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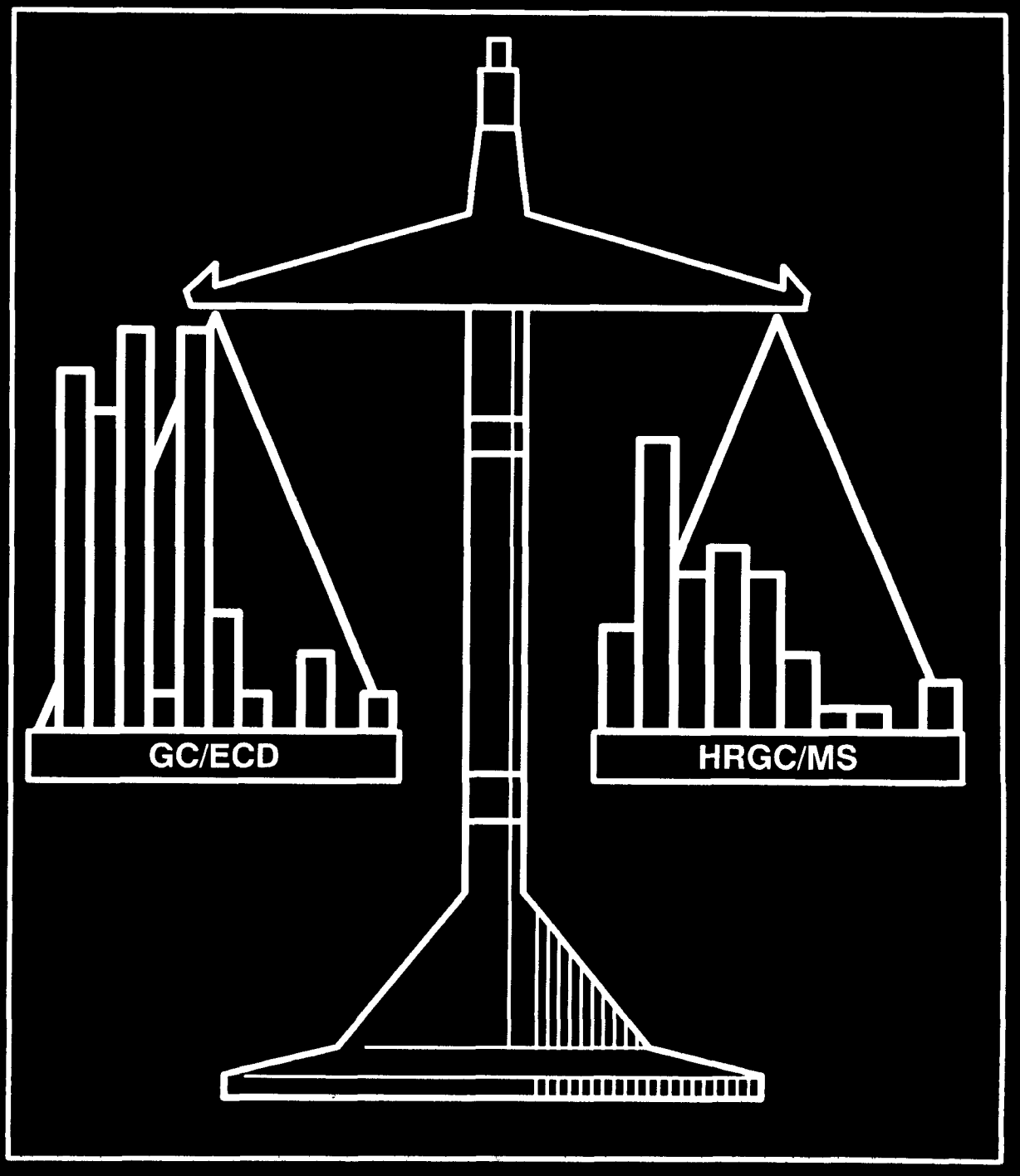
EPA NHATS COMPARABILITY STUDY



