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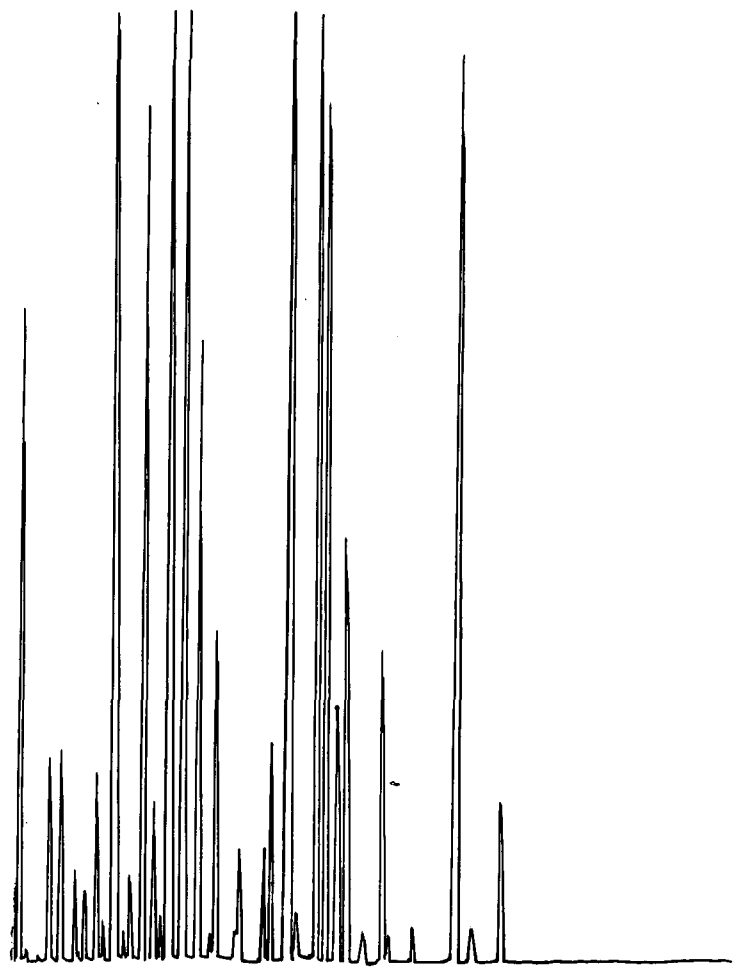
PCB Residue Levels in Human Adipose Tissue,

A Statistical Evaluation by Racial Grouping



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PCB Residue Levels in Human Adipose Tissue, A Statistical Evaluation by Racial Grouping

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1. INTRODUCTION

1.1. The National Human Monitoring Program

The National Human Monitoring Program (NHMP) was established in 1967 to monitor incidences of pesticide residues in the general United States population and to assess changes and trends in these levels. The program was initiated by the United States Public Health Service and was transferred in 1970 to the newly created United States Environmental Protection Agency.

The sample design of the National Human Adipose Tissue Survey, one of the major ongoing programs operated by the NHMP, involves several stages of selection. The conterminous 48 states were stratified into several geographic regions. Within each stratum, cities with populations greater than 25,000 were randomly selected for fiscal years 1972-76. In fiscal year 1977, the first-stage units were changed to Standard Metropolitan Statistical Areas (SMSA's). Hospitals were then selected within each sample city or SMSA. Cooperating pathologists and medical examiners supplied adipose tissue specimens.

Tissue specimens were analyzed by laboratories designated by the NHMP. Chemical analysis was accomplished by thin layer chromatography (TLC) until November 1974 when a gas chromatographic technique was adopted. These techniques are discussed in more detail in Sections 4.2.4 and 4.2.1, respectively.

Polychlorinated biphenyls (PCB's) possess chemical characteristics similar to those of organochlorine insecticides; hence, they also may be detected by the same chemical analysis procedures used to analyze human adipose tissue for pesticides. Because quantitating PCB's is more difficult than quantitating many other substances, the PCB concentrations in parts per million (ppm) were reported as falling in one of four categories: not detected, trace (detected but <1 ppm), 1-3 ppm, and >3 ppm.

NHMP adipose tissue surveys indicate that measurable residue levels of PCB's occur in a large percentage of the general population. Preliminary data summaries suggest that the higher levels of these chemicals are more prevalent in the nonwhite population. The overall objective of this report is to present the results of an evaluation of these apparent racial differences.

1.2. Study Methodology

The following activities were undertaken to evaluate estimates of PCB residue levels in adipose tissue with regard to the statistical meaning of apparent differences between racial groups:

1. Estimate and discuss the statistical impact of the survey design employed by the NHMP on the PCB residue level estimates.

2. Evaluate the level of "reading" error in the interpretation of the PCB chromatograms.

3. Investigate the implications of duplicity and possible biases related to the use of autopsy and surgical materials.

4. Assuming various plausible levels of design and measurement error effects, compute the statistical precision of PCB residue level estimates and the probability that real residue level differences between racial groups would be detected.

5. Statistically analyze the computerized data files.

It was concluded from a preliminary examination of a sample of the chromatograms that any "reading" errors resulting from manually integrating the chromatograms would probably be small compared to other sources of measurement error, i.e., in the analytical techniques used to measure the chemical residues. The analytical techniques were investigated, and lower bounds for the relative estimation precision of the PCB residue concentrations were developed on the basis of previously published results and experience. Different levels of precision in estimating the PCB residue concentrations will also result in different levels of precision in estimating the proportion (or percentage) of the population falling in a given classification category (i.e., percentage >3 ppm). The possible effects of measurement precision on the statistical significance of differences in the proportions of racial groups are discussed in Section 3.3.

In estimating the impact of the statistical survey design employed by the NHMP, the discussion in Section 3.4 focuses on the highest PCB residue level classification (>3 ppm). That is, the proportions of nonwhites with greater than 3 ppm PCB residue levels are compared to the proportions of whites having greater than that level. In this report, these proportions are denoted by P_n and P_w, respectively. The discussion is limited to this category for two reasons: (1) any health effect

of PCB's would be most evident in the highest concentration category and (2) one category is sufficient to illustrate the possible effect of the statistical design and measurement error.

Estimates for the proportions of whites and nonwhites having PCB residues greater than 3 ppm were calculated for the 1972-76 fiscal years assuming no design effect or measurement error. Tables illustrate how various levels of sample design effect and measurement error can affect the statistical significances of these differences. Fiscal 1977 data on the computer accessible data files were excluded because the first-stage sampling units were changed from the previous years. This precluded relating first-stage units in 1977 and other aspects of the sample design with the previous years.

In the discussion of the survey design impact, an example is used to illustrate the possible effects of the purposive exclusion of subpopulations resulting from sampling only within cities with populations of more than 25,000. The emphasis is on the bias potential for estimating the difference P_w-P_n, where the bias is defined as the expected difference minus the true difference. A similar discussion addresses the final-stage sampling of cadavers and surgical patients and the methods used in obtaining the tissue specimens.

The NHMP computer accessible data files were analyzed using a statistical method especially adapted for multistage survey data. This technique allows investigation of differences between racial groups after adjusting for other factors such as sex, age, geographic region, and fiscal year. The total variance of the estimates is approximated including both the measurement and sampling components. The analysis was based on the following two assumptions: (1) quantitation and sampling biases are similar for each racial group and (2) sampling within sample cities produced a nearly simple random sample within each city.

2. CONCLUSIONS

2.1. Issue of Racial Differences

The issue of whether or not racial differences exist in the distribution of PCB residue levels was examined for the category ">3 ppm" and for percentage detected (percentage of population with a trace, 1-3 ppm, or >3 ppm).

A summary of the analysis of the data on the NHMP computer accessible data files labeled "PCB's" is given below. (The PCB label is put in quotes because of the concerns noted below about quantitating PCB using only a single isomer).

For percent "PCB" >3 ppm:

1. Over the fiscal years analyzed (1972-76), nonwhites averaged 7.15 percentage points higher than whites.

2. Over the fiscal years 1972-76, there was an increasing trend in the percentage of people with "PCB" >3 ppm in their adipose tissue. The rate of increase is estimated to be 1.73 percentage points per year. The percentages of individuals with >3 ppm ranged from 2.58 percent in 1972 to 7.34 percent in 1976 for whites and 7.55 percent in 1972 to 16.67 percent in 1976 for nonwhites.

3. The trends over time (increase per year) for whites and nonwhites are 1.19 and 2.28 percentage points per year, respectively.

For percent positive detections:

1. Over the fiscal years 1972-76, there was an increasing trend in the percentage of people with some detectable "PCB" in their adipose tissue. The rate of increase is estimated to be 2.99 percentage points per year and means ranged from 84.58 percent in 1972 to 96.54 percent in 1976.

2. The trends over time for whites and nonwhites are 2.80 and 3.18 percentage points per year, respectively.

These findings listed above may not be precise or accurate measurements of PCB residues in the overall population because of potential inadequacies in the sampling and chemical analysis. However, based on the following assumptions: (1) quantitation and sampling biases are similar for each racial group and (2) sampling within sample cities produced a nearly simple random sample within each city, several significant differences were detected. The percentage of nonwhites having

greater than 3 ppm "PCB" is significantly higher than whites (probability of no difference <.001). There was no evidence of a difference in the percentage of whites and nonwhites with detected "PCB". The percentage in both categories investigated demonstrated a significant increase over time (probability of no time trend <.01). However, the apparent trend may be due in part to the change in chemical analysis methodology during the period studied. The trends appear to be the same for whites and nonwhites.

The apparent differences between racial groups could neither be confirmed nor denied because of the following two important reservations: (1) there are uncertainties about the accuracy and precision of using a single isomer to quantitate aggregate PCB's (see Section 4), and (2) the data appears to contain some inconsistencies and show greater fluctuations than normally expected in surveys of this size (see Section 5). The first reservation admits the possibility of bias in estimating differences between racial groups. The second is an indication of possible measurement or sampling bias. Because of these reservations, the data in their present form should not be used to make epidemiological inferences about aggregate PCB residues.

2.2. Statistical Design

The geographic stratification used in the NHMP sample design appears to have only a slight effect on the precision with which the proportions of each racial group having PCB levels >3 ppm or percent detected are estimated. This might be expected because of population mobility and the widespread use of PCB's. The clustering of sample individuals by cities is expected to decrease the precision of estimates, but this component of the variance was not estimated separately. Further, the effect on the variance of subsampling within the clusters cannot be estimated with any assured degree of accuracy because this stage of sampling was not conducted within a probability framework. The assumption of simple random sampling within cities is required for any statistical analysis of these data. The exclusion of some individuals in rural areas and the use of both surgical patients and cadavers qualify statistical inferences to the general U.S. population.

2.3. Chemical Analysis

The peak in the gas chromatograms labeled "PCB" has been tentatively identified as a heptachlorobiphenyl isomer (Appendix A). It is also possible that other halogenated organics may coelute, yielding false high values.* The "PCB" values were obtained by measuring one peak and correlating it with an Aroclor 1260 standard. This implies that the rest of the Aroclor 1260 pattern is "buried" under the pesticides and is present in a constant ratio to the measured peak. This procedure does not follow the recommended protocol (USEPA 1974 Section 5A[1]) and may not provide conclusive information on racial differences in PCB residues. Some chemists knowledgeable in the area of human tissue analysis doubt the reliability of the technique employed by the NHMP.

The TLC data (pre-November 1974) are estimated to have ±50 percent precision (Appendix B). Current data are insufficient to confirm or refute this estimate. As far as can be ascertained, some potential interferences (halogenated aromatics) may yield false high values.

2.4. Recommendations

2.4.1. Statistical Design

The purposive exclusion from the sampling frame of hospitals in cities with populations of less than 25,000 limits the inferences that can be drawn to the general U.S. population. This exclusion results in a substantial fraction of the total U. S. population having no chance of being selected in the sample. The additional selection of sample hospitals from cities of 2,500 to 25,000 persons would essentially eliminate this inferential limitation. Special arrangements may have to be made for hospitals in these smaller cities that do not have the medical facilities to secure and store sample tissues. The technique of selecting alternate first-stage sample sites also needs improvement. Repeating the process by which the original sites were selected would be satisfactory (see Section 5.1).

The limitations resulting from the use of judgment or convenience sampling are well known (Cochran 1977; Kish 1965). Hence, the method of selecting hospitals (or pathologists and medical examiners) should be conducted as nearly as possible within a probability framework. For instance, sampling frames for hospitals could be constructed for each

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*Personal Communications, G. W. Sovocool, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, October 1979, and H. Enos, University of Miami, Miami, Florida, October 1979.

sample city. The hospitals could then be randomly selected with a known probability. The judgment sampling of tissue specimens by cooperating professionals (pathologists or medical examiners) should also be modified. Simple protocols for selecting patients or cadavers and tissue specimens should be developed. Incorporating probability sampling methods or explicit procedures (where probability sampling is not feasible) would significantly improve the inferential value of the survey data.

For the majority of patients and cadavers, the zip code of residence can be recorded and, hence, the approximate geographic location of their residences can be ascertained. If the zip code of residence is not available, then the zip code of the hospital could be substituted.

The anatomical site from which the tissue sample is taken should be recorded. This may allow one to investigate and account for the difference in residue concentrations within the body. Variation of residues within the body does not preclude residue estimation, but it necessitates the use of a well-defined methodology for selecting or at least recording anatomical sites.

The above comments should be accompanied by an awareness of special problems that led to the purposive selection of certain hospitals and to the exclusion of hospitals in cities with populations of 25,000 or less. For example, long-term care facilities and mental hospitals in which surgery and postmortem examinations are not usually performed were excluded. The smaller cities were excluded because many of the hospitals within these cities do not have health care facilities with laboratory or pathology departments adequate for tissue sampling and storage. Some of these hospitals routinely embalm cadavers before autopsy and thus contaminate the adipose tissue.

2.4.2. Chemical Analysis

2.4.2.1 Evaluation of Current and Past Techniques

Data for PCB's obtained by gas chromatographic analysis in the NHMP should not be used as a basis for epidemiological inferences unless one of two validations is made as discussed below:

(1) The data collected over the past several years may prove useful if the peak being quantitated can be confirmed as a specific heptachlorobiphenyl isomer and shown to be free from interference by all halogenated organics known to be in general

tissue samples. The response of this isomer then could be measured and the concentration calculated. This would result in reporting a concentration (or range) of a given isomer that is observed but would not assume the other isomers are present when not observed.

(2) The second corrective measure would involve demonstrating (with a statistically valid sample) that the PCB profiles in the "unseen" part of the chromatogram are similar between the racial groupings and, therefore, that the heptachlorobiphenyl peak being measured is, in fact, indicative of "total PCB" concentrations in adipose tissue.

Even with these precautions, the data should be assumed to have an error of at least ±50 percent (see Section 4.2.2) unless better precision can be demonstrated.

The TLC data should be further validated to check for potential interference from such compounds as polychlorinated naphthalenes, terphenyls, benzene, and other aromatics and polybrominated aromatics. If used in the future, a selected portion (e.g., 10 percent) of the TLC determination should be confirmed by an independent technique such as GC/ECD or GC/MS.

2.4.2.2. Alternate Techniques for Future Work

The selection of analytical methods is a compromise between sensitivity and selectivity. GC/ECD is not only highly selective for halogenated compounds but also is one of the most sensitive techniques available with detection limits approaching the picogram (10^{-12}g) level. Several techniques are listed below in approximately increasing order of complexity and information content.

1. The easiest modification of current procedures would be simple confirmation of selected specimens (e.g., 10 percent) by an independent technique such as GC/HECD GC/MS, TLC, or perchlorination.

2. The method recommended in the EPA Pesticide Manual (USEPA 1974 Section 9 C) should be followed. The separation of PCB's from pesticides will produce more reliable and accurate data and will allow detection of early-eluting PCB peaks.

3. Modern chromatographic techniques should be considered. In particular, glass capillary GC will permit resolution of many more components and will significantly reduce chances of coelutions. Modern ECD's can be temperature-programmed. This increases the effective resolution of the chromatography. Precise retention times (Hewlett-Packard claims ±0.002 minimum precision) would also improve identification certainties.

4. Detection of PCB's could be significantly improved with mass spectrometric techniques. The "normal" electron impact MS detection limit of about 1 ng would produce problems for the detection of PCB's in NHMP tissue samples. This detection limit may be improved 10-100 fold by using selected ion monitoring (SIM), which is available on all quadrupole and many magnetic sector instruments. However, techniques such as negative ion MS have achieved extremely good sensitivities--better than ECD. If sufficient tissue specimen is available, high resolution MS will provide precise mass measurements to greatly aid in determining molecular formulae.

5. A combination of the above suggestions--cleanup by silicic acid chromatography and analysis by glass capillary GC/MS--is probably the most powerful routine PCB analytical system. Despite its high capital costs, capillary GC/MS is considered routine by many laboratories and is feasible for this program.

The above suggestions all move toward the ability to report individual PCB isomers (i.e., "70 pg 2,2',3,3',4'4'5-heptachlorobiphenyl" instead of "1.5 ng Aroclor 1260"). While this increases the burden of calculation for both the chemist and the statistician, the extrapolation to the health effects of PCB's is much more scientifically sound, particularly when it has been shown that toxicity and storage vary with individual isomers (Matthews and Anderson 1976; Biocca et al. 1976; McKinney 1976). Steps 1 and 2, above, along with a rigorous quality assurance program, should be considered a <u>minimum</u> for future analyses for PCB's in tissue.

3. STATISTICAL SURVEY DESIGN

3.1. Overview

The statistical design used to collect data for the Adipose Tissue Survey of the NHMP has several stages of sample selection. The conterminous 48 states were stratified into several geographic regions. Sample cities were selected from a list of eligible places (cities >25,000) proportional to their population. The hospitals were purposively selected according to type and size. Because sampling was conducted in selected hospitals in the sample cities, the sampling areas may be considered to be the union of the service area of the sample hospitals within each city. The specimens of adipose tissue from cadavers and surgical patients were obtained through the cooperation of pathologists and medical examiners. The cooperating professionals judgmentally selected the specimens. Guidelines were given to aid in the selection of cadavers, surgical patients, and anatomical sites (see Appendix F).

Certain sampling components differ between fiscal years 1970-72 and 1973-76. Before fiscal year 1973, the nation was stratified into the four census regions for purposes of sample allocation. The number of sampling sites within each region was assigned in proportion to the region's population. Population sizes used to set age, sex, and race quotas and for selection of cities were based on the 1960 census (Yobs 1971). Beginning in fiscal year 1973, the sample design was modified to reflect the demographic distribution of the 1970 census. The number of strata was increased from the four census regions to the nine census divisions. The quotas were adjusted to reflect the demographic distribution in each stratum (USEPA 1972b). Appendices D and E contain a listing of the census regions, divisions, and states and an example of the quotas assigned for fiscal years 1973-76. Figure 3.1 illustrates the various stages of sampling for the Middle Atlantic census division in fiscal year 1973. The sampling was conducted in an analogous manner for each stratum and in each fiscal year.

