Ex. 17

The Use and Effect of Mixed Standards on the Quantitation of Polychlorinated Biphenyls

by F. Lee Beezhold and Virginia F. Stout
Pacific Fishery Products Technology Center
NOAA, National Marine Fishery Products Center
U.S. Department of Commerce
Seattle, Washington

Polychlorinated biphenyls (PCB's) have been recognized and well documented as global pollutants. An excellent summary of the distribution of PCB's in the global ecosystem has been prepared by RISEBROUGH (1968), and a bibliography covering PCB investigations from 1881 to 1971 has been compiled by QUINBY (1972 for the Oak Ridge National Laboratory.

Despite this history of investigations, the analytical quantitation of PCB's is still largely a matter of approximation. Gas chromatography, the most common analytical procedure for these compounds, is hampered by the lack of primary standards of individual PCB components. PCB's have most often been quantitated by measuring peak heights of prominent GLC peaks, as RISEBROUGH (1969), ZITKO (1971a), and HANSEN (1971), or by measuring total area of all PCB peaks as ARMOUR and BURKE (1970) have; and relating these to a suitable standard PCB mixture, such as Aroclor 1254 or 1260. This method may work in some cases as it did for SKRENTNY (1971), but it has severe shortcomings.

If the material being analyzed for PCB's contained only one particular Aroclor, such as 1254, and if the chromatogram of the sample matched that of Aroclor 1254, then measurement of peak heights or peak areas would be satisfactory in the quantitation procedure. Unfortunately, the samples we have been analyzing (fish and fish products) often contain widely varying mixtures of PCB's, and unless the chromatograms match those of a readily available standard, such as one of the Aroclors, an accurate quantitation of the chromatograms is very difficult. Even in cases where a specific Aroclor is fed to an experimental animal, KOEMAN (1969) and MULHERN (1971) found that the profiles of the PCB's retained often do not match those of the original Aroclor.

This in itself, would not be a problem except that, as reported by ZITKO (1971b), and as substantiated by us, the GLC response is not constant for the variously chlorinated isomers found in a PCB mixture. As a result, the values found when analyzing for PCB's can vary by a considerable amount depending on which Aroclor, or mixture of Aroclors, is used as a standard.

The purpose of this paper is to (a) illustrate the problem created by samples containing mixtures of PCB's not readily comparable to a particular Aroclor; (b) to present some figures to illustrate, in numerical terms, the effect that the choice of standards has on the final result; and (c) to offer as a solution to the problem the use of standards of mixed Aroclors. For this reason, a detailed section on Standards has been included in the following section.

Bulletin of Environmental Contamination & Toxicology,
Vol. 10, No. 1 © 1973 by Springer-Verlag New York Inc.

MONS 084252

METHODS

Extraction and Cleanup

Samples were extracted into hexane and cleaned up on a Florisil (PR grade)* column, as reported by REINERT (1970). Elution from Florisil with neat hexane effected better cleanup than that achieved with the mixed solvent described by Reinert.

PCB Separation

PCB's were separated from DDT and its analogs by means of a silica gel column according to the method of REINERT (1971) with a slight modification. Reinert recommended using completely anhydrous silica gel activated at 200°C for 8 hours prior to use, but treating the silica gel this way resulted in the recovery of only 30-40% of the PCB's. Activation for 17 hours and addition of 2% H_2O by weight to the activated silica gel gave 85-100% separation of PCB's and pesticides.

Chromatography Conditions

A Wilkens 600D gas chromatograph with a tritium detector and a 5'x 1/8" silanized glass column containing equal parts of 10% DC-200 on 80/100 mesh Gas-Chrom Q** and 15% QF-1 on 80/100 mesh Gas-Chrom Q** was used for quantitation. Column temperature was maintained at 195°C by a Varian Aerograph 328A Isothermal Unit. Injector temperature was 240°C and the N_2 gas flow 50-60 ml/min.