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NHATS COMPARABILITY STUDY

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This Comparability Study is one part of the National Human Adipose Tissue Survey (NHATS). The study was conducted through the cooperative efforts of EPA and contract support staff. EPA participation was from the Exposure Evaluation Division within the Office of Pollution Prevention and Toxics (OPPT). Within the Exposure Evaluation Division, the Design and Development Branch and the Field Studies Branch were responsible for the conduct of the study and the preparation of the final report. Contract support to OPPT included Westat, Battelle, Midwest Research Institute (MRI), and the Institute of Rural Environmental Health at Colorado State University (CSU).

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EXECUTIVE SUMMARY

The National Human Adipose Tissue Survey (NHATS) is a chemical monitoring program operated by EPA from 1970 through 1992. The objectives of the NHATS program were: (1) to detect and quantify the concentrations and prevalences of selected toxic substances in the adipose tissue of the general U.S. population; (2) to measure trends in these concentrations over time; (3) to assess the effects of regulatory actions; and (4) to provide baseline body burden data for chemicals of interest to EPA.

The data for NHATS were generated by collecting and analyzing adipose tissue specimens for selected toxic substances. The adipose tissue specimens were obtained from cooperating hospitals and medical examiners across the continental United States.

Initially, the NHATS program focused on organochlorine pesticides and polychlorinated biphenyls (PCBs) using the Modified Mills Olney Gaither (MOG) protocol and packed column gas chromatograph/electron capture detection (GC/ECD) method. However, in 1982 the program was expanded to include a wider range of lipophilic compounds. In 1982 and 1984, the samples were analyzed using a method based on a high resolution gas chromatograph/mass spectrometer (HRGC/MS).

A comparability study was initiated to compare the measurements from the two analytical methods for similar adipose tissue samples. The determination of data comparability between the methods is essential to allow valid assessments of the data when combining results for GC/ECD and HRGC/MS for trend analysis and baseline estimates. The specific objectives of the Comparability Study were to: 1) characterize the detection levels, recovery, and precision of the measurements, and to compare these for the two methods; 2) assess whether there are significant differences between the measurements from the two analytical methods; and 3)

describe the relationship between the HRGC/MS and GC/ECD measurements and assess its usefulness for converting measurements from one method to the other.

The study objectives were met by analyzing paired composite samples using both the HRGC/MS and GC/ECD methods. Individual FY84 specimens were combined to create larger composite samples to reduce analysis costs and to provide adequate sample quantities to meet the sensitivity requirements for both HRGC/MS and GC/ECD analyses. The composite samples were grouped into batches for processing and analysis. Each sample was split to create paired samples, one for GC/ECD analysis and the other for HRGC/MS analysis. Method blanks, quality control, and multisplit samples were analyzed, in addition to the paired samples, to assess the quality of the data and to estimate the precision and recovery of the two methods.

The HRGC/MS method can detect a wider range of compounds than the GC/ECD, which is limited because of its specificity to compounds with high electron capture cross-sections. Of the 20 compounds measured by both methods, only the following nine compounds were positively identified and quantified by both the GC/ECD and HRGC/MS methods: p,p'-DDT; p,p'-DDE; beta-BHC; dieldrin, heptachlor epoxide; oxychlordane; trans-nonachlor; hexachlorobenzene; and PCBs.[1] These are the same compounds that have been routinely detected in previous GC/ECD efforts. These compounds are referred to as the primary compounds for the comparability study. Because the results for dieldrin were limited, the comparison of the two analytical methods is based primarily on the measurements of: p,p'-DDT; p,p'-DDE; beta-BHC; heptachlor epoxide; oxychlordane; trans-nonachlor; hexachlorobenzene; and PCBs.