Data summaries for fiscal years 1972-76 were provided by EPA (USEPA 1977b). These summaries presented frequencies and unweighted relative frequencies for four residue concentration categories for each of several racial groups. Included are tables for each stratum (census division or region) and a national summary for each fiscal year. Appendix G con-

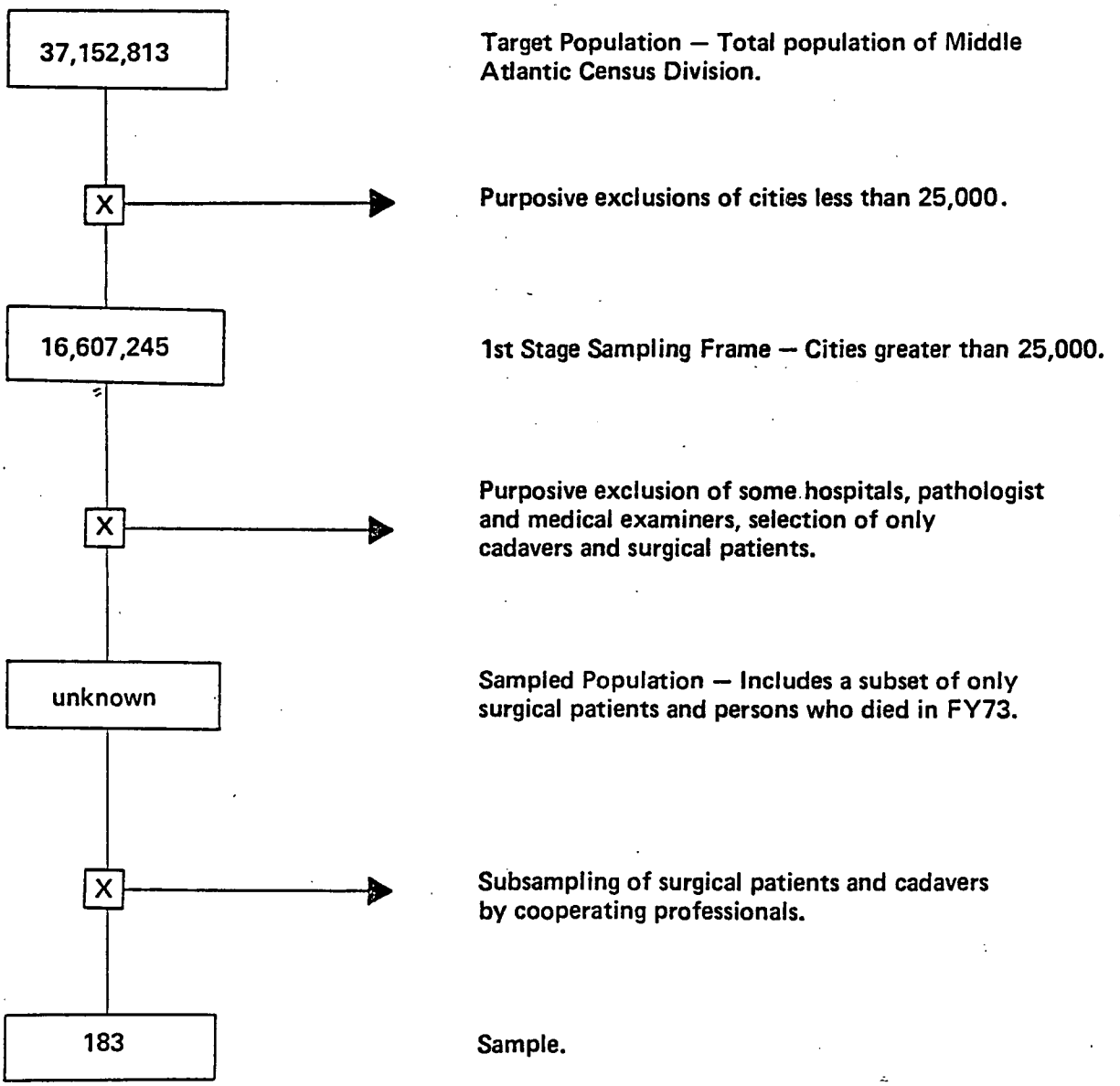

Figure 3.1 Sampling frame and selection proceudres for Middle Atlantic Census Division in FY73.

Source: Based on information contained in references (USBC 1972, USEPA 1972a and USEPA 1977b).

tains the national summaries. In a majority of the census regions and for each national summary, the proportion of nonwhites falling in the high concentration categories is greater than the proportion of whites. However, no statistical tests concerning the equality or inequality of the proportion of whites and nonwhites having concentration of PCB's greater than 3 ppm were performed by EPA on these data.

Selected statistical analyses (USEPA 1977c) using Statistical Packages for the Social Sciences (SPSS) were supplied by EPA. These analyses were run on data aggregated over several years and without weighting the data. The results supported the apparent PCB residue level differences between racial groups. However, because these analyses assumed simple random sampling and did not account for sources of error such as measurement variance, the statistical significance of these findings is questionable. In the next section the impact of various levels of measurement variance, sampling and measurement bias on the apparent significance levels is discussed.

3.2. Significance of Racial Differences Assuming No Design Effect or Measurement Error

The hypothesis of no difference between racial groups for the category >3 ppm ($P_n - P_w = 0$) is tested using the national data summary in Appendix F for the fiscal years 1972-76. Because of the large sample sizes, it is adequate to use the normal distribution to approximate the significance level of the test statistic

$$t_c = \frac{\hat{P}_n - \hat{P}_w}{[\widehat{VAR}\ (\hat{P}_n - \hat{P}_w)]^{\frac{1}{2}}} \quad .$$

where

n_w = Number of whites in the sample,

n_n = Number of nonwhites in the sample,

$\hat{P}_w$ = $\frac{\text{Number whites in the sample with >3 ppm}}{n_w}$,

$\hat{P}_w$ = $\frac{\text{Number nonwhites in the sample with >3 ppm}}{n_n}$,

and

$$\hat{\text{Var}}[\hat{P}_w - \hat{P}_n] = \frac{\hat{P}_w(1-\hat{P}_w)}{n_w} + \frac{\hat{P}_n(1-\hat{P}_n)}{n_n} \quad .$$

(The above variance formula is calculated by conditioning on n_w and n_n, hence no covariance term appears in the expression.)

Under the assumptions of simple random sampling, no measurement variance, and no measurement or sampling bias, the rightmost column of Table 3-1 can be thought of as the probability of no difference between the racial groups. Under these assumptions, fiscal years 1973 and 1976 exhibit what might be a "statistically significant" difference between races (the proportion of nonwhites is "significantly" larger than that of whites). In addition, there is a lesser indication that such a difference might exist in fiscal years 1972, 1974, and 1975.

In the following sections, tables illustrate the effects of various levels of measurement error, bias, and the sample design on the significance levels in Table 3-1 and, hence, on the validity of the claims of statistically significant differences.

3.3. Comments on the Impact of Measurement Error

It is difficult to relate the relative standard deviation of the measurement techniques (see Appendix B) to the error in estimating the proportion of the sample falling in a particular concentration range. A rigorous derivation requires knowledge of or assumptions on the distribution of the residue in the sampled population. Intuitively, it seems that any uncertainty in classifying tissue specimens correctly would increase the estimation variance. This is illustrated in the following example.

Consider the population consisting of five elements $\{e_1, e_2, e_3, e_4, e_5\}$. Elements e_1, e_2, and e_3 have real value one and elements e_4 and e_5 have real value zero. However, assume that e_3 and e_5 are classified correctly 50 percent of the time and incorrectly 50 percent of the time. Let P denote the proportion of the population with the value one. Hence, in this example, $P = 0.6$. The mean and variance of the estimator of $\hat{P}$ for a sample of size two is calculated for $\hat{P}$ considering the case above using first principles (Mood et al. 1974) and when no classification error occurs (error-free case) using the formula for the mean of

Table 3-1. Summary of Results for Testing the Null Hypothesis that $P_w - P_n = 0$ for FY72-FY76

FY	n_w	n_n	$\hat{P}_w$	$\hat{P}_n$	$\hat{P}_n - \hat{P}_w$	$\hat{SE}$	t_c	Significance level*
72	1469	303	.0530	.0825	.0295	.0169	1.74	.082
73	981	132	.0428	.1667	.1238	.0331	3.74	.000
74	798	119	.0438	.0924	.0486	.0275	1.77	.077
75	680	105	.0956	.1619	.0663	.0377	1.76	.078
76	569	103	.0738	.1942	.1204	.0405	2.97	.003

The information in this table is given in Appendix F, or calculated using the following equations.

$$\hat{P}_w = \frac{\text{Number whites in the sample with > 3 ppm}}{n_w}$$

$$\hat{P}_n = \frac{\text{Number nonwhites in the sample with > 3 ppm}}{n_n}$$

$$\hat{SE} = [\hat{Var}(\hat{P}_n - \hat{P}_w)]^{\frac{1}{2}}$$

$$t_c = \frac{\hat{P}_n - \hat{P}_w}{[\hat{Var}(\hat{P}_n - \hat{P}_w)]^{\frac{1}{2}}}$$

* Probability of a value occurring greater in absolute value than t_c, calculated by the equation

$$P = 1 - \int_{-|t|_c}^{|t|_c} \frac{1}{\sqrt{2\Pi}} e^{-\frac{1}{2}x^2} dx \quad \text{(Mood et al. 1974).}$$

the hypergeometric probability distribution (Mood et al. 1974). In both cases, the estimator $\hat{P}$ has an expected value of 0.6. Hence, P may be estimated in an unbiased manner even in the classification error case. In the error-free case the variance is 0.09 and for the classification error case the variance is 0.1025. Even in the situation where the measurement errors tend to be compensating, the variance of the estimated proportion is increased, in this case by more than 10 percent.

With the summary data available (USEPA 1972a and USEPA 1977c), it is not possible to estimate the reduction in precision of the estimated difference due to the presence of measurement errors. Techniques are available to obtain an overall estimate of the variance of the difference estimator (Hansen et al. 1953). This would involve obtaining the estimates of the proportions for each sample site from the NHMP data files. These techniques are based on the assumption that the sample site estimators are unbiased (as stated earlier, however, this requires assumptions about the data). An analysis technique discussed in Chapter 5 does approximate the overall variance of the proportions (or percentages).

Table 3-2 demonstrates the increase in the significance level for various levels of increase in variance due to measurement error. The relative standard error (γ) is defined as

$$\gamma = (SE^2 + \sigma_m^2)^{\frac{1}{2}}/SE ,$$

where SE is the standard error of the estimator $\hat{P}_w - \hat{P}_n$ assuming no increase in variance due to measurement error, and σ_m^2 is the additional variance due to measurement error. Small increases do not greatly affect the significance level; however, when the measurement variance equals SE^2 ($\gamma = 2$), the significance level is drastically increased in all cases. An increase of variance of this magnitude or greater due to the presence of measurement errors is not beyond the realm of possibility, considering the suspected measurement errors in the chemical analysis.

Table 3-2 can be used to estimate the true confidence of the usual expression for a 95 percent confidence interval ($\hat{\Theta}$-1.96SE, $\hat{\Theta}$+1.96SE) when measurement errors exist. For example, the true confidence for such an interval would be about 88 percent for a 25 percent increase in the standard error due to measurement error and 81 percent for a 50 percent increase in the standard error.

Table 3-2. Effect of Measurement Variance on the Significance Level of Test Statistics

Relative SE γ^*	True significance level for $\alpha^{**} = 0.05$	True significance level for $\alpha = 0.01$	True significance level for $\alpha = 0.001$
1.00	.0500	.0100	.0010
1.01	.0523	.0108	.0011
1.05	.0648	.0142	.0017
1.10	.0748	.0192	.0028
1.25	.1169	.0393	.0085
1.50	.1913	.0859	.0283
1.75	.2627	.1410	.0601
2.00	.3271	.1977	.1000
3.00	.5135	.3905	.2728

* $\gamma = \frac{(SE^2 + \sigma_m^2)^{\frac{1}{2}}}{SE}$ where SE is the standard error of the estimator $\hat{P}_w - \hat{P}_n$ assuming no increase in variance due to measurement error, and σ_m^2 is additional variance due to measurement error.

** α is the probability of making a Type I error, that is, concluding there is a difference when in fact no difference exists.

The entries in this table are calculated using the equation

$$P = 1 - \int_{-z_\alpha/\gamma}^{z_\alpha/\gamma} \frac{1}{\sqrt{2\Pi}} e^{-\frac{1}{2}x^2} dx, \qquad \text{(Mood et al. 1974)}$$

where z_α equals 1.96, 2.57, and 3.29 for α equal 0.05, 0.01, and 0.001, respectively.

Table 3-3 summarizes the effect of the increase in the standard error due to the presence of measurement error on the probability of detecting real differences between racial groups, if the probability of making a Type I error is 0.05 (the probability of concluding there is a difference between racial groups when in fact none exist). This is done for various levels of the relative standard error γ and the relative difference θ/SE where θ is the value of the true difference between P_w and P_n. If the difference is as large as the standard error ($\theta/SE = 1$), then the probability of detecting a difference of this size decreases from 0.17 (for $\gamma = 1.00$) to 0.0628 (for $\gamma = 3.0$). If $\theta/SE = 2$, the decrease is more dramatic, from 0.5160 to 0.1022.

3.4. Impact of the Sample Design

3.4.1. Restriction of Cities

The sample hospitals were selected from cities with populations greater than 25,000. The purposive exclusion of smaller cities may introduce bias in estimating proportions of interest. It is also possible that the bias could result in the appearance of differences between racial groups when no such differences exist.

Consider the following situation. The eligible cities constitute approximately 42 percent (.42) and the excluded areas 58 percent (.58) of the population. Let P_{sw} and P_{sn} denote the true proportions of the white and nonwhites in the sampled subpopulation, respectively. Let P_{uw} and P_{un} denote the true proportions in the unsampled subpopulation. Hence, the proportions must satisfy the relationships

$$P_w = 0.42\, P_{sw} + 0.58\, P_{uw}$$

$$P_n = 0.42\, P_{sn} + 0.58\, P_{un}.$$

It is possible for P_w to equal P_n and for P_{sw} and P_{sn} to be quite different; that, is the subpopulation sampled is actually different from the target population. The resulting bias in the estimation may give misleading results. To demonstrate, let $P_{sw} = 0.0428$ and $P_{sn} = 0.1667$. (See Table 3-1 for fiscal year 1973.) If $P_w = P_n = 0.10$, then the above equations can be solved for P_{uw} and P_{un} yielding 0.1414 and 0.0517 respectively. Hence by sampling only a subset of the target population, bias may be introduced into estimates.

Table 3-3. Effect of Measurement Variance on the Probability of Detecting Real Differences

		θ/SE**						
γ*	α†	0.25	0.50	1.00	1.50	2.00	3.00	4.00
1.00	.05	.0572	.0790	.1700	.3231	.5160	.8508	.9793
1.05	.05	.0565	.0763	.1584	.2980	.4781	.8152	.9678
1.10	.05	.0560	.0740	.1488	.2795	.4437	.7785	.9532
1.25	.05	.0546	.0685	.1259	.2244	.3596	.6700	.8925
1.50	.05	.0532	.0628	.1022	.1700	.2659	.5160	.7601
1.75	.05	.0523	.0594	.0882	.1374	.2079	.4031	.6277
2.00	.05	.0518	.0572	.0790	.1165	.1700	.3231	.5160
3.00	.05	.0508	.0532	.0628	.0790	.1022	.1700	.2659

* $\gamma = \frac{(SE^2 + \sigma_m^2)^{\frac{1}{2}}}{SE}$ where SE is the standard error of the estimator $\hat{P}_n - \hat{P}_w$ assuming no increase in variance due to measurement error, and σ_m^2 is additional variance due to measurement error.

** θ/SE is the value of the true difference between P_w and P_n divided by the standard error.

† α is the probability of making a Type I error, that is, concluding there is a difference, when in fact no such difference exists.

The entries in the table are calculated using the equation

$$P = 1 - \int_{\frac{-1.96 + \theta/SE}{\gamma}}^{\frac{1.96 + \theta/SE}{\gamma}} \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}x^2} dx \quad \text{(Mood et al. 1974)} .$$

The standardized bias of the estimator of $P_w - P_n$ is a function of the bias, $P_{sw} - P_{sn}$, and given by

$$\frac{\text{Bias}}{\text{SE}} = \frac{-0.1234}{0.0331} = -3.74 \quad .$$

Tables 3-4 and 3-5 allow one to gauge the impact of this standardized bias on the probability of concluding there is a difference between the proportions for the racial groups. They indicate that between 85 and 97 percent of the time one would erroneously conclude that the racial groups were different. Admittedly, this may be an extreme case. It is recognized that hospitals do indeed serve outlying areas beyond the sample city boundaries. However, in many states, particularly in the Rocky Mountain region, a significant portion of the population is, in fact, excluded from the sampled population because they live in rural areas.

Table 3-5 must be evaluated in proper perspective. At first glance, one may erroneously conclude that bias may even increase the likelihood of detecting real differences. In some situations, bias may in fact accentuate differences. In others, bias may conceal differences. In either case, bias can be misleading to the investigator in both the magnitude and direction of differences.

3.4.2. Sampling Cadavers and Surgical Patients

By restricting the sample to cadavers and surgical patients, one limits the sampled population to elements not actually belonging to the target population (cadavers) and to individuals that constitute a unique subset of the entire population (surgical patients). Bias similar to that discussed in the previous section may be involved if the proportions for each race of the sampled population and unsampled populations differ. Any inferences made about the general population are limited by the assumption that the sampled and unsampled populations do not differ substantially.

Another possible source of bias in using surgical specimens is the chance for repeated observations on the same individual, which will result in disproportionate sampling of persons who are prone to surgery. Precautions should be taken to prevent this from happening, but adjustments could be made in analysis to take this fact into account if it is recorded.

Table 3-4†. Probability of Detecting Real Differences for Various Levels of Positive Bias

θ/SE*	**BIAS/SE							
	0.00	0.25	0.60	1.00	1.50	2.00	3.00	4.00
0.00	.0500	.0572	.0790	.1700	.3231	.5160	.8508	.9793
0.25	.0572	.0790	.1165	.2396	.4169	.6141	.9015	.9890
0.50	.0790	.1165	.1700	.3231	.5160	.7054	.9382	.9945
1.00	.1700	.2396	.3231	.5160	.7054	.8508	.9793	.9988
1.50	.3231	.4169	.5160	.7054	.8508	.9382	.9945	.9998
2.00	.5160	.6141	.7054	.8508	.9382	.9793	.9988	1.0000
3.00	.8508	.9015	.9382	.9793	.9945	.9988	1.0000	1.0000
4.00	.9793	.9890	.9945	.9988	.9998	1.0000	1.0000	1.0000

* Standardized true difference.

** Standardized bias in estimating the difference θ.

† This table can also be used for the probability of detecting real differences for θ and bias both negative.

The entries in this table are calculated using the equation

$$P = 1 - \int_{-1.96 + \theta/SE + BIAS/SE}^{1.96 + \theta/SE + BIAS/SE} \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}x^2} dx \quad \text{(Mood et al. 1974)}.$$

Table 3-5[†]. Probability of Detecting Real Differences for Various Levels of Negative Bias

θ/SE*	**BIAS/SE							
	0.00	-0.25	-0.50	-1.00	-1.50	-2.00	-3.00	-4.00
0.00	.0500	.0572	.0790	.1700	.3231	.5160	.8508	.9793
0.25	.0572	.0500	.0572	.1165	.2396	.4169	.7852	.9633
0.50	.0790	.0572	.0500	.0790	.1700	.3231	.7054	.9382
1.00	.1700	.1165	.0790	.0500	.0790	.1700	.5160	.8508
1.50	.3231	.2396	.1700	.0790	.0500	.0790	.3231	.7054
2.00	.5160	.4169	.3231	.1700	.0790	.0500	.1700	.5160
3.00	.8508	.7852	.7054	.5160	.3231	.1700	.0500	.1700
4.00	.9793	.9633	.9382	.8508	.7054	.5160	.1700	.0500

* Standardized true difference.

** Standardized bias in estimating the difference θ.

† This table can also be used for determining the probability of detecting real differences for θ negative and bias positive.

