associated with quantitation of unaltered Aroclor mixtures; it only approximates the results one might obtain with altered Aroclors. In the latter case, bias for individual peaks could increase or decrease depending on peak composition.

Bias in the Aroclor Method

In the past, many laboratories (including SCCWRP) performed GC/ECD calibration with individual Aroclors. One or more chromatographic peaks were selected to represent the Aroclor of interest. The areas under these peaks were directly compared with the areas of peaks eluting in the same region in the sample chromatogram. Quantitation of ∑PCB as specific Aroclors may be biased because: 1) two or more congeners with different RRFs coelute (multi-component problem), and 2) the same congeners occur in more than one Aroclor (overlapping assemblage problem).

There is a significant amount of overlap among Aroclors 1242, 1254, and 1260, particularly for chromatographic peaks of PCBs with four to seven chlorines (Figure 4). Aroclors 1242 and 1260 appear to have numerous resolved peaks free of major interferences from the other

Figure 4.
Contributions of Aroclors 1242, 1254, and 1260 to individual peaks in a hypothetical 1:1:1 mixture: A) 1242, B) 1254, C) 1260. Letters "a", "b", and "c" are quantitation peaks for Aroclor 1254 (cf. Table 3).



Aroclors. The interference-free peaks that contain only one congener are candidates for calibration with the Aroclor method. In contrast, Aroclor 1254, one of the most commonly reported mixtures in environmental samples, suffers from extensive coelution with components of the other Aroclors.

Computation of the bias associated with quantitation of 1254 in the presence of other Aroclors may reveal problems associated with historical environmental data based on the Aroclor method. We selected three peaks (congeners 77/110, 153/132/105, and 138/160/158; (Figure 4) that have been used by SCCWRP in the past to quantify Aroclor 1254. Data from Mullin *et al.* (1984), Cooper *et al.* (1985), and Shulz *et al.* (1989) were used to calculate the bias (Table 2) associated with quantifying a 1:1:1 mixture of Aroclors 1242, 1254, and 1260 (Table 3).

Bias ranges from 60% to 230% and varies from one quantitation peak to the next. The variation is caused by differences in peak composition of each

Table 2.
Estimated bias due to quantitation of Aroclor 1254 in the presence of other Aroclors.

$$[\text{conc}_i]_{mix} = \sum_{k=1}^{m} \left\{ [\text{conc}_i]_k \cdot [W_f]_k \right\} \quad (2)$$

where:
$[\text{conc}_i]_{mix}$ = concentration of congener i of the quantitation peak in the Aroclor mixture;
$[\text{conc}_i]_k$ = weight percent of congener in Aroclor k;
m = number of overlapping Aroclors that contain congener i; and
$[W_f]_k$ = weight fraction of Aroclor k.