The GC/ECD method is more sensitive than the HRGC/MS method in measuring all primary compounds except PCBs. Because the de-

[1]Total PCBs are counted as one compound here, however, for the HRGC/MS method, the concentrations of individual PCB homologs are reported.

tection limits vary among samples, the average detection limits for each compound were used for comparison. The average GC/ECD detection limits are consistently below 0.01 ug/g, compared to the average HRGC/MS detection limits which range from 0.01 to 0.35 ug/g for different compounds.[2] However, for PCBs, the GC/ECD method is much less sensitive than the HRGC/MS method, with average detection levels of 0.43 ug/g and 0.01 ug/g respectively.[3]

Method recovery was determined from the analysis of samples spiked with known amounts of target analytes. The recovery estimates vary, depending on the compound. The GC/ECD recoveries for spiked lipid material range from 52% to 109%. The HRGC/MS recovery estimates are less precise and range from 26% to 62%, with the exception of beta-BHC with an estimated recovery of 99%. These general results are consistent with the ratio of the GC/ECD to HRGC/MS measurements in the paired samples. The GC/ECD recovery estimates are consistently greater than the HRGC/MS estimates for all compounds except beta-BHC for which the estimates are similar.

For all compounds except dieldrin and PCBs, a strong linear relationship existed between the HRGC/MS and GC/ECD measurements in the paired samples, with the ratio of the HRGC/MS to GC/ECD measurements being roughly constant. For some compounds, the ratio of the HRGC/MS to GC/ECD measurements varied among analysis batches and depended on the concentration in the samples. The ratio of the average GC/ECD measurement to the average HRGC/MS measurement in the paired samples ranged from 1.25 to 3.88 for different compounds. For p,p'-DDT, p,p'-DDE, heptachlor epoxide, oxychlordane, trans-nonachlor, and hexachlorobenzene, the ratios are statistically significantly greater than 1.0.

For the statistical analysis, PCBs were analyzed separately because the GC/ECD concentrations in the paired samples were

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[2]The measurements are reported in micrograms per gram lipid weight, unless otherwise noted.

[3]The ECD method provides data on total PCBs while the MS method is capable of providing data on individual PCB compounds.

reported on an interval scale[4] rather than as a concentration, which limited the ability to compare the HRGC/MS and GC/ECD measurements. The results for the PCB measurements were similar to other primary compounds in that the GC/ECD measurements tended to be higher than the corresponding HRGC/MS measurements. The ratio of the GC/ECD and HRGC/MS measurements could not be determined due to the interval nature of the GC/ECD data.

The precision with which a concentration can be measured depends on the analysis method and the concentration in the sample, with the standard deviation of the measurements increasing as the concentration increases. For the primary compounds other than dieldrin the GC/ECD measurements were more precise than the HRGC/MS measurements. For these compounds, the following rules of thumb can be used to describe the precision: 1) the coefficient of variation of the HRGC/MS measurements was three times larger than for the GC/ECD measurements; and 2) 95% of GC/ECD measurements were within 22% of the actual concentrations and 95% of HRGC/MS measurements were within 63% of the actual concentrations in the samples.

The final objective of the Comparability Study was to assess whether the relationship between the HRGC/MS and GC/ECD measurements in the FY84 composite samples is useful for comparing average concentrations measured by different methods in different years. To make this assessment, the ratios of the average GC/ECD to HRGC/MS measurements were used to adjust the 1982 HRGC/MS measurements for comparison to the trends in the GC/ECD measurements in neighboring years. Given the likely prediction errors, the adjusted HRGC/MS averages from 1982 were consistent with the trends in the GC/ECD averages prior to 1982 and in 1983. Use of this adjustment method assumes that the relationship between the GC/ECD and HRGC/MS measurements are unaffected by changes in the

[4]Concentrations were reported as being in one of the intervals 0 to .33 ug/g, .33 to 1 ug/g, 1 to 3 ug/g, or greater than 3 ug/g.

procedures over time, the laboratory used, or the concentration levels in the samples.