The entries in this table are calculated using the equation

$$P = 1 - \int_{-1.96 + \theta/SE + BIAS/SE}^{1.96 + \theta/SE + BIAS/SE} \frac{1}{\sqrt{2\Pi}} e^{-\frac{1}{2}x^2} dx \quad \text{(Mood et al. 1974)}.$$

When a method other than probability sampling is used to select individuals, sampling bias in the resultant data becomes a real possibility. The purposive elimination of particular subpopulations should be avoided or, if unavoidable, the resulting limitations should be clearly used to qualify the results. Biases such as those discussed previously resulting from the purposive exclusion of small cities might also arise with purposive exclusion of certain types of hospitals.

3.4.3. Remarks on the Bias in Estimating Racial Differences

There is no prior evidence that any of the possible sources of bias discussed earlier affect the racial groups differently. If this is the real situation, the biasing effects would tend to cancel out when differences are estimated. Hence, the biases of the estimated difference between subpopulation proportions could be small. Also, bias introduced by the method of chemical analysis may be similar for each racial group. If true, this type of bias would also tend to cancel out when looking at differences between groups.

4. EVALUATION OF CHEMICAL ANALYSIS AND DATA INTERPRETATION

4.1. Objective

The objective of this section is to comment on the reliability of the raw PCB data reported as part of the NHMP.

4.2. Discussion

4.2.1. Qualitative Assessment

Chromatograms of the original data were obtained through the courtesy of Mr. John D. Tessari, Supervising Chemist and Laboratory Director, Colorado Epidemiologic Pesticide Studies Center, Colorado State University, Fort Collins, Colorado. A copy of the cover letter discussing the measurement calculation method is included as Appendix B.

Tissue specimens were extracted according to the modified Mills-Olney-Gaither procedure (USEPA 1974 Section 5A[1]) and analyzed by gas chromatography with electron capture detection (GC/ECD) on two columns, as discussed in Mr. Tessari's letter. The pesticides and PCB's were not separated using silicic acid chromatography as recommended (USEPA 1974 Section 9 C). Without this separation, some of the pesticides interfere with the GC/ECD analysis of some of the isomers of PCB's. It should be noted that the primary objective of this program was pesticide monitoring; the PCB analysis was appended with a directive that minimal additional effort be expended. Three chromatograms are presented in Figures 4-1, 4-2, and 4-3. Figure 4-1 represents a "high" PCB level (>3 ppm) sample, Figure 4-2 a "medium" level (1-3 ppm), and Figure 4-3 a "low" level (<1 ppm). Figure 4-4 shows the standard Aroclor 1260 chromatograms obtained under conditions similar to those in Figures 4-1, 4-2, and 4-3, on October 2, 1979, at Colorado State University. Comparison of Figures 4-1, 4-2 and 4-3 with the standard (Figure 4-4) indicates that a clear Aroclor 1260 pattern is not discernible among the pesticide peaks. Other PCB peaks (in addition to the later-eluting peak used in quantitation) seem to be present and lend support to the PCB identification, but there is no clear "fingerprint." This is reasonable because the environmental fate, absorption and excretion dynamics, and metabolic rate differences of the various PCB isomers dictate that a PCB pattern in tissue will probably not be consistent with a given commercial mixture as shown in Figure 4-5.

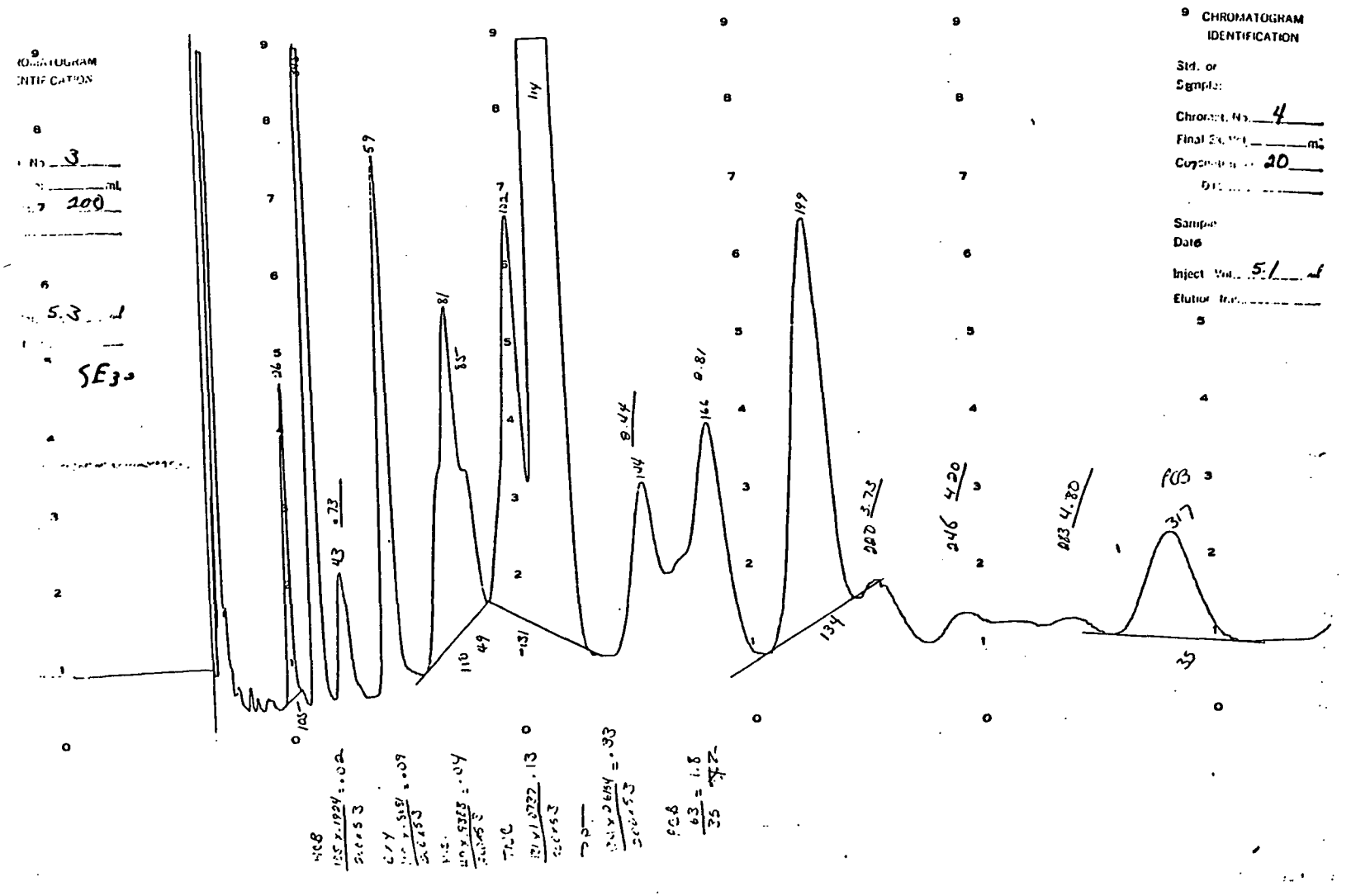

Figure 4.1 Chromatogram of a tissue specimen on 4% SE30/6% OV-210 column, reported as ">3ppm PCB" (factor calculated as 18. along bottom)
See Appendix A for source

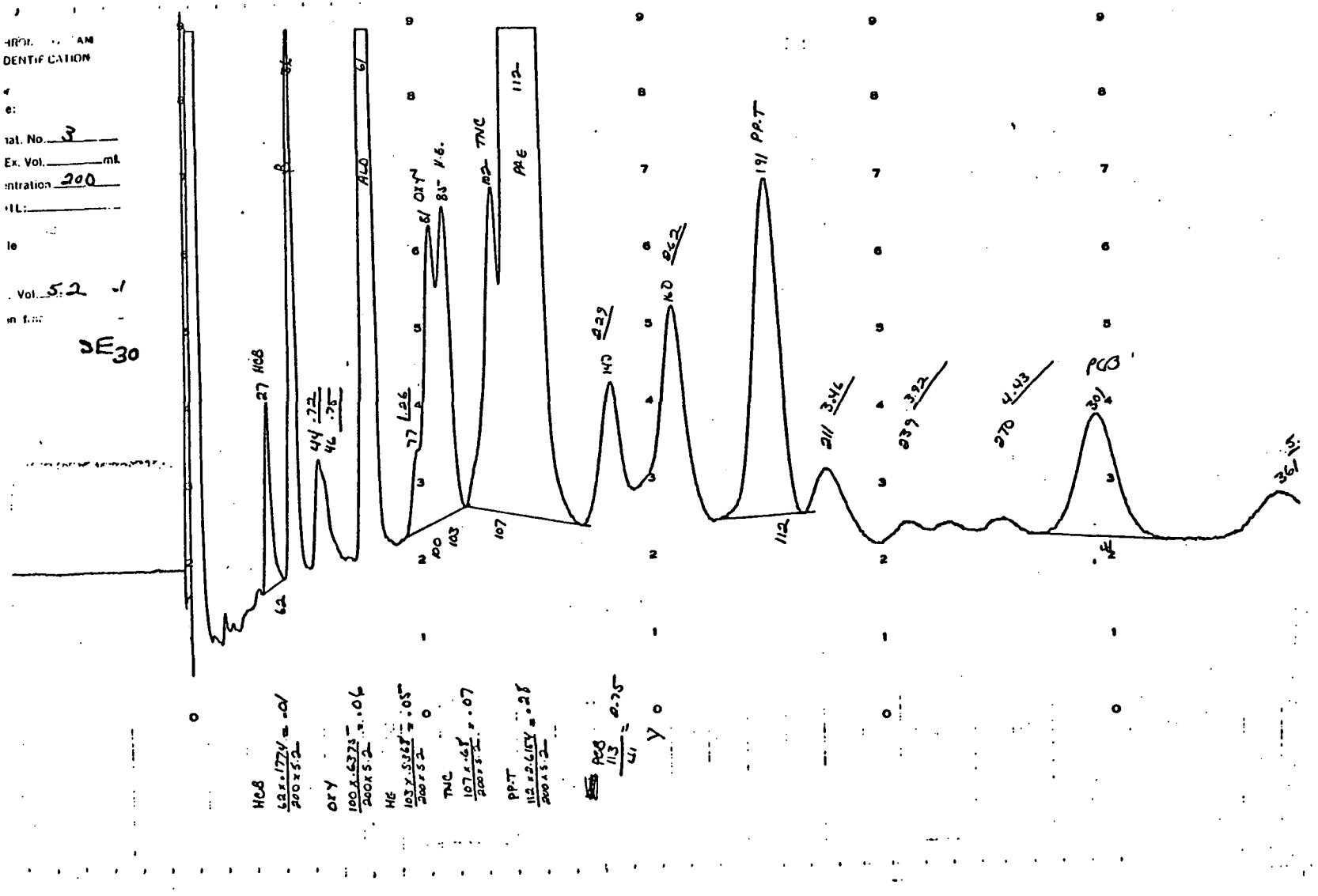

Figure 4.2 Chromatogram of a tissue specimen on 4% of SE30/6% OV-210 column, reported as "1-3 ppm PCB" (factor calculated along bottom as 2.75). See Appendix A for source.

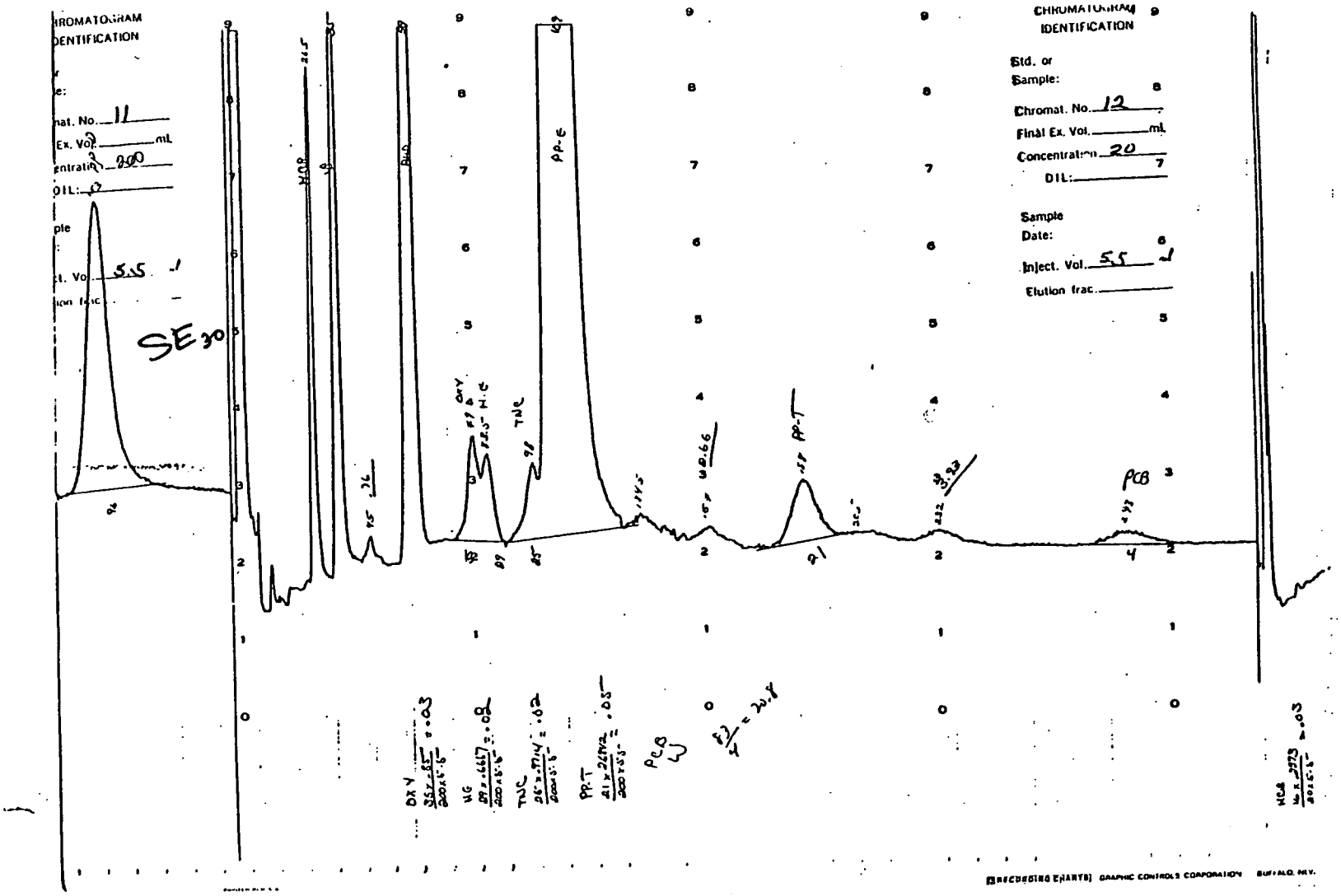

Figure 4.3 Chromatogram of a tissue specimen on 4% SE30/6% OV-210 column, reported as "<1ppm PCB" (factor calculated as 20.8 along bottom). See Appendix A for source.

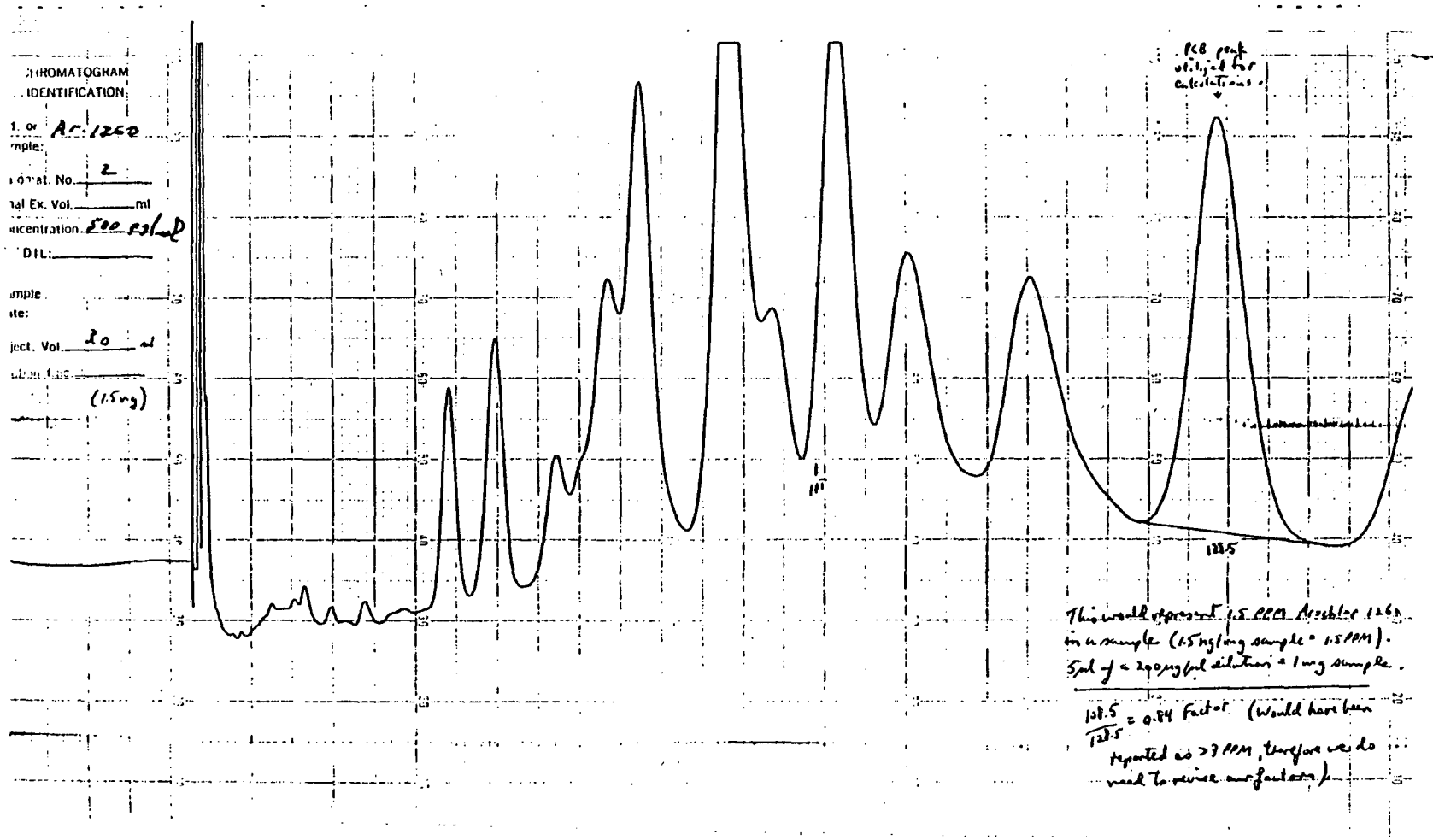

Figure 4.4 Chromatogram of equivalent to 1.5 ppm Aroclor 1260 standard
Note comment on chromatogram. See Appendix A for source

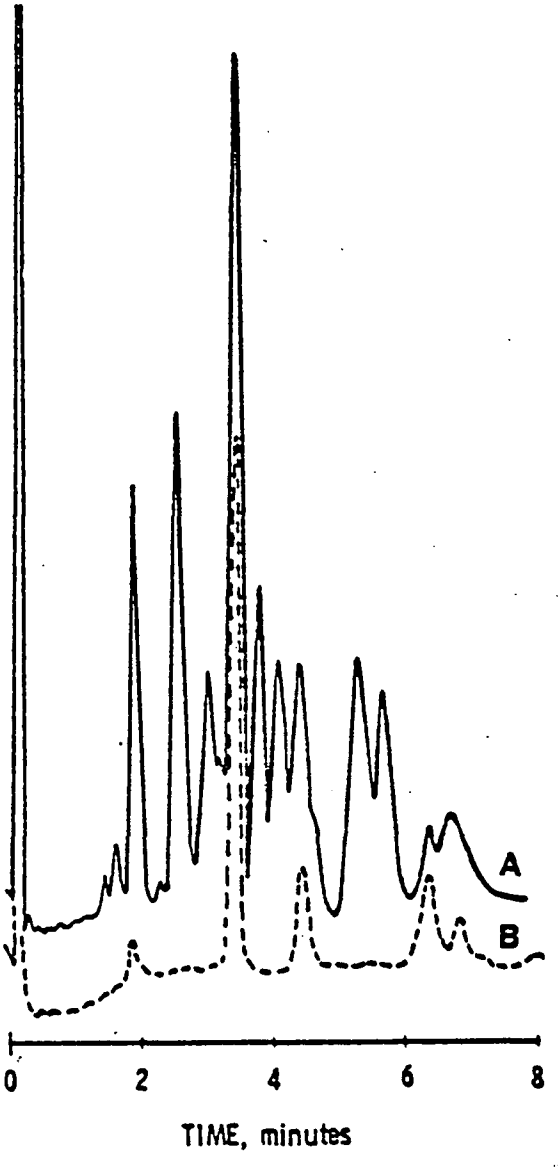

Figure 4.5 Electron capture gas chromatograms of (A) Aroclor 1016 standard and (B) PCB-residue extracted from brain of rat fed on diet containing Aroclor 1016 for one year. Reproduced from (Lewis 1977).