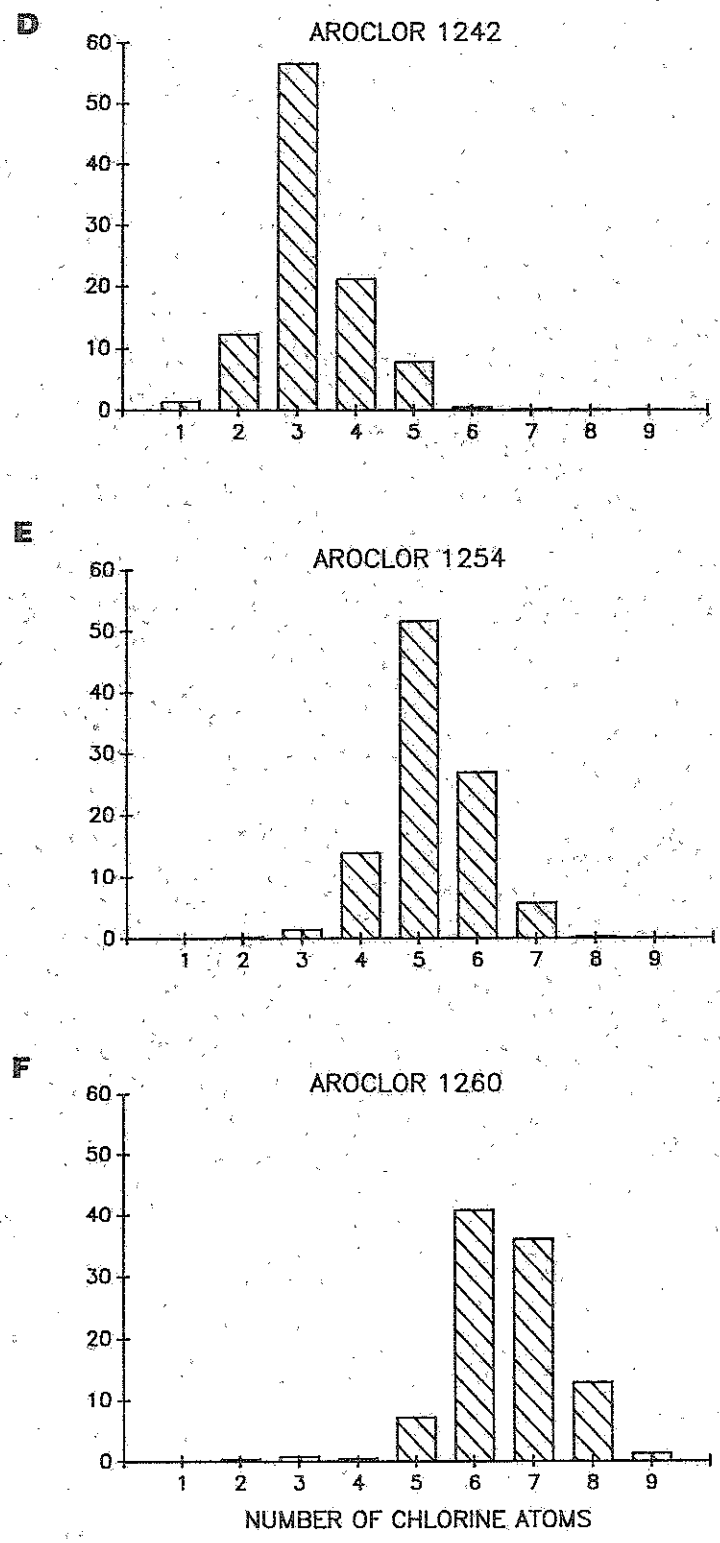
$$\text{Bias} = \frac{\left[\sum_{i=1}^{n} \left\{ [\text{conc}_i]_{mix} \cdot \text{RRF}_i \right\} \right] \cdot \sum_{i=1}^{n} [\text{conc}_i]_{k=q}}{\sum_{i=1}^{n} [\text{conc}_i]_{k=q} \cdot \text{RRF}_i} - \frac{\sum_{i=1}^{n} [\text{conc}_i]_{k=q} \cdot (W_f)_{k=q}}{\sum_{i=1}^{n} [\text{conc}_i]_{k=q} \cdot (W_f)_{k=q}} \cdot 100 \quad (3)$$

where:
RRF_i = relative response factor for congener i in the quantitation peak;
n = total number of congeners in each peak; and
q = Aroclor quantitated.

Table 3.
Estimates of bias associated with quantitation of Aroclor 1254 using the "Aroclor Method" for a 1:1:1 mixture of Aroclors 1242, 1254 and 1260.

	Bias (%)		Aroclor abundance[d] (%)		
Multicomponent Peak[a]	Mullin[b]	Cooper[c]	1242	1254	1260
77,110	63	—	20.3	60.1	19.5
105,132,153	146	176	7.0	38.0	55.0
138,158,160	208	227	4.4	32.4	63.2
Mean (SD[e])	139 (73)	202 (36)			

[a]Peaks containing several congeners based on data of Shulz *et al.* (1989).
[b]Based on RRF data of Mullin *et al.* (1984).
[c]Based on RRF data of Cooper *et al.* (1985).
[d]Contribution of each Aroclor to ΣPCB in each quantitation peak.
[e]Standard deviation.



peaks selected for quantitation (Figure 4), the composition of each sample (equations 2 and 3), and the analyst's assumptions about which Aroclors are present. Although it is unlikely that the hypothetical compositions upon which we based our estimates of bias occur in the environment, it is clear that the bias associated with the Aroclor method can be large.

Conclusions

There is little justification for using the Aroclor method to quantify ΣPCB. The potential bias associated with this approach is variable in sign and magnitude. The major factors that determine the magnitude of bias include: the peaks selected for quantitation, the relative abundances of the contributing Aroclors, and the composition of overlapping Aroclors (for multicomponent peaks). The magnitude of bias can vary between laboratories because of instrument-specific differences in the GC/ECD response of individual congeners and differences in peak selection procedures.

The *post hoc* estimation of bias is complicated because several factors contribute to it during calibration. No method is available that can completely resolve all 209 PCB congeners on a single high resolution capillary column. However, using congeners that represent the dominant components of multicomponent peaks during calibration reduces the error for ΣPCB to extremely small levels. This approach is recommended because it yields more accurate results and more meaningful data for toxicological and biogeochemical studies. ■

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