An alternate procedure for comparing the HRGC/MS and GC/ECD data is to correct all measurements for recovery. Because the recovery depends on the sample matrix, this would require recovery measurements on lipid samples for each year of data. An adequate number of samples for estimation of recovery is recommended to limit the possible error in the estimated recoveries and the corresponding averages. When adequate recovery information is available, correcting the HRGC/MS data for recovery may be preferable to using the ratios from FY84 data because the recovery correction requires making fewer assumptions.

The conclusions from the Comparability Study are:

The measurements from the HRGC/MS and GC/ECD methods cannot be compared without accounting for differences in recovery, precision, and detection limits.

- The GC/ECD method has generally more precise estimates, higher recovery, and lower detection limits than the HRGC/MS method when measuring the primary compounds.

  The GC/ECD method was more precise than the HRGC/MS method. The GC/ECD method has higher recovery than the HRGC/MS method for all compounds except beta-BHC for which the recovery estimates are similar. The GC/ECD method has lower detection limits than the HRGC/MS method for all compounds except PCBs.

- The HRGC/MS measurements are lower than the GC/ECD measurements for all primary compounds. The differences are statistically significant for some compounds.

- Compared to the GC/ECD method, the HRGC/MS method can be used to study a wider range of target compounds and was chosen in recent years in order to expand the list of chemicals monitored by NHATS.

For comparison of measurements across methods and years, the measurements can be adjusted using either the ratio of the GC/ECD to HRGC/MS measurements or by correcting for recovery. A correction for recovery requires making fewer assumptions.

- Using the ratios of the average GC/ECD and HRGC/MS measurements in FY84 samples to convert from one method to the other proved reasonable for the 1982 data. However, this conversion method has limitations. The relationship between the GC/ECD and HRGC/MS measurements may change with time, the laboratory used, or the concentration levels in the samples.

- A combination of theoretical arguments and data analysis suggests that the comparability of the measurements from the two methods can be improved by correcting for recovery within each sample year and laboratory. Additional analysis beyond the scope of this study is required to evaluate the best method for making a recovery correction and how it compares to use of a ratio correction based on the Comparability Study.

1 INTRODUCTION

The National Human Adipose Tissue Survey (NHATS) has been the main operative program of the National Human Monitoring Program (NHMP). NHMP is a chemical monitoring program designed to fulfill the human and environmental monitoring mandates of both the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended. NHATS was first established by the U. S. Public Health Service in 1967, and was transferred to the U. S. Environmental Protection Agency (EPA) in 1970. NHATS was conducted by EPA through 1992. The Agency now is in the process of developing alternate human tissue monitoring activities as part of the National Human Exposure Assessment Survey (NHEXAS).

The data for NHATS were generated by collecting and analyzing adipose tissue specimens for selected toxic substances. The adipose tissue specimens were obtained from cooperating hospitals and medical examiners within a statistically representative sample of metropolitan statistical areas across the continental United States. Cooperating pathologists and medical examiners collected and sent adipose tissue specimens to EPA on a continuing basis throughout each fiscal year. The pathologists and medical examiners also supplied EPA with a limited amount of demographic, occupational, and medical information for each specimen. This information allows reporting of the residue levels by subpopulations of interest, namely sex, race, age group, and geographic region.

Historically, the NHATS program monitored human adipose tissue for the presence and levels of 19 organochlorine pesticides and polychlorinated biphenyl compounds (PCBs). Nine of these 20 compounds have been regularly detected in more than 90% of the annual NHATS specimens (Robinson, Mack, Remmers, Mohadjer, 1990). The NHATS program has documented a decrease in the concentrations

of several of these compounds over time. NHATS data showed a decrease in the percentage of samples with PCB levels above 3 ug/g wet weight[1] starting in 1978. This decrease began 2 years after Congressional legislation in 1976 banned production and use of PCBs.