GC/MS confirmation of a composite (Figure 4-6) and overwhelming evidence from previous studies suggest that PCB's are in nearly all human specimens. In a two-year intensive study, PCB's were confirmed in 322 tissues by GC/MS.* In addition, recent evidence supplied by the Office of Pesticides and Toxic Substances of the U.S. Environmental Protecton Agency (OPTS-EPA) (Appendix A) confirms this peak as heptachlorobiphenyl. Although the evidence indicates that the peak being measured is heptachlorobiphenyl, interferences may be present in some specimens.

4.2.2. Quantitative Assessment

The single late-eluting peak was quantitated as discussed in Appendix B. A comparison of five integration methods (USFDA) showed that peak heights, disc integration, triangulation, peak height x width-at-half height, and retention time x peak height were not significantly different. Thus, the precision of integration is not a major issue in this evaluation.

Assessing the quantitative accuracy and precision of the PCB values reported is problematic (Appendix B). Disagreement exists among scientists in the field as to the accuracy of the technique employed. Mr. Tessari estimates precision of about ±50 percent. Furthermore, Larry Griffin, also of Colorado State University, in notes on the standard chromatograms (Figure 4-4), illustrates the calculations of the factors (see Appendix B). His calculations show factors of 1.00 and 0.84 for injections of the equivalent to 1.5 ppm Aroclor 1260 on the two GC columns. This represents a 16 percent uncertainty in the GC analysis alone. As he further noted, these factors "would have been reported as > 3 ppm, therefore we do need to revise our factors."

Dr. R. G. Lewis and several of his coworkers at the Research Triangle Park laboratory of the Environmental Protection Agency (US EPA-RTP) were consulted about this problem. They indicated that a precision of ±50 percent was the best to be expected in cases where the pattern matches an Aroclor and where four or more peaks are quantitated. When presented with the quantitative method used by NHMP, they felt it would be a reasonable estimate to consider the precision at best ±100 percent.

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*Personal Communications, H. Enos, University of Miami, Miami, Florida, October 1979.

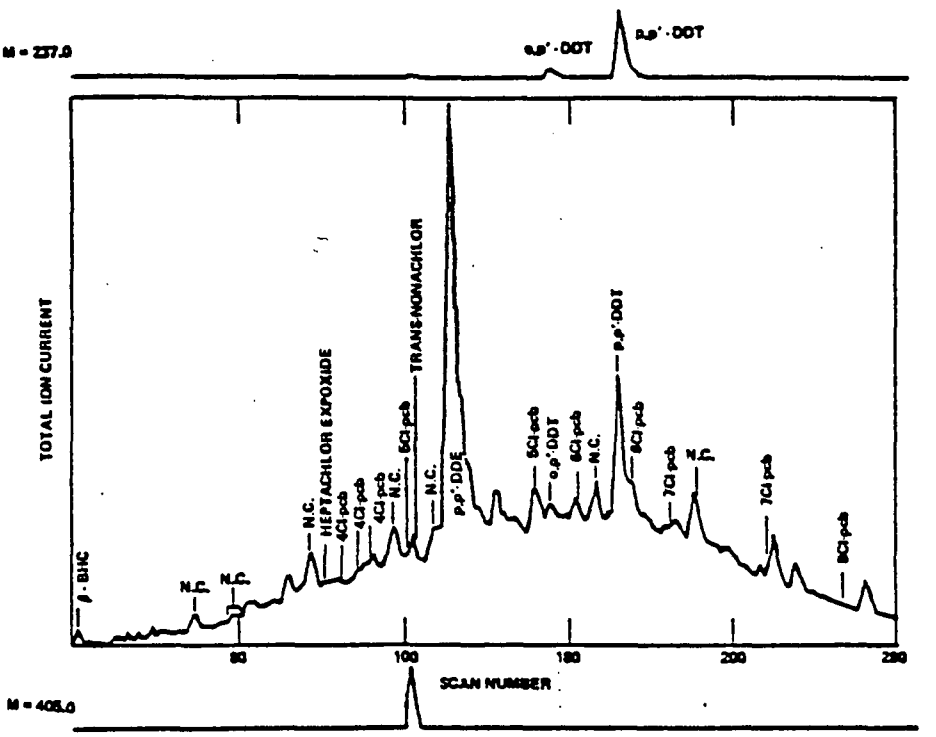

Figure 4.6 Computer Reconstructed Total ION and Mass Chromatograms of a Composite Human Adipose Tissue Extract. Column: 45.7 m Scot Column coated with SE-30. Programmed from 170-240°C at 2°C/ min (N.C. - not chlorinated). Reproduced from (Lewis 1977).

The chromatograms and procedures were described over the telephone to Dr. Henry Enos, an expert in this field, who also felt that a precision of ±100 percent would be a reasonable estimate. He noted that although this procedure may be valid for Aroclor 1260, it does not detect any of the lower PCB's that do not contain heptachlorobiphenyl due to the "masking of those peaks by pesticides.

A further indication of accuracy and precision is presented in the interlaboratory PCB analysis results supplied by R.G. Lewis in Appendix C. Excluding two outliers, thirteen laboratories obtained a mean of 81 ±68 percent on 10 µg/L of Aroclor 1254 in water. A more recent interlaboratory check with spiked fat reported 96 percent accuracy and ±33.8 percent precision. However, if the data are recalculated including both the excluded "outlier" and the missed identification as "zero", the mean recovery is 65 percent (accuracy) and ±96 percent precision. These results are also provided in Appendix C. It should be noted that all participating laboratories were aware this specimen was a check and therefore should have devoted special attention to achieving their "best".

Thus, estimates of ±50 percent precision are optimistic. Given the above arguments of precision and that a clear Aroclor 1260 pattern was not evident in the sample, it appears that PCB quantitation based solely on the peak labeled as "PCB" by the methods used is not reasonable and even "semiquantitation" is questionable.

4.2.3. Potential Interference

Many other compounds could coelute with PCB peaks giving erroneously high readings. Conversely, the early-eluting PCB isomers could and do interfere with quantitation of the pesticides. This may be seen by comparing retention times of some of the pesticides and the Aroclor 1260 standard in Figures 4.1 through 4.4. A more graphic representation is shown in Figure 4.7 (USEPA 1974 Section 9E). These chromatograms were obtained under instrumental conditions similar to those shown in Figures 4.1 through 4.4.

4.2.4. Assessment of TLC Methodology

Prior to November 1974, PCB values in NHMP were obtained by a thin layer chromatographic (TLC) technique (USEPA 1977a and Mulhern et al. 1971). This semiquantitative technique is reported to have a precision

4%SE-30/6%OV-210

Chromatograms of three AROCLORS on column of 4% SE-30 / 6% OV-210. Column temp. 200°C., carrier flow 60 ml/min., ^{3}H detector, electrom. attenuation on an E-2 10 x 16; dotted line a mixture of chlorinated pesticides, identity and injection concentration given below:

1.	Diazinon -- 1.5 ng	7.	o,p'-DDT	--	0.24 ng
2.	Heptachlor -- 0.03	8.	p,p'-DDD	--	.24
3.	Aldrin -- .045	9.	p,p'-DDT	--	.30
4.	Hept.Epox. -- .09	10.	Dilan	--	.75
5.	p,p'-DDE -- .09	11.	Methoxychlor		.60
6.	Dieldrin -- .12				

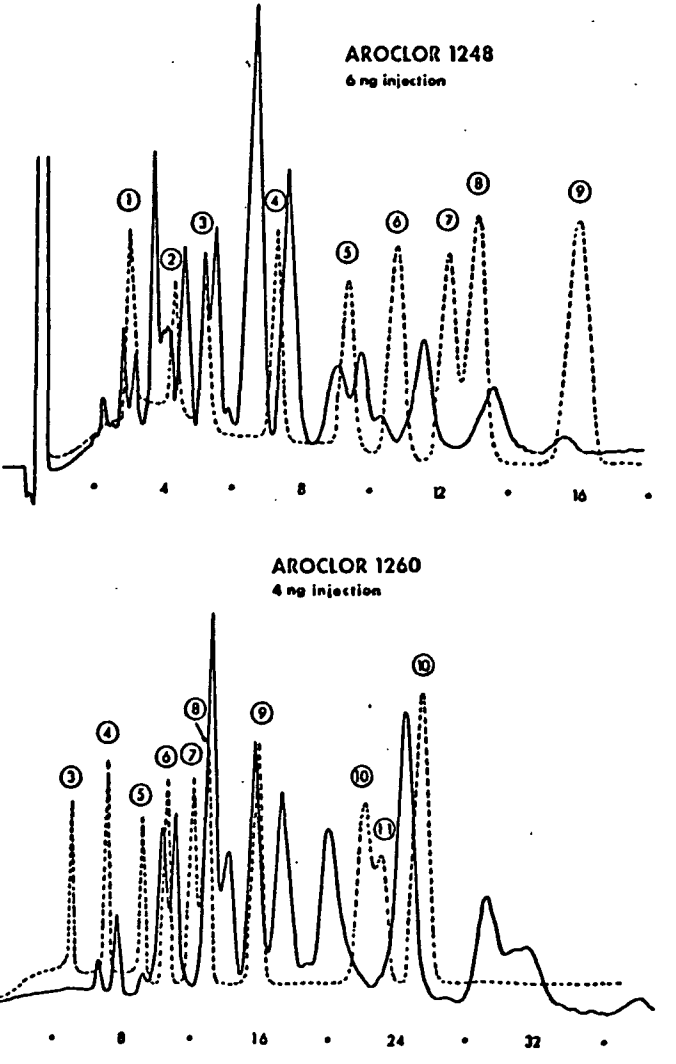

Figure 4.7 Chromatograms of Aroclors and Pesticide standards illustrating potential interferences.
Reproduced from (USEPA 1974 Section 9 E).

of ±50 percent (Appendix B). This technique is advantageous in that all tested PCB isomers or, more correctly, Aroclor mixtures have similar R_f values (i.e., they elute to nearly the same position on the TLC plate), so the PCB value reported is an integration of all isomers present. This technique, however, lacks specificity for individual PCB isomers.

The analytical conditions used by the NHMP elute PCB's essentially at the solvent front with R_f values ranging from 0.91 to 0.94 (Mulhern et al. 1971). The chromatographic resolution is poor and other compounds such as polychlorinated naphthalenes (PCN's), polybrominated biphenyls (PBB's), polychlorinated terphenyls (PCT's) and nonpolar pesticides may coelute. The best resolution on TLC is obtained at an R_f of about 0.2 to 0.8 (Stahl 1969). As an example, DDE elutes with the PCB's and must be removed by oxidation prior to TLC analysis (Mulhern et al. 1971). That proper quality control procedures were followed,* including GC/MS confirmation of the pooled extracts, adds confidence to the reported values.

4.2.5. Correlation of Data Generated by the Two Methods

Since the "PCB" values reported by the two chemical analytical methods are used in parallel for statistical analysis, it is appropriate to comment on the comparability of the data. The TLC method reportedly detects all PCB's, while the GC method uses one isomer (of 209 total possible) and extrapolates the rest. Evidence that the methods generate comparable data is discussed by Mr. Tessari in Appendix B. However, because no quantitative estimation of the relative bias of the two methods was available, their relative equivalence is unknown. For the purpose of discussion in this report, the differences in the data were assumed to be negligible.

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*Personal Communication, J. Tessari, Colorado State University, Fort Collins, Colorado, October 1979.

5. WEIGHTED ANALYSIS OF COMPUTER ACCESSIBLE DATA FILES

5.1. Introduction and Assumptions

This chapter includes a technical discussion of the statistical analysis of NHMP computer accessible data files. The following analysis employs a regression technique especially adapted for multistage survey data. This technique approximates the total variance of the estimates including the measurement and sampling components and also allows adjustments for other factors such as sex, age, census region, and fiscal year. The analysis is based on the following two important assumptions: (1) quantitation and sampling biases are similar for each racial group and (2) sampling within sample cities produced a nearly simple random sample within each city. The calculation of the sample weights was based on assumption (2) and the significance levels of all tests of hypotheses depend on the validity of both (1) and (2).

5.2. Sample Weights

Because some stages of selection involve nonprobability sampling, the true probability of selection cannot be calculated. Even if one assumes that the selection probabilities of a specimen within sample cities are approximately equal, the sample design of the NHMP adipose tissue network does not give equal "probabilities" of selection to all elements in the sample. Because of this situation, a sample weight was calculated for each observation that reflects its approximate probability of selection. Including these weights in the analysis may reduce bias in estimating means or proportions. In the following paragraphs, the procedures used for computing approximate weights for the NHMP data are described.

5.2.1 Calculation of Weights

For the purpose of calculating weights, two stages of selection are considered. In the first stage, cities were selected within each stratum with probability approximately proportional to population. In the second stage, samples of cadavers and surgical patients were selected in a nonprobabilistic manner from a hospital(s) or other facility located in the sample cities. Equal probabilities of selection were assumed for this stage.

The sample weights were calculated as the inverse product of the probabilities of selection for each stage. This can be expressed algebraically as

$$W_{hij} = [P_{hi} P_{j|hi}]^{-1} ,$$

where W_{hij} denotes the weight of the j-th specimen in the i-th city and h-th stratum; P_{hi} denotes the probability of selecting the i-th city in the h-th stratum (for large cities selected with probability 1 this is more accurately described as the expected number of times the city is selected in the sample); and $P_{j\ hi}$ denotes the probability of selecting the j-th specimen given the i-th city in the h-th stratum was selected. To calculate W_{hij}, it is necessary to calculate P_{hi} and $P_{j|hi}$.

The cities were selected independently within each stratum. For each stratum, the cumulative total population of eligible cities was divided by the number of cities to be selected in the h-th stratum. This calculation gives the sample selection interval. A random number is then selected between "1" and the value of the selection interval; this gives the random start. The method then involves listing the cities in a random order, calculating the cumulative totals and then selecting cities by matching the cumulative totals to the random start and integer multiples of the selection interval plus the random start (USEPA 1973).

The above can be expressed algebraically in the following manner. For each stratum, the selection interval is

$$I_h = N_h/m_h ,$$

where I_h, N_h, and m_h denote the selection interval, cumulative population total, and number to be selected for the h-th stratum, respectively. The cities selected in the sample are those for which the cumulative totals match $r_h + K \times I_h$ for $K = 0,\ldots,m_h-1$, where r_h is the random start for the h-th stratum.

This method assigns a probability of selection to each city equal to the population of the city divided by the selection interval. This can be expressed algebraically as

$$P_{hi} = N_{hi}/I_h ,$$

where N_{hi} denotes the population of the i-th city in the h-th stratum and P_{hi} and I_h are defined above.

Under the assumption made above for random selection of samples within a city, the calculation is straightforward. The probability of selection is given by dividing the number of specimens selected in each city by the population of the city. This can be expressed algebraically as

$$P_{j|hi} = n_{hi}/N_{hi}$$

for $j = 1,\ldots,n_{hi}$, where n_{hi} denotes the number of samples collected in the i-th city in the h-th stratum and $P_{j|hi}$ and N_{hi} are defined above.

The weight can be written as

$$W_{hij} = [N_{hi}/I_h \times n_{hi}/N_{hi}]^{-1} ,$$

which simplifies to

$$W_{hij} = I_h/n_{hi} .$$

Hence, the approximate sampling weight for the i-th city in the h-th stratum is the same for all samples collected in the city and is calculated by dividing the selection interval for the h-th stratum by the number of specimens collected in the city.

The method described above does yield selection probabilities proportional to the population of the cities when certain precautions are followed. Adequate methods for selecting alternate (substitute) cities were not taken. Hence, the procedures followed in the study alter the probability of selection for some cities, but the degree, although probably minor, is undetermined. For the purpose of calculating approximate weights, these special situations were treated the same as all others.

5.2.2. Sample Sizes Used for Calculating Weights

As noted earlier, the weighted analysis was performed using only fiscal years 1972-76. Fiscal year 1977 was excluded for the following two reasons: (1) the initial data summaries received from EPA did not include 1977 and (2) the sampling frames and primary sampling units (PSU, the unit selected in the first stage of sampling) were changed in

1977. In 1977 the PSU's were changed from cities with populations greater than 25,000 to Standard Metropolitan Statistical Areas (SMSA's).

Not all data for fiscal years 1972-76 were used in the analysis. Out of a total of 8,372 observations, only 5,880 were used. The exclusion of 2,492 observations resulted for several reasons. Of these, 2,380 observations were volunteer contributions to the survey from cities not even in the sample. These records were not members of the survey design, and hence, their probability of selection cannot be reasonably approximated. The other 112 observations were excluded for one of several possible reasons; e.g., the percent extractable lipid may have been to small or the confidence codes on the data file indicated uncertainties about data quality. The precise rules were the following:

- If the record indicated that a technical error had been made for a particular residue amount, that residue amount was considered missing.

- If the confidence code for a particular residue amount was blank, that residue amount was considered missing.

- If the record indicated that there was less than 10 percent lipid extractable material, all residue amounts were considered missing for that record.

5.3. Racial Comparisons by Weighted Analysis

5.3.1. Overview

This section presents results from weighted regression analyses that compare whites with nonwhites over time (fiscal years 1972-1976) adjusting for age (Age 1 $\leq$ 14, 15 $\leq$ Age 2 $\leq$ 44, Age 3 $\geq$ 45), census region (CR1 = North East, CR2 = North Central, CR3 = South, CR4 = West), and sex. Because the strata in fiscal year 1972 were census regions, the census divisions within each census region were grouped together for fiscal years 1973-76. Racial comparisons were performed for each of two variables; they are

- Percent of individuals with greater than 3 ppm "PCB's" (percent >3 ppm)

- Percent of individuals with detected "PCB's" (percent positive detections (trace, 1-3 ppm and >3 ppm))

In summary, the analysis showed:

For percent >3 ppm "PCB":

(i) Over the fiscal years 1972-76, there was an increasing trend in the percentage of people with greater than 3 ppm PCB in their adipose tissue. The rate of increase is estimated to be 1.73 percentage points per year, with a standard error of the estimate of 0.54. A test of the hypothesis of no time trend was rejected at the 1 percent significance (99 percent confidence) level by the estimated trend and its standard error.