Quantitation

The choice of appropriate mixed standard was made by visual comparison of gas chromatograms of the extract from the sample and of varying proportions of PCB standards. Quantitation was achieved by cutting out the chromatograms and weighing them on an analytical balance. Such an integrated measurement of total peak area offers the best possibility at present of compensating for the differences between a sample containing most of a particular Aroclor (some components having been either lost or differentially metabolized) and a standard of that particular Aroclor.

Standards

A detailed description may prove useful. Once separate standards of Aroclors 1254 and 1260 had been prepared, mixtures of the two were prepared at increments of about 10% in Aroclor 1260 content. The total (1254 + 1260) concentration for each mixture was kept approximately equal so that the chromatograms could be compared easily and an assessment could be made of the changes

*Mention of trade names and specific companies does not imply endorsement

**Prepared by Applied Science Laboratories, Inc., State College, PA

MONS 084253

in total peak area with the changes in Aroclor composition. Figure 1 illustrates the change in total peak area as the composition of the standard changes.

Once all the mixed standards had been run on the GLC, the chromatograms were cut out, weighed (to give Figure 1), and placed in a reference file. This allowed the GLC operator to match rapidly the profile obtained from a sample with that of

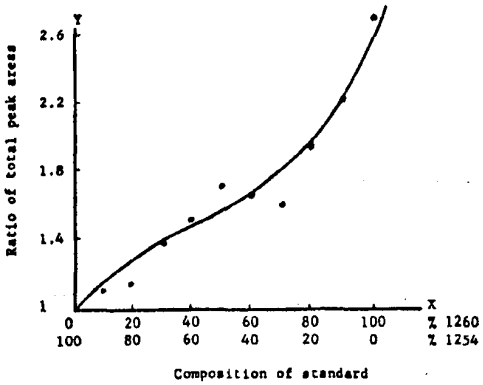

Figure 1. Dependence of total peak area on the relative amounts of Aroclors 1254 and 1260 in a mixed standard. (Total peak area = g paper/ng. PCB's injected).

the appropriate mixed standard. It was then relatively easy to make several appropriate dilutions of the mixed standard to obtain a standard curve. The same procedure can also be used for preparing mixed standards of other Aroclors, such as 1242 and 1254. Mixed standards of three or more Aroclors are feasible but obviously more complicated.

RESULTS AND DISCUSSION

Figure 2 illustrates the problem created by samples containing mixtures of PCB's not readily comparable to a specific Aroclor. Chromatograms of extracts of menhaden (Brevoortia tyrannus) meal, channel catfish (Ictalurus punctatus), and silky shark (Carcharhinus falciformis) liver*** are placed to indicate

***From the laboratory of Dr. George Harvey, Woods Hole Oceanographic Institution

MONS 084254

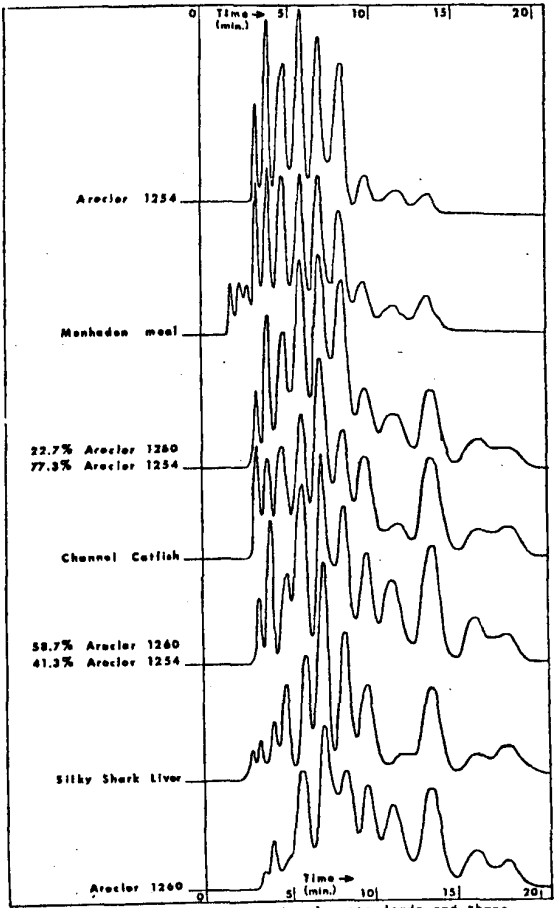

Figure 2. Gas chromatograms of Aroclor standards and three fishery products. Single vertical line represents solvent peaks.