The success in monitoring the effect of regulation and the desire to broaden knowledge of the range of chemicals to which humans are exposed led EPA to expand the list of chemicals monitored by NHATS to include a wider range of lipophilic compounds. The expansion of the chemical list led to a new chemical measurement protocol in 1982. From 1970 through 1981 and in 1983, the NHATS samples were analyzed by the Modified Mills Olney Gaither (MOG) protocol using a packed column gas chromatograph with electron capture detection (GC/ECD)(Sherma and Beroza 1980). In 1982 the samples were analyzed using a method that applies high resolution gas chromatography/mass spectrometry (HRGC/MS) for detection of analytes (Stanley 1985). In 1984, samples were split and analyzed using both methods for the Comparability Study.

Since a primary objective of the NHATS program has been to estimate baseline levels and trends over time, possible changes in the measurements associated with changes in the analytical technique are of interest. Therefore, this Comparability Study was initiated to compare the measurements from the two analytical methods for similar adipose tissue samples. Comparability of the measurements from the two methods has focused on eight individual organochlorine pesticides and one class of chemicals which have been consistently detected in human adipose tissue with the GC/ECD method. These compounds are p,p' DDT, p,p' DDE, beta-BHC, heptachlor epoxide, oxychlordane, trans-nonachlor, hexachlorobenzene, dieldrin, and PCBs. For the GC/ECD method, the hexachlorobenzene measurements were reported as measured (referred to as uncorrected

[1]The measurements are reported in micrograms per gram lipid weight, unless otherwise noted.

hexachlorobenzene) and corrected for recovery based on historical recovery estimates (referred to as corrected hexachlorobenzene).

The specific objectives of the Comparability Study were to:

- Describe and compare the detection level, recovery, and precision of the measurements using the HRGC/MS and GC/ECD analysis methods;
- Determine if there are significant statistical differences between the HRGC/MS and GC/ECD measurements on paired samples; and
- Describe the relationship between the HRGC/MS and GC/ECD measurements and evaluate its usefulness for converting measurements from one method to the other.

These objectives have been met by analyzing split composite adipose tissue samples collected in 1984 using both analytical procedures. NHATS samples from 690 individuals were composited into 46 composite tissue samples. These samples were split into two portions, or paired samples, one for analysis using the GC/ECD method and 1 for analysis using the HRGC/MS method. One of the paired samples for GC/ECD analysis was lost during preparation, leaving 45 paired samples for the Comparability Study. Three of the 45 composite samples were larger than the others and are called multisplit composite samples Each of these three composite samples was split into 10 portions, five portions for GC/ECD analysis and five for the HRGC/MS analysis. Four of the five portions for each method were spiked with the target analytes in order to measure recovery. Additional quality control (QC) and blank samples were prepared to bring the total number of samples for each method to 80. This report summarizes the measurements, presents the results of the statistical analyses used to determine the recovery for each analytical method, and compares the performance of the HRGC/MS and GC/ECD procedures.

The Comparability Study was limited to a comparison of the data from the 1984 paired samples, the multisplit samples, associated quality control samples, and summary data from other years.

This study did not attempt to analyze trends over time or provide national or regional estimates.

The rest of this report describes of the sample collection, analysis, and the results and conclusions of the Comparability Study. The report is divided into the following chapters and appendices:

Chapter 2 Summary and Conclusions;

Chapter 3 Sample Collection, Preparation, and Analysis Procedures;

Chapter 4 Description of the Data;

Chapter 5 Overview of the Statistical Analysis;

Chapter 6 Analysis of Detection Limits and Percent Recovery;

Chapter 7 Relationship Between the GC/ECD and HRGC/MS Methods;

Chapter 8 Comparison of GC/ECD and HRGC/MS Measurements Across Years;

Chapter 9 PCB Measurements;

Chapter 10 Analysis of Precision and Components of Variance;

Chapter 11 References;

Appendix A Summary Data Tables;

Appendix B Conversion from Standard Deviation of Log Transformed Data to Coefficient of Variation;

Appendix C Recovery from Multisplit Samples;

Appendix D Description of the Analytical Procedures; and

Appendix E Discussion of the Variance Components.