(ii) Over the fiscal years analyzed (1972-76), nonwhites averaged 7.15 percentage points higher than whites. The standard error of the estimate is 1.41 and the racial difference is significant at the 0.1 percent (99.9 percent confidence) level.

(iii) The hypothesis that there is a differential trend for whites and nonwhites is not supported by the data. That is, the white-nonwhite differential is reasonably constant over time.

For percent positive detections:

(i) There is an increasing trend (fiscal years 1972-76) of an estimated 2.98 percentage points per year with a standard error of 0.82. The estimated trend and its standard error reject the hypothesis of no trend at the 0.1 percent (99.9 percent confidence) level.

(ii) Over time, differences between whites and nonwhites are not significantly different from zero in either absolute level or trend.

5.3.2. Statistical Method

Each individual for which a sampling weight could be calculated in fiscal years 1972-76 was used in the analysis (except for quality-code related exclusions). The analyses proceeded by defining an indicator variable for each sample member. Specifically, the variables Y_{1i}, Y_{2i} were created for the i-th individual such that:

$$Y_{1i} = \begin{cases} 1 \text{ if the i-th sample member's adipose tissue had} \\ \quad >3 \text{ ppm PCB's and} \\ 0 \text{ if } \leq 3 \text{ ppm PCB's,} \end{cases}$$

$$Y_{2i} = \begin{cases} 1 \text{ if any PCB's detected and} \\ 0 \text{ if no PCB's detected.} \end{cases}$$

For each of these dependent (Y) variables, a linear model was specified, which attempted to relate the Y variables to the sample member's age, race, sex, census region, and year of tissue collection. Specifically, Y was approximated by:

$$Y \doteq \mu + \sum_{i=1}^{2} A_i X_i + R_W X_W + S_M X_M + \sum_{j=1}^{3} CR_j \ell_j$$

$$+ FY(\beta_0 + \sum_{i=1}^{2} \beta_i X_i + \beta_W X_W + \beta_M X_M + \sum_{j=1}^{3} \beta_j^{*} \ell_j); \qquad (1)$$

where

$$X_i = \begin{cases} 1 \text{ if an individual is in age group } i \ (i = 1,2), \\ 0 \text{ otherwise.} \end{cases}$$

$$X_W = \begin{cases} 1 \text{ if an individual is white,} \\ 0 \text{ otherwise.} \end{cases}$$

$$X_M = \begin{cases} 1 \text{ if an individual is a male,} \\ 0 \text{ otherwise.} \end{cases}$$

$$\ell_j = \begin{cases} 1 \text{ if an individual is in census region } j \ (j = 1,2,3,4) \\ 0 \text{ otherwise.} \end{cases}$$

FY = Fiscal Year - 1970 (coding by subtracting 1970 from fiscal year).

In the sample, the $X_i, \ldots,$ FY are known and the coefficients $(\mu, \ldots, \beta_j^{*})$ must be estimated from the data and represent the "effects" of an individual being a member of a particular age, race, sex combination living in a specific census region at a specific time.

In general, the model is simply a generalization of a covariance model (with FY being the covariate) that allows a function linear in FY to be fitted simultaneously for each combination of age, race, sex and

census region. As an example, suppose two individuals are in the same age group (age group 1, say), both are males, and both live in census region 1 however, one is white and one is nonwhite. Then, for the white individual:

$$Y_W \doteq \mu + A_1 + R_W + S_M + CR_1 + FY\ (\beta_0 + \beta_1 + \beta_W + \beta_M + \beta_1^*), \quad (2)$$

and for the nonwhite individual:

$$Y_{NW} \doteq \mu + A_1 + 0 + S_M + CR_1 + FY\ (\beta_0 + \beta_1 + 0 + \beta_M + \beta_1^*). \quad (3)$$

Combining terms common to both individuals gives:

$$Y_W \doteq M_{111} + R_W + (B_{111} + \beta_W)FY\ , \quad (4)$$

$$Y_{NW} \doteq M_{111} + B_{111}FY\ , \quad (5)$$

where M_{111} and B_{111} are the sum of the terms common to both individuals and (111) indexes the age group, sex, and census region of the individuals. Hence, the model is simply a formulation of a covariance model that allows the estimation and testing of a differential FY slope (time trend) by different subgroups of the population. Hence, by appropriate manipulation of the slopes and intercepts of the fitted lines, differences between whites and nonwhites may be estimated and tested statistically.

The parameters (the coefficients above) were estimated by using weighted least squares, where an individual's weight was the sampling weight discussed in Subsection 5.2. The variance-covariance matrix of the estimated coefficients was computed by the use of Taylorized deviations (Shah et al. 1978).

For the two variables analyzed (percent >3 ppm "PCB", percent positive detections) the estimated coefficients are given in Table 5.1.

Following the notation developed in (4) and (5) above, for every combination of age, sex and census region (denoted as i,j,k), an estimated line can be produced for each race. They are of the form:

Table 5-1* Model Coefficients (Percent)

Percent >3 ppm "PCB"				Percent Positive Detections			
Intercept Terms		Slope Terms		Intercept Terms		Slope Terms	
μ	-4.68	β_0	4.39	μ	79.84	β_0	3.36
A_1	3.15	β_1	-2.96	A_1	-5.65	β_1	-0.87
A_2	2.68	β_2	-1.89	A_2	1.96	β_2	-0.36
R_W	-2.79	β_W	-1.09	R_W	2.47	β_W	-0.38
S_M	1.20	β_M	0.04	S_M	6.00	β_M	-1.27
CR_1	3.40	β_1^*	1.29	CR_1	-5.57	β_1^*	1.22
CR_2	9.38	β_2^*	-2.03	CR_2	-6.06	β_2^*	1.27
CR_3	7.78	β_3^*	-1.32	CR_3	-5.32	β_3^*	0.95

*Based on data for fiscal years 1972-76 from NHMP computerized data files, using methodology defined in section 5.2.

For whites:

$$\hat{Y}_W(i,j,k) = \hat{M}_{ijk} + \hat{R}_W + (\hat{B}_{ijk} + \hat{\beta}_W)FY \quad . \tag{6}$$

For nonwhites:

$$\hat{Y}_{NW}(i,j,k) = \hat{M}_{ijk} + \hat{B}_{ijk}FY \quad . \tag{7}$$

Now, there are 3 age groups, 2 sexes and 4 census regions; therefore, averaging over the 24 distinct lines in (6) and (7) gives:

$$\bar{\hat{Y}}_W = \bar{\hat{M}} + \hat{R}_W + (\bar{\hat{B}} + \hat{\beta}_W)FY \quad , \tag{8}$$

$$\bar{\hat{Y}}_{NW} = \bar{\hat{M}} + \bar{\hat{B}}\,FY \quad . \tag{9}$$

where

$$\bar{\hat{M}} = \frac{1}{24} \sum_{i=1}^{3} \sum_{j=1}^{2} \sum_{k=1}^{4} \hat{M}\,(i, j, k)$$

$$\bar{\hat{B}} = \frac{1}{24} \sum_{i=1}^{3} \sum_{j=1}^{2} \sum_{k=1}^{4} \hat{B}\,(i, j, k)$$

For the data used in the analyses, (8) and (9) are:

For percent >3 ppm PCB,

$$\bar{\hat{Y}}_W = 2.99 - 2.79 + (2.28 - 1.09)FY \quad ,$$

$$= 0.20 + 1.19\,FY \quad , \tag{10}$$

$$\bar{\hat{Y}}_{NW} = 2.29 + 2.28\,FY \quad . \tag{11}$$

For percent Positive Detections,

$$\hat{\bar{Y}}_W = 77.37 + 2.47 + (3.18 - 0.38)FY$$

$$= 79.8 + 2.80\ FY\ , \qquad (12)$$

$$\hat{\bar{Y}}_{NW} = 77.37 + 3.18\ FY\ . \qquad (13)$$

To test for slope differences, it is sufficient to test $\beta_W = 0$. The tests are presented in Table 5-2.

Now assuming the slopes are not different for the two racial groups (a hypothesis supported by the tests in Table 5-2), the difference between the two levels may be estimated by averaging the yearly difference in the lines over the years. This is algebraically equivalent to testing the difference in the lines at FY = 4 (1974). Table 5-3 gives the results.

The tests given in Table 5-2 indicate that for both percent >3 ppm "PCB" and percent positive detections, no differences exist between whites and nonwhites with respect to their time trend (FY slope). Therefore, a common trend is given as the average of the two race-specific slopes. They are the following:

For percent >3 ppm PCB,

$$\hat{\beta}_{AVG} = \tfrac{1}{2}(2.28 + 1.19) = 1.73, \text{ with } SE(\hat{\beta}_{AVG}) = 0.54\ ,$$

For percent Positive Detections,

$$\hat{\beta}_{AVG} = \tfrac{1}{2}(2.80 + 3.18) = 2.99, \text{ with } SE(\hat{\beta}_{AVG}) = 0.82\ .$$

Both of these common slopes are significantly greater than zero ($p < .01$).

Figures 5-1 and 5-2 present the above estimates and analyses graphically. In addition to the trend lines discussed above, certain directly adjusted white and nonwhite means are plotted. These are plotted merely to present a sense of the data and are, roughly, what the race-specific lines are predicting. To compute these means, the following procedure was followed. First, weighted means were computed for each combination

Table 5-2.* Tests for Differential Slopes--White Versus Nonwhite

	Percent >3 ppm "PCB" Detections	Percent Positive
$\hat{\beta}_W$	-1.09	-.38
$SE(\hat{\beta}_W)$	2.96	1.22
$Z = \hat{\beta}_W/SE(\hat{\beta}_W)$	- .36	- .31
Significance	Not Significant	Not Significant

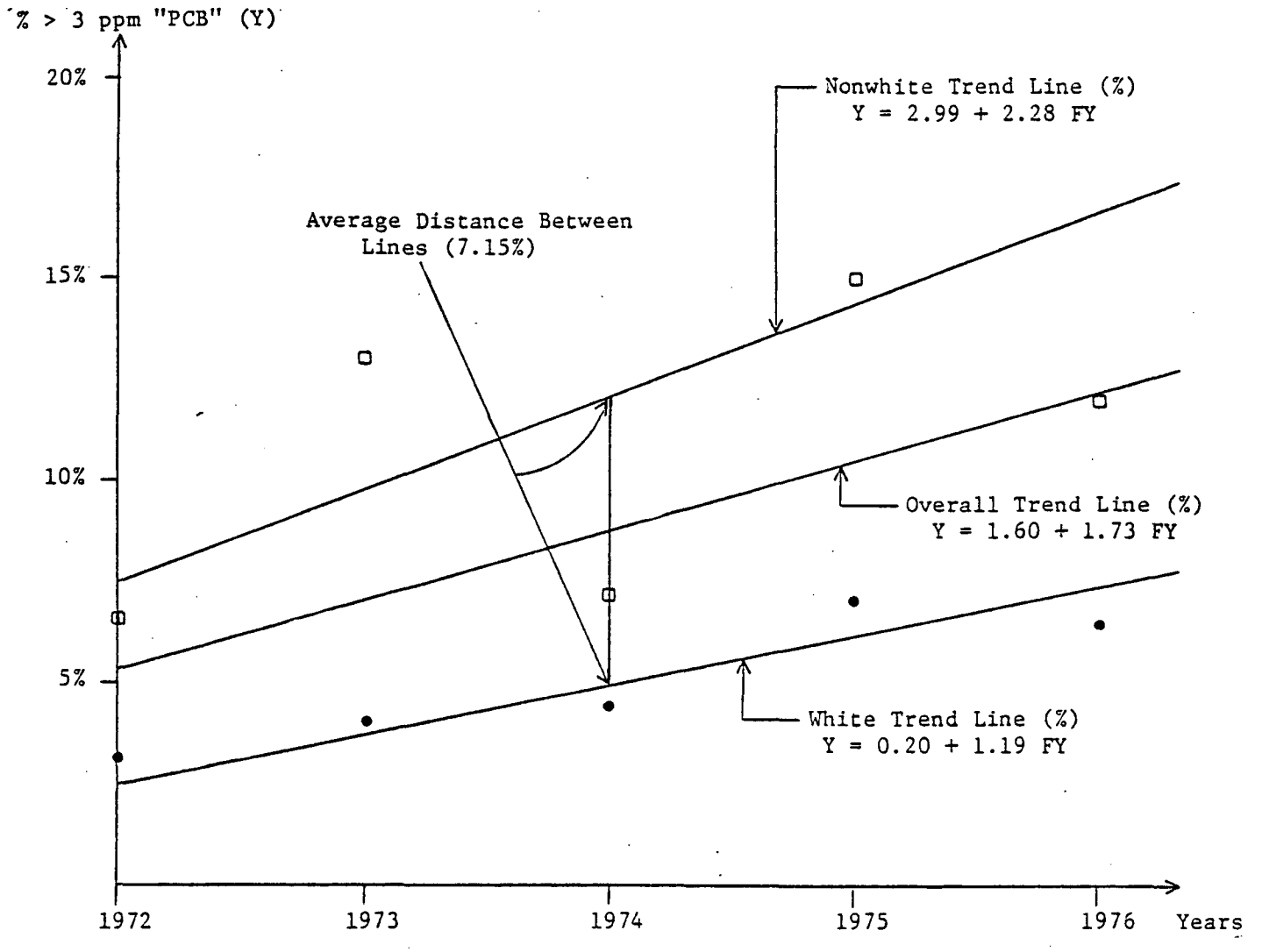
*Based on data for fiscal years 1972-76 from NHMP computer accessible data files, using methodology defined in Section 5.2.

Table 5-3.* Tests for Average Racial Group Differences

	Percent >3 ppm "PCB" Detections	Percent Positive
Difference Between Lines ($\hat{\Delta}$):White-Nonwhite	-7.15	.95
$SE(\hat{\Delta})$	1.41	1.89
$Z = \hat{\Delta}/SE(\hat{\Delta})$	-5.07	0.50
Significance	$p < .001$	Not Significant

*Based on data for fiscal years 1972-76 from NHMP computer accessible data files, using methodology defined in Section 5.2.

Figure 5-1.* Racial Comparisons Over Time: Percent > 3 ppm "PCB"



LEGEND

□ = Nonwhite estimated means adjusted for Age, Sex, and Census Region.

● = White estimated means adjusted for Age, Sex, and Census Region.

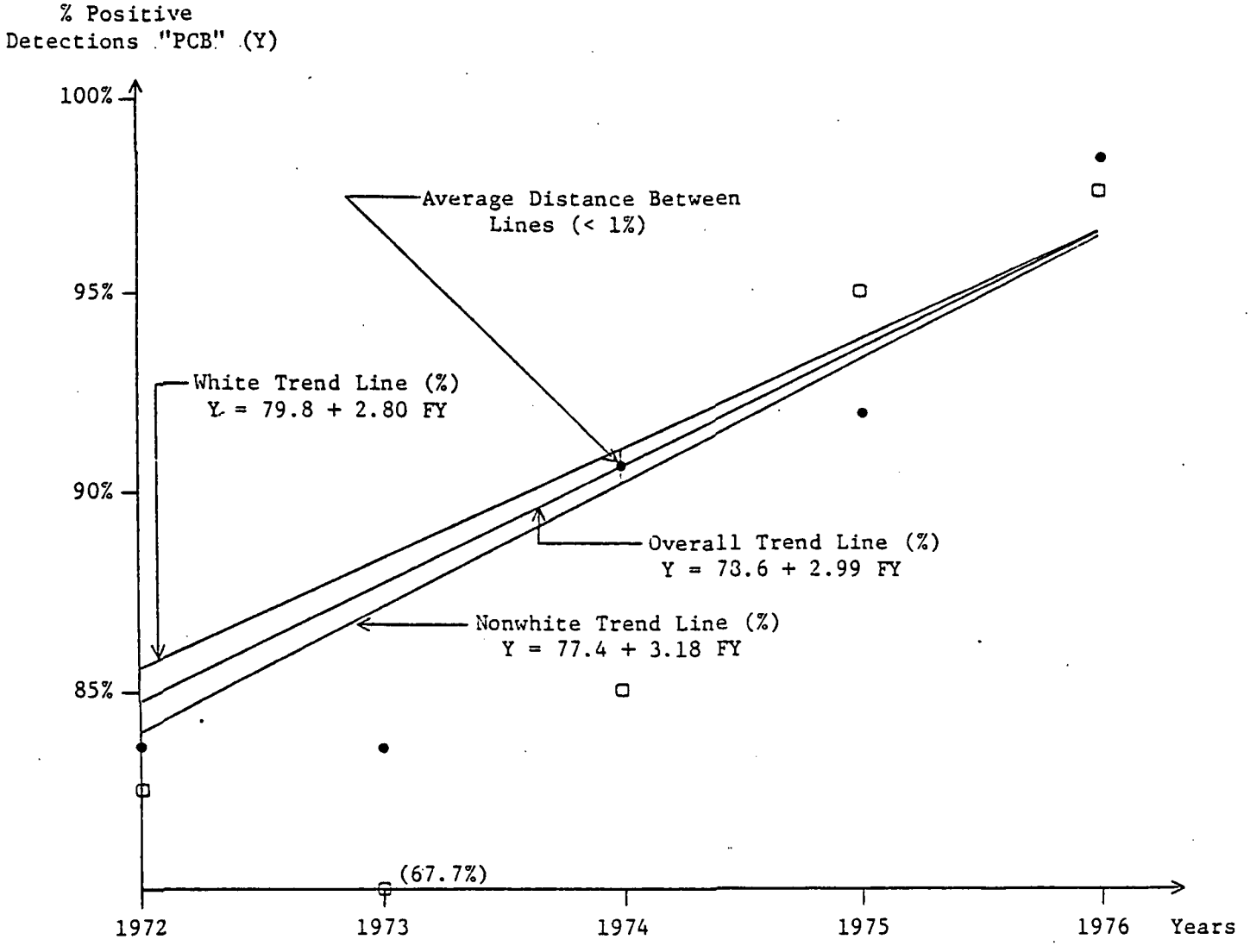
FY = Years - 1970

STATISTICAL TESTS

Test	Estimate	Standard Error	Significance
1. Slope Differences White Versus Nonwhite	-1.09	2.96	Not Significant
2. Average Distance Between Lines White and Nonwhite	7.15	1.41	Highly Significant ($p < .001$)
3. Trend Summary Average of White and Nonwhite slopes	1.73	0.54	Highly Significant ($p < .01$)

* Based on data for fiscal years 1972-76 from NHMP computer accessible data files using methodology defined in Section 5.2.

Figure 5-2.* Racial Comparison Over Time: Percent Positive "PCB" Detections



LEGEND

□ = Nonwhite estimated means adjusted by Age, Sex, and Census Region.

● = White estimated means adjusted by Age, Sex, and Census Region.

FY = Years - 1970

STATISTICAL TESTS

		Estimate	Standard Error	Significance
1.	Slope Differences White Versus Nonwhite	- .38	1.22	Not Significant
2.	Average Distance Between Lines White and Nonwhite	.95	1.89	Not Significant
3.	Trend Summary Average of White and Nonwhite Slopes	2.98	.82	Highly Significant ($p < .01$)

* Based on data for fiscal years 1972-76 from NHMP computer accessible data files using methodology defined in Section 5.2.

of age, race, sex, census region, and fiscal year. There are potentially 240 (= 3 x 2 x 2 x 4 x 5) such means. However, due to the absence in the sample of young nonwhites in certain years in census region 4, only 234 means were computed. In essence, a five-dimensional table with one entry per cell was produced (the weighted means). Next, for each race and year, the simple average of the weighted averages was computed. These averages are rough estimates of the means by race and year. It is not difficult to show that the race-specific lines drawn in Figures 5-1 and 5-2 and these means estimate the same quantity if the assumed model form (1) is correct. Finally, these means are plotted along with the estimated lines in the two figures.