MONS 084255

TABLE

Variation of total PCB residues (Aroclor 1254 and 1260) as the composition of the quantitating standard changes

% 1260	0	10	20	30	40	60	70	80	90	100
Species	Total PCB content (ppm)									
Silky shark liver	17.0	--	--	--	--	10.2	10.9*	8.5	7.5	--
Channel catfish+	6.7	6.2	6.1	4.9	4.4	3.5*	3.6	2.9	2.6	--
Goatfish+	5.4	--	--	--	--	3.4	3.5	2.8*	2.5	2.4
Menhaden meal	0.94*	--	--	0.80	--	--	--	--	--	0.43

*Best approximation of true value
--Not calculated
+Edible portion: muscle and skin

MONS 084256

the approximate composition in terms of Aroclors present. Although the chromatograms are somewhat crowded on the page, it can be seen that neither the channel catfish nor the silky shark can be quantitated as Aroclor 1254 or 1260. The profiles more closely resemble those of the mixed Aroclors.

The table illustrates what happens when a sample is quantitated by Aroclors 1254, 1260, and various mixtures of the two. The asterisks indicate the best approximation of the true values as calculated by the standards judged most similar in profile to the samples. If one were to plot the values with the changing compositions of the standards, the curves would resemble those of Figure 1. Both Figure 1 and the table illustrate the fact that the selection of a standard can alter the analytical values by more than a factor of 2. In the case of the channel catfish, it can be seen that the standard one chooses for quantitation could determine whether or not it is over the 5.0 ppm temporary tolerance proposed by FDA for fish.

SUMMARY

A study was made of the problems encountered when the chromatogram of a sample being analyzed for PCB's via GLC cannot readily be compared to a particular Aroclor standard. The use of mixed Aroclor standards was proposed and a study made of the effect of mixed standards on the analytical results. It was found that the choice of a standard could alter the values by more than a factor of 2. In addition, the particular mixture used as a standard should be reported along with resulting values.

References

ARMOUR, J.A. and BURKE, J.A., J. Ass. Offic. Anal. Chem. 53, 761 (1970)
HANSEN, D.J. et al., Bull. Environ. Contam. Toxicol. 6,113 (1971)
KOEMAN, J.H. et al., Nature 221,1126 (1969)
MULHERN, B. et al., J. Ass. Offic. Anal. Chem. 54, 548 (1971)
QUINBY, G.E. ed., Polychlorobiphenyls (PCB's) and Related Chlorophenyls: Effects on Environment, Vol. I, Bibliography 1881-1971, (1972), Oak Ridge National Laboratory, Oak Ridge, Tennessee (Oak Ridge Order No. TIRC-1, ORNL-EIS-72-20)
REINERT, R.E., Pestic. Monit. J. 3,233 (1970)
REINERT, R.E. and SNYDER, D., Bull. Environ. Contam. Toxicol. 6,385 (1971)
RISEBROUGH, R.W. et al., Nature 220,1098 (1968)
RISEBROUGH, R.W. et al., Bull. Environ. Contam. Toxicol. 4,192 (1969)
SKRENTNY, R.F., Bull. Environ. Contam. Toxicol. 6,409 (1971)
ZITKO, V., Bull. Environ. Contam. Toxicol. 6,464 (1971a)
ZITKO, V., Bull. Environ. Contam. Toxicol. 6,160 (1971b)

MONS 084257