Reports that describe the NHATS program, the sample collection procedures, and the preparation and analysis procedures for the tissue samples are listed in the references.

2 SUMMARY AND CONCLUSIONS

The NHATS Comparability Study compared the performance of the HRGC/MS and GC/ECD analytical methods for measuring pesticides and PCBs in human adipose tissue samples. The results are based on data from 45 paired composite FY84 NHATS samples and associated multisplit spiked, quality control, and blank samples, and an analysis of summary data from other years. The conclusions, based on measurements of p,p'-DDT, p,p'-DDE, beta-BHC, dieldrin, heptachlor epoxide, oxychlordane, trans-nonachlor, hexachlorobenzene, and PCBs, are:

- **Average detection limits for the GC/ECD method were lower than for the HRGC/MS method, except for PCBs.**

  For the organochlorine pesticides, the average GC/ECD detection limits are less than .01 ug/g. The corresponding HRGC/MS detection limits range from .01 ug/g to .35 ug/g for different compounds. For PCBs, the average GC/ECD detection limit of .43 ug/g is much greater than the average for the HRGC/MS method of .01 ug/g.

- **Method Recovery in lipid samples was higher for the GC/ECD method than for the HRGC/MS method for all compounds except beta-BHC, for which the recovery estimates were similar.** These general results are consistent with the ratio of the GC/ECD to HRGC/MS measurements in the paired samples. The method recovery depended on the sample matrix.

  The GC/ECD recoveries ranged from 52% to 109%, except for uncorrected hexachlorobenzene, with recovery from 33% to 61%. The HRGC/MS recoveries in the lipid samples ranged from 26% to 62%, except for beta-BHC with a recovery estimate of 99%. The ratio of the GC/ECD to the HRGC/MS recovery ranged from 0.90 to 3.15 in the multisplit samples.

- **The percentage of composite samples with detected concentrations was greater for the GC/ECD method than for the HRGC/MS method.**

  The primary compounds were detected in all GC/ECD samples. The percent of HRGC/MS samples with detected concentrations ranged from 82% to 98% for different compounds, with the exception of dieldrin which was detected in 42% of the samples tested. The percentage of samples with detected concentrations depends on the method detection limit, the recovery and the concentration in the samples.

- **In paired samples, the measurements using the GC/ECD method were greater than those using the HRGC/MS method, with the ratio of the GC/ECD to HRGC/MS measurements being roughly constant. For some compounds, the ratio depended on the concentration in the samples or differed among batches.**

  When compared using geometric means, the GC/ECD measurements were greater than the HRGC/MS measurements. The ratio of the GC/ECD to HRGC/MS measurement ranged from 1.25 to 3.88. The ratio is statistically significantly greater than 1.0 for p,p'-DDT, p,p'-DDE, heptachlor epoxide, trans-nonachlor, and corrected hexachlorobenzene.

  Differences in the ratio among batches were statistically significant for four compounds: p,p'-DDE, beta-BHC, oxychlordane, and trans-nonachlor. Changes in the ratio of the GC/ECD to HRGC/MS measurements with changing concentrations were statistically significant for three compounds: p,p'-DDT and beta-BHC, and hexachlorobenzene. For these compounds the ratio of the GC/ECD to HRGC/MS measurements decreases as the concentration in the samples increases.

- **For PCBs, the HRGC/MS measurements were similar to or lower than the corresponding GC/ECD measurements.**

- **The standard deviation of the HRGC/MS and GC/ECD measurements increases as the concentration being measured increases such that the coefficient of variation of the measurements is constant. GC/ECD measurements have coefficients of variation less than that for the HRGC/MS measurements. For the primary compounds other than dieldrin the GC/ECD measurements were more precise than the HRGC/MS measurements.**

  Approximate 95% prediction intervals for the GC/ECD and HRGC/MS measurements of Fraction 1 compounds[2] (all primary compounds except dieldrin) are 22% and 63% respectively. There are not enough measurements for Fraction 2 compounds (including dieldrin) to reliably compare the measurement precision for the HRGC/MS and the GC/ECD methods for Fraction 2 compounds.