5.4. Comments on Assumptions

Several apparent inconsistencies in the data cast doubt on the assumptions. The most striking is the very low value of percentage detected for nonwhites in 1973. The 67.7 percent is more than three standard deviations below the predicted mean value of 87.6 percent (see Figure 5-2). Thus this low value is not likely to be due to random variation in the data. The change in the chemical analysis methods in 1974 may contribute to the higher percentages for later fiscal years than for early fiscal years, and the trend over time may be in part due to the change in chemical analyses.

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APPENDIX A

Letter from M. Aaronson
(Reproduced with permission of S. Strassman-Sundy)

January 7, 1980

Mrs. Sandra Strassman
Project Officer
National Human Monitoring Program
Field Studies Branch
Survey and Analysis Division (PS-793)
U.S. E.P.A.
401 "M" Street S.W.
Washington, D.C. 20460

Dear Sandy:

Enclosed please find a copy of the following items:

1) Reconstructed ion chromatogram (RIC) of Tissue Pool sample 79-A,6%

2) The mass spectrum of scan #266

3) The library search of scan #266

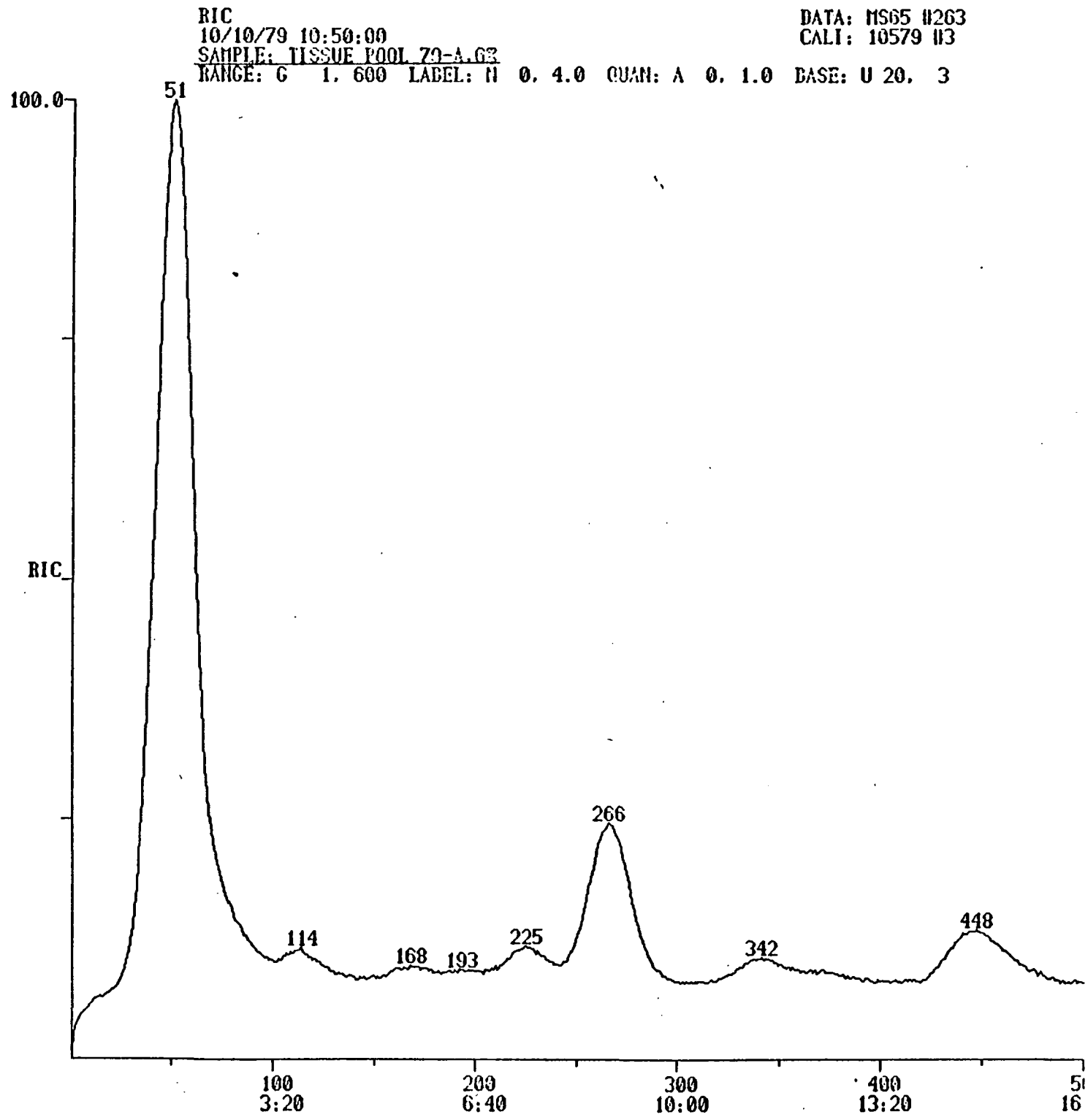
4) A comparison of the mass spectrum of scan #266 to that of heptachlorobiphenyl

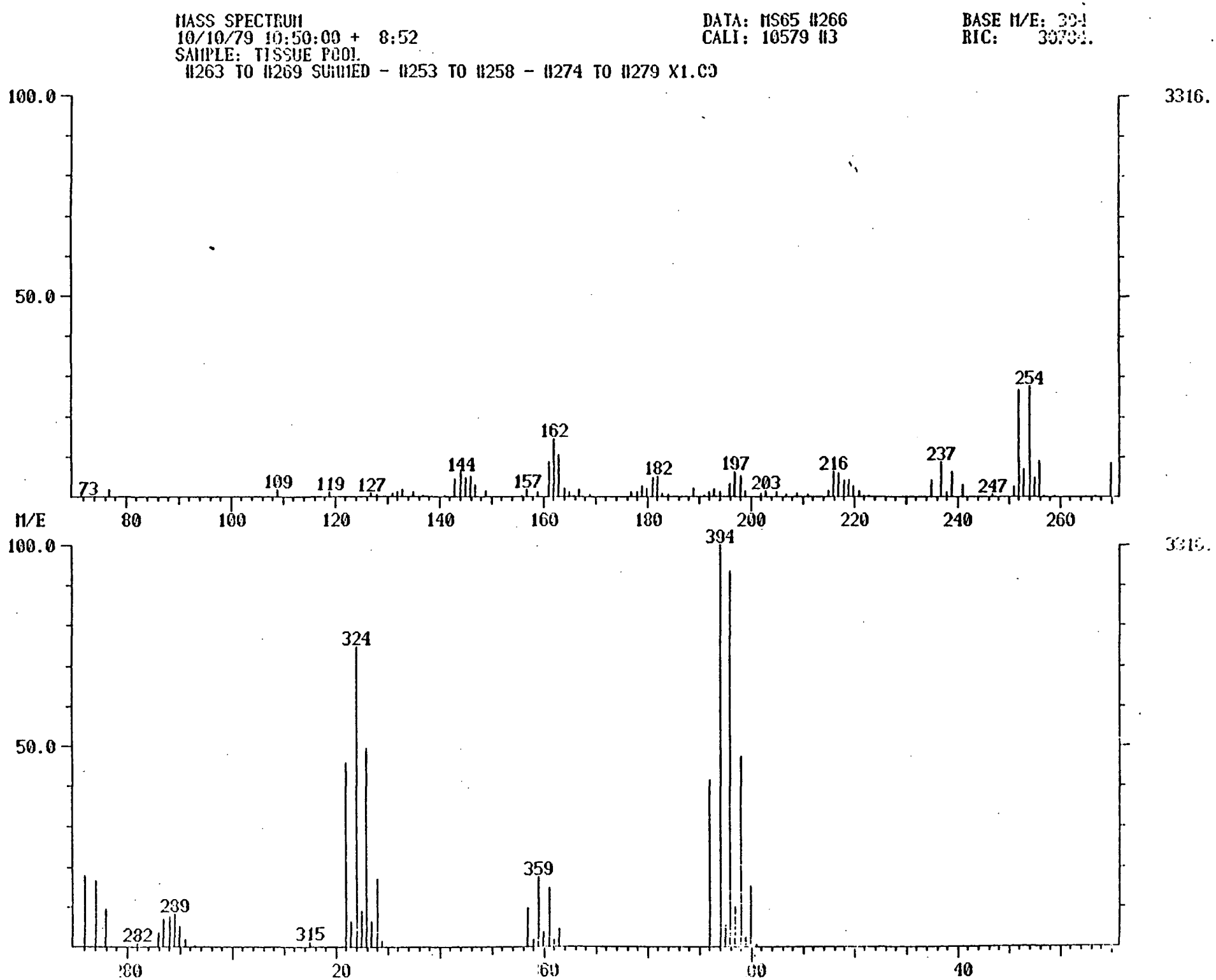
The RIC begins at pp-DDT (scan #51) and runs for twenty minutes. Scan #266 is the peak utilized by the laboratory for the semi-quantitative estimation of PCB's in tissue extracts. Further examination of the peak represented by scan #266 tentatively identifies it as a heptachlorobiphenyl. The actual heptachlorobiphenyl standard would be required to produce a more definitive identification.

If you have any further questions or if I can be of more assistance do not hesitate to call.

Sincerely

Michael J. Aaronson


RIC
10/10/79 10:50:00
SAMPLE: TISSUE POOL 79-A.63
RANGE: G 1. 600 LABEL: N 0. 4.0 QUAN: A 0. 1.0 BASE: U 20. 3
DATA: MS65 #263
CALI: 10579 #3
100.0
RIC
51
114
168
193
225
266
342
448
100
3:20
200
6:40
300
10:00
400
13:20


MASS SPECTRUM
10/10/79 10:50:00 + 8:52
SAMPLE: TISSUE POOL
#263 TO #269 SUMMED - #253 TO #258 - #274 TO #279 X1.00
DATA: MS65 #266
CALI: 10579 #3
BASE M/E: 394
RIC: 3316.
100.0
50.0
3316.
73
109
119
127
144
157
162
182
197
203
216
237
247
254
M/E
80
100
120
140
160
180
200
220
240
260
100.0
50.0
3316.
282
289
315
324
359
394
280
20
60
00
40

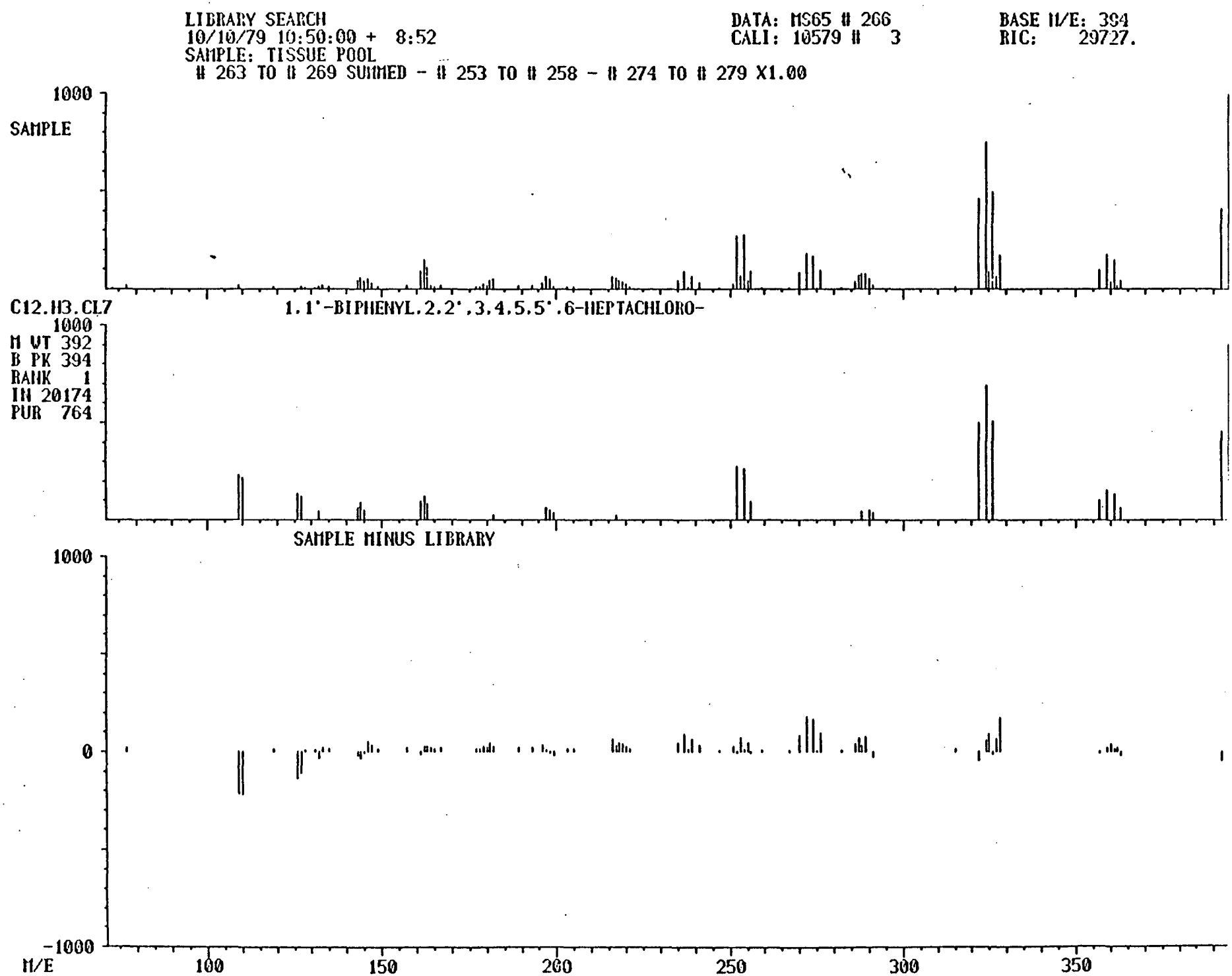
LIBRARY SEARCH DATA: MS65 * 266 BASE M/E: 394
10/10/79 10:50:00 + 8:52 CALI: 10579 * 3 RIC: 29727.
SAMPLE: TISSUE POOL #9
* 263 TO * 269 SUMMED - * 253 TO * 258 - * 274 TO * 279 X1.00

25409 SPECTRA IN LIBRARYNB SEARCHED FOR MAXIMUM PURITY
54 MATCHED AT LEAST 3 OF THE 16 LARGEST PEAKS IN THE UNKNOWN

RANK IN NAME
1 20174 1,1'-BIPHENYL,2,2',3,4,5,5',6-HEPTACHLORO-
2 9039 1,3,5-TRIAZINE,2,4,6-TRIS(TRICHLOROMETHYL)-
3 23034 BENZENE,1,1'-THIOBIS-,TETRACHLORODERIV.
4 17181 FERROCENE,1,1',2,2'-TETRACHLORO-
5 16584 1,1'-BIPHENYL,2,3',4,4',5-PENTACHLORO-

RANK	FORMULA	M.WT	B.PK	PURITY	FIT	RFIT
1	C12.H3.CL7	392	394	764	946	795
2	C6.N3.CL9	429	398	338	568	445
3	C12.H6.S.CL4	322	324	309	935	327
4	C10.H6.CL4.FE	322	324	215	780	261
5	C12.H5.CL5	324	326	185	743	199

MASS	INTEN	1	2	3	4	5
36			150			
39					75	
47			231			
49			68			
50				14		
51				4		
56					78	
63				23	543	
73	0		97	21	95	
74				35	41	
75				34	49	
77	17					
82			210			
84			129			
91					171	
93					54	
94			196			
97					55	
99					49	
108			552	15		
109	19	231		19		
110		215	340			
111				7		
117			275		43	
119	13		265			
125					73	
126		133			274	
127	10	119				
128	5					
131	8					
132	12	47				
133	18				108	
134			107			
135	13				67	
136			80			
142				84		
143	44	61		37		
144	60	90		66		
145	48	52				
146	51					
147	30					
149	16					
157	19					
160						


LIBRARY SEARCH
10/10/79 10:50:00 + 8:52
SAMPLE: TISSUE POOL
263 TO # 269 SUMMED - # 253 TO # 258 - # 274 TO # 279 X1.00
DATA: MS65 # 266
CALI: 10579 # 3
BASE M/E: 394
RIC: 29727.
1000
SAMPLE
C12.H3.CL7
1.1'-BIPHENYL.2.2',3.4.5.5'.6-HEPTACHLORO-
1000
M WT 392
B PK 394
RANK 1
IN 20174
PUR 764
SAMPLE MINUS LIBRARY
1000
0
-1000
M/E
100
150
200
250
300
350

APPENDIX B

Letter from John D. Tessari
(Reproduced with Permission)

College of Veterinary Medicine
and Biomedical Sciences
Institute of Rural Environmental Health
Colorado Epidemiologic Pesticide Studies Center
Spruce Hall

Colorado State University
Fort Collins, Colorado
80523

September 21, 1979

Dr. Mitchel Erickson
P.O. Box 12194
Drafysus Lab
Research Triangle Institute
RTP North Carolina 27709

Dear Dr. Erickson:

Enclosed find the gas chromatographic charts you requested with our estimate of the PCB content of those samples. We randomly chose 38 of our most recently analyzed samples. Also find enclosed a chart showing sample numbers and data.

The calculation method utilizes the isolated large PCB peak (Arochlor 1260) eluting 8-12 cm beyond p,p'-DDT on 1.5% OV-17/ 1.95% OV-210 (6.0-6.9 relative retention time to aldrin) and on 4% SE-30/6% OV-210 (4.6-5.0 relative retention time to aldrin). A comparison is made between the peak height of 40 pg of aldrin (5 ul of an 8 pg/ul aldrin standard) and the peak height of this PCB peak in 1 mg of sample (5 ul of a 200 ug/ul dilution of sample) according to the following relationship:

$$\frac{\text{peak height (mm) of 40 pg aldrin}}{\text{peak height (mm) of PCB peak in mg of sample}} = \text{FACTOR}$$

The factor determines the amount of PCB's present, which is reported to the EPA by code letter, according to the following:

FACTOR	PCB's Present	Code Letter
No peak present	0 PPM	V
> 3.5	< 1 PPM	W
3.5-2.6	1-3 PPM	Y
< 2.6	> 3 PPM	Z

This method is based on the TLC method of Mulhorn et al. (1) which has a precision of ± 50% when using Arochlor 1260 as the reference standard. A series of representative adipose samples were analyzed for PCB's by this method, and for all chlorinated pesticides by the more accurate standard adipose tissue method (2). A direct correlation was noted between the PCB result by TLC and the height of the afore mentioned peak. Finally, a relationship was established between the ratio of a specified

amount of aldrin and the PCB peak height and concentration in order to produce the factors. Thus, our method essentially relates the relative peak height of the PCB peak to aldrin to obtain a PCB concentration.

Due to the semi-quantitative nature of the method, certainly no better than the reference method which claims ± 50% precision, discrepancies between the two GLC columns occassionally arise. For example, 1-3 PPM may be noted on one column and <1 PPM on another. Judgement is applied to the individual situation to determine which result to report. A factor of 3.5 (just in the 1-3 PPM range) from one column and a factor of 4.0 (<1 PPM) would probably be reported as <1 PPM. A factor of 3.0 (1-3 PPM) and a factor of 3.6 (just <1PPM) would probably be reported as 1-3 PPM. If the discrepancies are extreme (<1 PPM on one column, >3 PPM on the other), we attempt to resolve the difference. If no errors can be found, an average is usually reported (1-3 PPM).

If you have any questions please contact us.

John D. Tessari

John D. Tessari

Supervising Chemist
Laboratory Director

(1) Analysis of Pesticide Residues in Human and Environmental Samples, U.S. EPA, R.T.P. N.C., 1974, Sect 9,D.

(2) Ibid., Section 5,A,(1).

OV-17/OV-210			SE-30/OV-210			Value Reported	
Factor	PPM PCB's	Code Letter	Factor	PPM PCB's	Code Letter	PPM PCB's	Code L
17.6	<1	W	11.1	<1	W	<1	W
3.9	<1	W	3.1	1-3	Y	1-3	Y
30.0	<1	W	20.8	<1	W	<1	W
6.1	<1	W	4.0	<1	W	<1	W
6.5	<1	W	5.2	<1	W	<1	W
8.4	<1	W	8.1	<1	W	<1	W
1.6	>3	Z	2.2	>3	Z	>3	Z
2.0	>3	Z	1.2	>3	Z	>3	Z
12.3	<1	W	6.5	<1	W	<1	W
5.3	<1	W	3.6	<1	W	<1	W
8.9	<1	W	6.1	<1	W	<1	W
6.5	<1	W	3.5	1-3	Y	<1	W
5.8	<1	W	4.5	<1	W	<1	W
6.9	<1	W	3.9	<1	W	<1	W
5.0	<1	W	3.9	<1	W	<1	W
5.7	<1	W	3.6	<1	W	<1	W
4.4	<1	W	2.8	1-3	Y	<1	W
8.1	<1	W	5.1	<1	W	<1	W
6.3	<1	W	4.5	<1	W	<1	W
3.8	<1	W	2.8	1-3	Y	1-3	Y
3.2	1-3	Y	2.1	>3	Z	1-3	Y
22.7	<1	W	0.0	0	V	<1	W
8.2	<1	W	5.3	<1	W	<1	W
6.6	<1	W	4.2	<1	W	<1	W
5.9	<1	W	4.0	<1	W	<1	W
3.2	1-3	Y	2.2	>3	Z	1-3	Y
2.5	>3	Z	1.5	>3	Z	>3	Z
5.2	<1	W	2.7	1-3	Y	1-3	Y
4.7	<1	W	2.4	>3	Z	1-3	Y
6.5	<1	W	3.9	<1	W	<1	W
1.3	>3	Z	1.2	>3	Z	>3	Z
2.0	>3	Z	1.9	>3	Z	>3	Z
3.1	1-3	Y	3.0	1-3	Y	1-3	Y
2.3	>3	Z	1.9	>3	Z	>3	Z
<0.7	>3	Z	0.3	>3	Z	>3	Z
2.0	>3	Z	1.8	>3	Z	>3	Z
4.1	<1	W	3.7	<1	W	<1	W
3.8	<1	W	3.4	1-3	Y	<1	W

APPENDIX C

Interlaboratory PCB Analysis Results
(Reproduced with permission of R. G. Lewis)

ENVIRONMENTAL TOXICOLOGY DIVISION
HEALTH EFFECTS RESEARCH LABORATORY
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: August 17, 1977

SUBJECT: Water Blind Sample No. 49

FROM: Chief, Quality Assurance Section,
HERL, ETD, ACB (MD-69), RTP, NC 27711 J. F. Thompson

TO: All Participating Laboratories

On June 3 a water round robin sample (No. 49) was mailed to 25 laboratories who had previously signified they wished to participate in this interlaboratory excercise. Nineteen laboratories mailed in their analytical data, and a brief summary report was mailed back to all 25 laboratories on July 8 so that the formulation would be available to all, coincidental with the receipt of the 6-month water SPRM in acetone which was mailed June 28.

GENERAL COMMENTS:

We think that most of you will agree that this was not the easiest sample possible nor was it intended to be. We think, however, that it was probably no more difficult than many routine samples; in fact, it was probably somewhat less difficult than many monitoring samples of unknown composition because of the spike with intact parent compounds. Routine environmental samples are far more likely to contain partially altered compounds.

We were somewhat disappointed that five Labs failed to detect both DDT metabolites, and seven Labs failed on oxychlordane. Five Labs overlooked HCB, and to their credit all except three Labs identified the PCB (Aroclor 1254). Those three who overlooked this compound should be deeply concerned because the chromatographic fingerprint should have been unmistakable on just about any GC column one chose to use. Similarly, the HCB peak should have been most glaringly apparent. Two of the Labs who failed on Aroclor 1254 thought they saw all sorts of other things--dieldrin, Kepone, and what have you.

G.C. column selection was undoubtedly one key factor in the failure to detect certain compounds in the presence of the multitude of Aroclor peaks. But another, and we think more cogent factor, was the failure to apply a high degree of reasoning in the interpretation of the available chromatographic data. By way of illustration, p,p'-DDT and a major peak of Aroclor 1254 nearly completely superimpose on the OV-17/OV-210 column operated at 200°C. One laboratory had a peak in their sample extract which calculated an RRT_A of 4.20, about on target for either/or p,p'-DDT and the Aroclor peak. The peak width at baseline measured 24 mm. In their pure Aroclor chromatogram the peak RRT_A calculated 4.14, slightly earlier than the p,p'-DDT RRT_A, but most significantly

EPA Form 1320-6 (Rev. 3-76)

this Aroclor peak had a base width of 19 mm, a most clear indication that something else besides the Aroclor was eluting from the sample extract at this retention site. The prime suspect, of course, would be p,p'-DDT.

We could document a dozen more similar illustrations, but we believe the point should be clear that in the process of interpretation, a number of factors must be weighed, to a much greater extent than simply measuring retentions.

QUANTITATION:

The most glaring irregularity in quantitation was experienced by Lab Code 16 wherein the reported values were apparently in error by an approximate factor of x 100. This laboratory was requested to review their reported data but the values reported back were still far off.

The very best that could be done with HCB is about 85% recovery. Therefore, no Lab should be expected to report a value in excess of 0.25 ppb. It was found in our Lab that the 4% SE-30/6% OV-210 was most suitable for quantitating the HCB as this was the only column producing baseline separation from the solvent peak.

Rejecting those outlier values designated by asterisks in Table 1, the mean recovery values from all Labs, with respect to formulation, were as follows:

HCB	67%
Oxychlordane	83
p,p'-DDE	85
p,p'-DDT	86
Aroclor 1254	81

Considering the relative difficulty of the sample, we think the overall performance was not too bad. In terms of interlaboratory precision, relative standard deviation values in the range of the calculated values were quite acceptable. On less complex formulations "Total Error" values in the under 50% range are generally considered satisfactory. On this particular sample, however, the calculated values could not be considered too far out of line.

Our apologies to Lab Code 23 for overlooking their reported value for p,p'-DDE. This correction is reflected in the attached corrected summary (Table 1).

RELATIVE PERFORMANCE:

Table 2 shows the relative overall performance of the 19 participating laboratories. The method for this computation may be found in our Quality Control Manual, Chapter 2.

Those laboratories with scores over 170 (out of a possible 200) had minor problems, if any. Below this, there was a sharp breakdown to a 115.9 score. Laboratories in the range less than this had problems and the lower the score, the greater the problems. The zero score of the cellar position was due to the sum of the penalty points exceeding the positive points.

Enclosures

cc: Dr. W. S. Murray
Dr. Jack Griffith
Dr. F. W. Kutz
Dr. C. W. Miller
Dr. Lee Leiserson
Dr. John Kliewer
Dr. W. F. Durham

bc: Randy Watts

Reports to: 1A, 3A, 4A, 4B, 5A, 5B, 6A, 7A, 7B, 8A, 8B, 9A, 10A, 10B, 10C, 11A, 12A, 12B, 13A, 15A, 15B, 16A, 19A, 20A, 20B, 22A, 23A, 24A, 24B, 25A, 25B, 25C, 26A, 26B, 26C, 31A, 32A, 34A, 36A

NOTE: Individual critiques sent to the A, B, & C's of each and to the appropriate EPA Field Studies Coordinator

TABLE 1

INTERLABORATORY CHECK SAMPLE NO. 49, WATER - SUMMARY OF RESULTS

LAB CODE	PESTICIDES REPORTED IN MICROGRAMS PER LITER (OR PARTS PER BILLION)					
	HCB	Oxychlordane	p,p'-DDE	p,p'-DDT	Aroclor 1254	
Formulation	0.30	0.40	0.60	1.60	10	
8	0.20	0.30	0.40	1.30	9.1	
16**	---	45.*	81.0*	151.*	627.*	
15	0.18	0.33	0.53	1.50	10.9	
6	---	---	---	---	7.9	
7	0.20	---	---	2.12	13.4	
11	0.13	0.31	0.43	1.20	5.6	
34	0.14	0.17	0.23*	0.97	5.1	
9	0.18	0.40	0.50	1.18	---	p,p'-DDD---0.47, T-Nonachlor---0.28, Kepone---3.3, o,p'-DDT--0.23
36	0.18	0.65*	0.53	1.60	7.5	o,p'-DDT--0.15
26	0.20	0.30	---	---	8.6	
13	0.26	0.36	---	---	3.9	
3	0.25	0.36	0.47	1.51	8.4	
23	---	---	0.83	2.68*	---	o,p'-DDT--0.45, Hept. Epox.--0.42, o,p'-DDE--0.69, Dieldrin--0.95
1	0.25	---	---	---	10	
24	0.16	---	---	---	16.7*	
25	0.22	0.44	0.56	1.41	7.3	
5	0.24	0.36	0.56	1.50	6.8	
12	---	---	0.45	1.20	---	Aldrin--0.04
10	---	---	0.35	1.00	8.8	Aroclor 1248--13.4
Overall Mean	0.20	0.33	0.51	1.37	8.09	
Standard Deviation	0.04	0.07	0.13	0.31	2.43	
Relative Std. Deviat., %	20	22	26	23	30	
Total Error, %	60	53	58	53	68	

*Rejected as outliers

**Reporting units questioned and verified.

Table 2

CHECK SAMPLE NO. 49, WATER--RELATIVE PERFORMANCE RANKING

Lab Code	Compounds Missed	False Identificat.	No. of[1] Rejects	Total[2] Score
5	---	---	---	195.72
3	---	---	---	195.37
25	---	---	---	195.14
15	---	---	---	194.31
8	---	---	---	191.87
11	---	---	---	188.93
34	---	---	1	183.37
36	---	1	1	171.40
13	2	---	---	115.92
26	2*	---	---	115.49
7	2	---	---	114.42
10	2	1	---	94.0
16	1	---	4	80.0
1	3	---	---	78.75
9	1	4	---	74.22
24	3	---	1	73.74
12	3	1	---	56.57
6	4	---	---	39.14
23	3	4	1	0.00

* Later reported the presence of the two compounds

[1] Rejected as outliers

[2] Total possible score - 200 points

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK
NORTH CAROLINA 27711

DATE: January 29, 1980

SUBJECT: Fat Blind No. 70, Check Sample Report

FROM: Chief, Quality Assurance Section (MD-69) R.R. Watts
ACB/ETD/HERL
Research Triangle Park, NC 27711

TO: All Laboratories Receiving SPRM No. 70

On December 3, a fat blind sample was sent to the 22 laboratories which had previously signified they wished to participate in the inter-laboratory exercise. Eighteen laboratories returned reports of analytical results. One Epidemiology Studies laboratory did not participate.

General Comments

This check sample study was designed to measure proficiency of a laboratory in recognizing and quantitatively determining PCB contamination in an adipose sample containing common organochlorine pesticide residues. The pesticides and fortification levels for SPRM No. 70 were derived from national surveys of residues in human adipose tissue. This exercise therefore represented a realistic analytical problem that might be encountered in a residue laboratory.

Specific comments concerning submitted sample report sheets and chromatograms may be found in individual reports.

Results and Discussion

Table 1 presents a summary of the standard pesticide reference material (SPRM) formulation and analytical results. Laboratories above the double line are Epidemiology/Human Monitoring contract laboratories.

This sample proved to be a difficult challenge for several of the participants. Performance scores of 190 points or above (out of 200 possible) were obtained by only five laboratories. Eight of the eighteen total respondents and four out of the eleven "epidemiology/human monitoring" laboratories failed to recognize the PCB residue pattern. Analysts that did not recognize the PCB pattern often misidentified PCB peaks as pesticides.

The percent total error (TE) figures in Table 1 demonstrate a generally unacceptable level of performance for this analysis. Five of the nine TE figures were greater than 50% and therefore unacceptable (for explanation of total error see EPA "Manual of Analytical Quality Control for Pesticides in Human and Environmental Media" section 2K). Total error figures for only the EPA contract laboratories are as follows: HCB, 53.1%; β-BHC, 16.4%; oxychlordane, 45.7%; *t*-nonachlor, 22.3%; Hept. Epox., 43.5%; p,p'-DDE, 46.7%; p,p'-DDT, 30.1%; dieldrin, 42.7%; and PCB 1254, 120%.

GC column pairs used for analysis and confirmation were generally OV-17/OV-210 and SE-30/OV-210. These are both good columns but generally do not make a good complementary pair for qualitative and quantitative confirmation. A better suggestion for most residues would be either of the two mixed columns (SE-30/OV-210 or OV-17/OV-210) paired with the 5% OV-210. The apparent best selection for this sample No. 70 would have been OV-17/OV-210 paired with the OV-210. Pairing of either mixed phase with OV-210 unfortunately entails GC runs at 200 and 180°C and necessitates either two GC's or change of column temperature and rerun of sample extracts and standards.

Laboratories using silica columns on the 6% Florisil fraction to effect a separation of PCB's and chlorinated pesticides appeared to experience some difficulty with achieving proper fractionation. PCB's sometimes eluted in the pesticide fraction causing a PCB peak to be misidentified as a pesticide, i.e., o,p'-DDT.

At least two laboratory reports indicated some Florisil fractionation problem. These problems should be resolved as soon as possible. Florisil that is too retentive could result from (1) improper activation temperature, (2) improper percent of ethyl ether in pet. ether, and (3) ethyl ether that does not contain the required 2% ethanol (read the fine print analytical information on the can or bottle). Florisil that appears insufficiently retentive or inactive might result from (1) or (2) above, and (3) residual amounts of a polar solvent in the sample or standard being placed on the column. Likely candidates here could be acetonitrile from the sample partition cleanup (if the water-out steps where not performed properly) or incomplete removal of benzene (or other solvent more polar than hexane) from a standard solution placed on the column. Other sources of Florisil problems are undoubtedly possible.

Relative Performance

Table 2 shows the overall performance ranking of the eighteen participating laboratories. A possible score of 200 points is derived from 100 points each for qualitative and quantitative analytical results.

General Recommendations

Residue analysts should become familiar with PCB elution patterns from their common GC columns. Chromatograms could be exhibited on a wall or filed in some other convenient manner for easy referral.

The 5% OV-210 column should be used routinely for identity confirmations.

Laboratories should investigate the various schemes that have been offered in the literature for separation of PCB's from other chlorinated pesticide residues. Silicic acid column chromatography of the 6% Florisil fraction to achieve this separation is being used by the EPA human milk monitoring program.

CC: Dr. V. R. Hunt
Dr. F. W. Kutz
Ms. S. C. Strassman-Sundy
Ms. Madeline Dean
Dr. Hale Vandermer
Dr. C. W. Miller
Dr. Lee Leiserson
Dr. John Kliewer
Dr. W. F. Durham

TABLE 1

INTERLABORATORY CHECK SAMPLE NO. 70, FAT-SUMMARY of RESULTS

LAB CODE	Formulation	PESTICIDES REPORTED IN PPM									
		HCB	β-BHC	OXYCHLOR-DANE	trans-NONACHLOR	HEPT. EPOXIDE	P,P'-DDE	P,P'-DDT	DIELDRIN	AROCLOR 1254	NON-SPIKE
		0.061	0.25	0.10	0.15	0.081	3.50	0.60	0.13	1.00	
4		0.038	0.278	0.114	0.156	0.092	3.39	0.675	0.162	1.99*	
5		N O	R E P O R T								
7		0.035	0.269	0.079	0.175	0.091	3.289	0.705	0.149	+	O,P'-DDT = 0.057
8		0.038	0.22	0.10	0.16	0.105	3.31	0.59	0.133	1.40	
10		0.041	0.244	0.094	0.154	0.095	3.212	0.581	0.081	-	O,P'-DDT 0.062 Aldrin 0.014
11		0.032	0.23	0.075	0.12	0.086	3.3	0.68	0.13	-	
12		0.042	0.278	0.117	0.148	0.096	2.148	0.741	0.132	-	Aldrin 0.018
14		0.032	0.234	0.057	0.151	0.062	2.097	0.481	0.082	-	
24		0.049	0.180	0.074	0.130	0.080	2.40	0.470	-	0.505	O,P'-DDT 0.052
25		0.042	0.258	0.105	0.152	0.090	3.964	0.559	0.152	1.328	O,P'-DDT 0.040 P,P'-DDD 0.045
26		0.022	0.237	0.105	0.13	0.059	2.96	0.588	0.148	0.943	
51		0.04	0.25	0.09	0.15	0.10	3.10	0.60	0.13	0.96	
1		0.06	-	0.16	0.11	0.03	3.36	0.80	0.13	-	O,P'-DDT 0.14 Ald. 0.02 Lindane 0.05
6		0.048	-	-	0.12	0.064	3.40	0.58	0.12	1.00	
9		0.042	0.264	0.182*	0.208*	0.086	2.577	0.819	0.102	-	
13		0.024	0.242	0.083	0.143	0.094	2.061	0.575	0.029	-	
16		0.045	0.212	0.122	0.165	0.073	2.98	1.01*	0.124	-	
38		0.043	0.223	0.058	0.116	0.053	2.992	0.503	0.118	1.00	
52		0.014*	0.119*	0.036	0.115	0.015*	2.166	0.403	0.019*	0.519	
Overall Mean		0.040	0.24	0.092	0.14	0.080	2.93	0.61	0.12	0.96	
Standard Deviation		0.009	0.03	0.030	0.02	0.02	0.56	0.12	0.03	0.32	
Relative Std. Deviat., %		23.0	11.0	32.6	14.0	25.1	19.0	18.9	27.7	33.8	
Total Error, %		65.0	24.8	68.0	32.4	51.1	48.1	39.9	58.8	69.1	
Ave. % Recovery		66.7	96.0	92.0	93.3	98.8	83.7	101.7	92.3	96.0	
(*Outlier)											

TABLE 2

CHECK SAMPLE NO. 70, FAT-RELATIVE PERFORMANCE RANKING

LAB CODE	COMPOUNDS MISSED	FALSE IDENTIFICATION	IDENTIFICATION SCORE	QUANTITATION SCORE	TOTAL SCORE
51*	0	0	100	95.57	195.57
8*	0	0	100	92.89	192.89
26*	0	0	100	91.17	191.17
38	0	0	100	90.37	190.37
4*	0	0	100	90.24	190.24
52	0	0	100	73.80	173.80
25*	0	2	77.78	94.03	171.81
11*	1	0	88.89	81.30	170.19
7*	0	1	88.89	80.99	169.88
16	1	0	88.89	79.14	168.03
14*	1	0	88.89	77.68	166.57
13	1	0	88.89	77.12	166.01
9	1	0	88.89	75.95	164.84
12*	1	1	77.78	80.62	158.40
24*	1	1	77.78	78.38	156.16
6	2	0	77.78	73.33	151.11
10*	1	2	66.67	83.21	149.88
1	2	3	44.44	69.10	113.54

*Epidemiology/Human Monitoring Contract Laboratory

Recalculation of Fat Check Sample No. 70.

1) All quantitations including Lab #4 which was termed "outlier"

n	9
Mean	1.07
SD	46
RSD (%)	43

$$\text{Accuracy } \frac{\text{Mean}}{\text{Actual}} \quad 107\%$$

2) All reporting laboratories with "zero" included for those who did not report Aroclor 1254

n	18
Mean	0.65
SD	0.62
RSD (%)	96

$$\text{Accuracy } \frac{\text{Mean}}{\text{Actual}} \quad 65\%$$

APPENDIX D

Breakdown of Census Regions and Divisions by State
(Provided by EPA)

Census Breakdowns of the United States

Region	Division	States
North East	New England	Connecticut
		Maine
		Massachusetts
		New Hampshire
		Rhode Island
		Vermont
	Middle Atlantic	New Jersey
		New York
		Pennsylvania
North Central	East North Central	Illinois
		Indiana
		Michigan
		Ohio
		Wisconsin
	West North Central	Iowa
		Kansas
		Minnesota
		Missouri
		Nebraska
		North Dakota
		South Dakota
South	South Atlantic	Delaware
		District of Columbia
		Florida
		Georgia
		Maryland
		North Carolina
		South Carolina
		Virginia
		West Virginia
	East South Central	Alabama
		Kentucky
		Mississippi
		Tennessee
	West South Central	Arkansas
		Louisiana
		Oklahoma
		Texas

Census Breakdowns of the United States (Continued)

Region	Division	States
West	Mountain	Arizona
		Colorado
		Idaho
		Montana
		Nevada
		New Mexico
		Utah
		Wyoming
	Pacific	Alaska
		California
		Hawaii
		Oregon
		Washington

APPENDIX E
Survey and Site Quotas for Fiscal Years 1972-1976
(Provided by EPA)

Census Divisions

National Human Monitoring Program
Age, Race, Sex Distributions

	Age Groups	Sex M	Sex F	Total
New England (1)				
	0-14	4	4	8
	15-44	5	5	10
	45+	4	5	9
	Total:	13	14	27
		# Negroes = 1		
Middle Atlantic (2)				
	0-14	4	3	7
	15-44	5	6	11
	45+	4	5	9
	Total:	13	14	27
		# Negroes = 3		
East North Central (3)				
	0-14	4	4	8
	15-44	5	6	11
	45+	4	4	8
	Total:	13	14	27
		# Negroes = 3		
West North Central (4)				
	0-14	4	4	8
	15-44	5	5	10
	45+	4	5	9
	Total:	13	14	27
		# Negroes = 1		
South Atlantic (5)				
	0-14	4	4	8
	15-44	5	6	11
	45+	4	4	8
	Total:	13	14	27
		# Negroes = 6		

Census Divisions

National Human Monitoring Program
Age, Race, Sex Distributions

	Age Groups	Sex M	Sex F	Total
East South Central (6)				
	0-14	4	4	8
	15-44	5	6	11
	45+	4	4	8
	Total:	13	14	27
		# Negroes = 5		
West South Central (7)				
	0-14	4	4	8
	15-44	6	6	12
	45+	3	4	8
	Total:	13	14	27
		# Negroes = 4		
Mountain (8)				
	0-14	4	4	8
	15-44	6	5	11
	45+	4	4	8
	Total:	14	13	27
		# Negroes = 1		
Pacific (9)				
	0-14	4	3	7
	15-44	6	6	12
	45+	4	4	8
	Total:	14	13	27
		# Negroes = 2		

National Human Monitoring Program
Collected by Census Division

	Age Groups	Sex M	Sex F	Total
New England (1) - 4 Collection Sites - 4%				
	0-14	16	16	32
	15-44	20	20	40
	45+	16	20	36
	Total:	52	56	108
		# Negroes = 4		
Middle Atlantic (2) - 14 Collection Sites - 19%				
	0-14	56	42	98
	15-44	70	84	154
	45+	56	70	126
	Total:	182	196	378
		# Negroes = 42		
East North Central (3) - 14 Collection Sites - 19%				
	0-14	56	56	112
	15-44	70	84	154
	45+	56	56	112
	Total:	182	196	378
		# Negroes = 42		
West North Central (4) - 5 Collection Sites - 7%				
	0-14	20	20	40
	15-44	25	25	50
	45+	20	25	45
	Total:	65	70	135
		# Negroes = 5		

National Human Monitoring Program
Collected by Census Division

	Age Groups	Sex M	Sex F	Total
South Atlantic (5) - 11 Collection Sites - 15%				
	0-14	44	44	88
	15-44	55	66	121
	45+	44	44	88
	Total:	143	154	297
		# Negroes = 66		
East South Central (6) - 7 Collection Sites - 9%				
	0-14	28	28	56
	15-44	35	42	77
	45+	28	28	56
	Total:	91	98	189
		# Negroes = 35		
West South Central (7) - 7 Collection Sites - 9%				
	0-14	28	28	56
	15-44	42	42	84
	45+	21	28	49
	Total:	91	98	189
		# Negroes = 28		
Mountain (8) - 3 Collection Sites - 4%				
	0-14	12	12	24
	15-44	18	15	33
	45+	12	12	24
	Total:	42	39	81
		# Negroes = 3		
Pacific (9) - 10 Collection Sites - 13%				
	0-14	40	30	70
	15-44	60	60	120
	45+	40	40	80
	Total:	140	130	270
		# Negroes = 20		

National Human Monitoring Program
Collected by Census Division

	Age Groups	Sex M	F	Total	Percent
Summary:					
	0-14	300	276	576	28.4
	15-44	395	438	833	41.1
	45+	293	323	616	30.4
	Total:	988	1037	2025	99.9

Negroes = 245

Percent = 12%

Total Males = 49%
Total Females = 51%
Total Negroes = 12%

APPENDIX F

EPA Guidelines and General Information About Collecting Adipose Tissue for the National Human Monitoring Program for Pesticides

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Guidelines and General Information About Collecting Adipose Tissue For the National Human Monitoring Program for Pesticides

The National Human Monitoring Program for Pesticides is responsible for determining, on a national basis, the incidences, levels and other evidences of exposure to pesticides in the general population of the United States. At present, the program collects and analyzes adipose tissues for selected pesticides and their metabolites known to be stored in the lipid portion of these tissues. The results from the program are used in evaluating various factors and conditions pertaining to human health and effective pesticide regulation.

The adipose tissue for this program is secured through the cooperation of participating pathologists and medical examiners located throughout the continental United States. The tissue is obtained from surgical specimens previously excised for pathological examination and from postmortem examinations. The specimens are sent to the program office in Washington, D. C., from which they are subsequently forwarded to contract laboratories for chemical analysis. Periodic reports of the laboratory results are sent to each participating pathologist for the tissues which were submitted under his auspices. Summaries comparing results with other regions of the country are also provided as they become available.

In order to develop valid information on a national basis, collections must be made according to an experimental design which dictates the number of samples required according to the demographic distribution of the population in the appropriate census division. You should have a copy of the annual quota of samples expected to be collected from your location on a fiscal year basis. All collections should be made according to this age/sex/race distribution. You should be able to collect the number of samples required in each category. Since our total sample is relatively small and the validity of the results depends on a high response rate, your participation is particularly important. If you feel that you will be unable to collect the number of samples required, please let us know.

Criteria for Selection of Patients to be Sampled

Since the program objective is to reflect pesticide incidences and levels in the general (man-on-the-street) population, a few suggestions are listed here for your guidance:

- The highest priority should be given to satisfying the number and demographic distribution of your annual quota. This quota should be completed as soon after the start of the fiscal year as possible.
- Patients having known or suspected pesticide poisoning should not be sampled. If you are involved with a potential pesticide poisoning, we would like to know about it. However, samples should not be taken for the National Human Monitoring Program for Pesticides.

- Patients exhibiting cachexia or who have been institutionalized for long periods should not be sampled for the national program.

Legal Considerations

The National Human Monitoring Program for Pesticides is both interested and deeply concerned about the legal ramification of this human research project. Since the program operates in about 40 states, it is not feasible for us to handle the variety of local or state interpretations from our location in Washington. Therefore, as a matter of policy, the legal requirements, i.e., informed consent, confidentiality, are matters for your consideration and resolution. Collections for this program must be made in conformance with the applicable HEW guidelines on the protection of human subjects of biomedical and behavioral research. We will, however, be pleased to assist you in any way possible.

We have completed several studies on these matters and do not believe that they present major obstacles to your participation. In most documents authorizing postmortem examinations, there is a clause granting the examining physician permission to remove tissues for research purposes. We consider this project to be included in that category. In the case of specimens recovered from your surgical practice, the use of a small amount of tissue from a previously excised specimen certainly does not place the patient at risk in any way whatsoever.

As you will notice in our discussion of data needed for each patient sampled, we do have several mechanisms to assure confidentiality. In fact, the disclosure or release of certain data is protected by federal statute. The fees paid to you by our program are solely intended to remunerate you or your designee for professional services rendered.

Collection of Surgical Adipose Tissue

Collect samples of adipose tissue from unfixed specimens which have been surgically excised for therapeutic reasons. Take special care to keep samples from different patients separate, correctly and securely labeled, and avoid their contact with other chemicals, such as paraffin, disinfectants, preservatives, or plastics.

At least five grams of good quality (subcutaneous, perirenal, or mesenteric) adipose tissue should be collected; avoid fibrous or connective tissue, i.e., omentum. Place the fat, without any fixatives or preservatives into the provided chemically-cleaned container; legibly complete and attach the self-adhesive label in ball-point pen or pencil. The bottle labels should be affixed before freezing. Store the specimens up-right in a freezer at -4°F (-20°C) until shipment.

Collection of Postmortem Adipose Tissue

Adipose tissue samples must be obtained only from unembalmed cadavers. The interval between death and the collection of tissue should be as short as possible and must not exceed 24 hours, assuming refrigeration during the interval. Samples of adipose tissue must weigh at least five grams and should be placed in the supplied, chemically-clean container with a completed label affixed. Specimens should be stored at -4°F (-20°C) without any fixative or preservative until shipment. Submit only good quality fat; do not submit omentum as it contains too much connective tissue for satisfactory analysis.

Adipose should be taken dry, and should not be rinsed before placing in the provided containers. Many water supplies contain materials which would interfere with chemical analysis.

Instruments should be well-rinsed with distilled water and dried before taking the adipose sample.

Completion of the Patient Summary Report

A Patient Summary Report should be completed for each patient from whom a sample was taken. Special attention should be given to the completeness of the data. All medical information submitted is protected from disclosure or release by U.S.C. 552, (b) (6); 45 CFR Part 5. First and last initials, in that order, should be used instead of the complete name to insure that confidentiality is maintained. The initials, along with the data of birth, sex, and race, are used in this office to compose the AMA identification number. The patient's identification number and/or the pathology department's accession number are for your information in referring back to the individual patient when you receive the results of the pesticide analysis.

Confirmed diagnosis should be detailed in the spaces provided. Only the major ones should be supplied.

Other information required should be completed as accurately as possible. The complete forms should be held and sent under the lid of the insulated container when shipment is made.

Packing and Shipping

Tighten all lids on the specimen bottles carefully. This is important since we are required to use special aluminum foil cap liners which make tightening a little difficult. Be certain that a completed bottle label is firmly attached to each specimen bottle. Wrap each bottle in gauze or paper to prevent breakage during shipment and to keep the label on the container. Place the specimen bottles in the insulated mailer and fill it with dry ice. If you have difficulty obtaining dry ice, please call us and we can arrange alternative methods of refrigeration for you.

A franked addressed label is on the reverse side of the address card. This card is marked AIR MAIL - SPECIAL DELIVERY. (Do not send Air Express, please). There is no cost to the sender because of the franked label. All insulated mailers should have a PERISHABLE-PACKED IN DRY ICE label visible from all sides on the outside.

Specimens should be mailed on a Monday or Tuesday of a week with no federal holidays. This assures that they will arrive before the end of the work week on Friday.

Patient Summary Reports should be sent in the carton with the specimens when possible. They can be folded and placed on the top of the polyfoam lids.

Only samples which meet our criteria and are handled according to the guidelines can be accepted. No substitute containers will be accepted.

For Further Information

If you have any questions or comments, please contact us. Telephone (collect): 202/755-8060.

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National Human Monitoring Program
for Pesticides (WH-569)

APPENDIX G

National Data Summaries for Fiscal Years 1972-1976
(Provided by EPA)

FY 1972

FREQUENCY AND LEVELS OF POLYCHLORINATED BIPHENYLS IN HUMAN ADIPOSE TISSUE, BY GEOGRAPHIC AREA AND RACE: WET. WT. BASIS

Stratification	Sample size	Percent not Detected	Percent less than 1 ppm	Percent 1-3 ppm	Percent greater than 3 ppm	File Type
NATIONAL	1778	10.24	22.05	61.87	5.85	DESIGN
RACIAL GROUP						
Caucasian	1469	9.12	23.48	62.08	5.30	DESIGN
Negro	303	15.18	15.18	61.38	8.25	DESIGN
Mexican American	--*					DESIGN
Puerto Rican	--					DESIGN
Oriental	3	0.00	0.00	66.66	33.33	DESIGN
Other	3	66.66	33.33	0.00	0.00	DESIGN
NATIONAL	4098	26.06	15.45	50.63	7.86	MASTER
RACIAL GROUP						
Caucasian	3370	25.16	18.52	51.57	6.73	MASTER
Negro	708	30.22	10.45	46.46	12.86	MASTER
Mexican American	--					MASTER
Puerto Rican	--					MASTER
Oriental	4	0.00	25.00	50.00	25.00	MASTER
Other	7	28.57	14.28	14.28	42.65	MASTER
American Indian	9	44.44	0.00	55.55	0.00	MASTER

*Indicates no samples in this group.

FY 1973

FREQUENCY AND LEVELS OF POLYCHLORINATED BIPHENYLS IN HUMAN ADIPOSE TISSUE, BY GEOGRAPHIC AREA AND RACE: WET. WT. BASIS

Stratification	Sample size	Percent not Detected	Percent less than 1 ppm	Percent 1-3 ppm	Percent greater than 3 ppm	File Type
NATIONAL	1120	21.43	41.61	31.16	5.80	DESIGN
RACIAL GROUP						
Caucasian	918	20.18	45.25	30.27	4.28	DESIGN
Negro	132	31.06	14.39	37.87	16.66	DESIGN
Mexican American	2	0.00	100.00	0.00	0.00	DESIGN
Puerto Rican	--					DESIGN
Oriental	1	0.00	0.00	0.00	100.00	DESIGN
Other	3	33.33	33.33	33.33	0.00	DESIGN
American Indian	1	0.00	0.00	100.00	0.00	DESIGN
NATIONAL	1279					MASTER
RACIAL GROUP						
Caucasian	1102	22.86	43.82	29.12	4.17	MASTER
Negro	169	36.09	17.75	32.54	13.60	MASTER
Mexican American	2	0.00	50.00	0.00	50.00	MASTER
Puerto Rican	--					MASTER
Oriental	2	0.00	50.00	0.00	50.00	MASTER
Other	3	33.33	33.33	33.33	0.00	MASTER
American Indian	1	0.00	0.00	100.00	0.00	MASTER

FY 1974

FREQUENCY AND LEVELS OF POLYCHLORINATED BIPHENYLS IN HUMAN ADIPOSE TISSUE, BY GEOGRAPHIC AREA AND RACE: WET. WT. BASIS

Stratification	Sample size	Percent not Detected	Percent less than 1 ppm	Percent 1-3 ppm	Percent greater than 3 ppm	File Type
NATIONAL	926	9.29	51.62	34.02	5.08	DESIGN
RACIAL GROUP						
Caucasion	798	8.27	54.38	32.95	4.38	DESIGN
Negro	119	14.28	33.61	42.85	9.24	DESIGN
Mexican American	1	0.00	100.00	0.00	0.00	DESIGN
Puerto Rican	--					DESIGN
Oriental	1	0.00	100.00	0.00	0.00	DESIGN
Other	7	42.85	25.57	14.28	14.28	DESIGN
NATIONAL	1051	9.04	50.62	35.49	4.85	MASTER
RACIAL GROUP						
Caucasian	891	8.30	53.87	33.55	4.26	MASTER
Negro	150	12.00	32.00	48.00	8.00	MASTER
Mexican American	1	0.00	100.00	0.00	0.00	MASTER
Puerto Rican	--					MASTER
Oriental	1	0.00	100.00	0.00	0.00	MASTER
Other	8	37.50	25.00	25.00	12.50	MASTER

FY 1975

FREQUENCY AND LEVELS OF POLYCHLORINATED BIPHENYLS IN HUMAN ADIPOSE TISSUE, BY GEOGRAPHIC AREA AND RACE: WET. WT. BASIS

Stratification	Sample size	Percent not Detected	Percent less than 1 ppm	Percent 1-3 ppm	Percent greater than 3 ppm	File Type
NATIONAL	793	5.80	56.49	27.24	10.47	DESIGN
RACIAL GROUP						
Caucasian	680	5.88	57.35	27.21	9.56	DESIGN
Negro	105	4.76	52.38	26.67	16.19	DESIGN
Mexican American	4	25.00	0.00	75.00	0.00	DESIGN
Puerto Rican	--					DESIGN
Oriental	2	0.00	100.00	0.00	0.00	DESIGN
Other	2	0.00	50.00	0.00	50.00	DESIGN
NATIONAL	910	5.82	55.93	27.58	10.66	MASTER
RACIAL GROUP						
Caucasian	756	5.95	57.01	26.98	10.05	MASTER
Negro	135	4.44	52.59	29.63	13.33	MASTER
Mexican American	13	15.38	30.77	46.15	7.69	MASTER
Puerto Rican	--					MASTER
Oriental	2	0.00	100.00	0.00	0.00	MASTER
Other	4	0.00	25.00	25.00	50.00	MASTER

FY 1976

FREQUENCY AND LEVELS OF POLYCHLORINATED BIPHENYLS IN HUMAN ADIPOSE TISSUE, BY GEOGRAPHIC AREA AND RACE: WET. WT. BASIS

Stratification	Sample size	Percent not Detected	Percent less than 1 ppm	Percent 1-3 ppm	Percent greater than 3 ppm	File Type
NATIONAL	684	2.03	59.36	29.32	9.29	DESIGN
RACIAL GROUP						
Caucasian	569	1.76	61.16	29.70	7.38	DESIGN
Negro	103	1.94	49.51	29.13	19.42	DESIGN
Mexican American	1	0.00	100.00	0.00	0.00	DESIGN
Puerto Rican	--					DESIGN
Oriental	2	0.00	100.00	0.00	0.00	DESIGN
Other	9	22.22	44.44	11.11	22.22	DESIGN
American Indian	5	0.00	60.00	28.54	9.68	DESIGN
NATIONAL	785	1.78	60.00	28.54	9.68	MASTER
RACIAL GROUP						
Caucasian	645	1.55	61.86	28.84	7.75	MASTER
Negro	120	1.67	50.00	28.33	0.00	MASTER
Mexican American	1	0.00	100.00	0.00	0.00	MASTER
Puerto Rican	--					MASTER
Oriental	2	0.00	100.00	0.00	0.00	MASTER
Other	12	16.67	50.00	16.67	16.67	MASTER
American Indian	5	0.00	60.00	40.00	0.00	MASTER