- **Given the likely prediction errors, the HRGC/MS averages from 1982, adjusted by the GC/ECD to HRGC/MS ratio found in the FY84 samples, were consistent with the trends in the GC/ECD averages in the years adjacent to 1982.**

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[2]The laboratory analysis procedures separate the target compounds into two portions, called Fraction 1 and Fraction 2.

For five compounds, the adjusted HRGC/MS average was close to the GC/ECD trend. For one other compound, the adjusted HRGC/MS average was close to the GC/ECD trend after removing two outliers from the HRGC/MS data. For one additional compound, the data and the ratio estimates were too variable to attribute the observed differences in the GC/ECD and HRGC/MS adjusted averages to the adjustment procedure. For one compound, no comparison was possible because there were no data from 1982.

- **For comparison of measurements across methods and years, the measurements can be adjusted using either the ratio of the GC/ECD to HRGC/MS measurements or by correcting for recovery.**

  Using the ratios of the average GC/ECD and HRGC/MS measurements in FY84 samples to convert from one method to the other proved reasonable for the 1982 data. However, this conversion method has limitations. The relationship between the GC/ECD and HRGC/MS measurements may change with time, the laboratory used, or the concentration levels in the samples. When recovery information, either average recovery or within sample recovery estimates based on surrogate compounds, is available, correcting the HRGC/MS data for recovery may be preferable to using the ratios from the Comparability Study. A combination of theoretical arguments and data analysis suggests that the comparability of the measurements from the two methods may be improved by correcting for recovery within each sample year and laboratory. Evaluating the use of recovery estimates to adjust the data across years was beyond the scope of this study.

3 SAMPLE COLLECTION, PREPARATION, AND ANALYSIS PROCEDURES

The sample and data processing steps for the Comparability Study, from the initial tissue collection to the final analysis, included:

(1) Collecting the individual adipose tissue samples;

(2) Preparing the sample design for the Comparability Study;

(3) Compositing the individual specimens into composite adipose tissue samples;

(4) Preparing the quality control and blank samples;

(5) Extracting the lipid from the composite adipose tissue samples;

(6) Dividing each lipid sample into a pair of samples, one to be analyzed using the GC/ECD method and one to be analyzed using the HRGC/MS method;

(7) Measuring the concentration of target and surrogate compounds using the GC/ECD and HRGC/MS procedures;

(8) Entering the concentration measurements into a computer file for statistical analysis; and

(9) Analyzing the data to determine the precision and recovery of the GC/ECD and HRGC/MS methods and compare the measurements in the paired composite samples.

Battelle Columbus Division participated in the sample collection design and prepared the sample design for the Comparability Study. Midwest Research Institute (MRI) collected and stored the tissue samples and prepared the composite samples and lipid extracts. The Institute of Rural Environmental Health at Colorado State University (CSU) performed the HRGC/MS and GC/ECD analysis on the lipid extract samples. Westat prepared the data files and performed the statistical analysis.

This chapter reviews the Comparability Study specimen collection procedures; the preparation of the composite, blank, and

spiked samples; and the analytical procedures for the HRGC/MS and GC/ECD methods. The data are summarized in chapter 4. Chapters 5 through 10 present the statistical analysis and results.

3.1 Collection and Storage of the Human Adipose Tissue Specimens

As part of the ongoing NHATS program, individual adipose tissue specimens are collected on a yearly basis from volunteer hospitals and medical examiners in selected cities throughout the continental United States. Specimens from the fiscal year 1984 collection were used to compare the GC/ECD and HRGC/MS analytical procedures.

The cities in the NHATS survey were selected using probability sampling. Within the selected cities, organizations (hospitals and medical examiners) were recruited to participate in the NHATS program. Each participating organization was assigned a quota of individual tissue specimens to be collected over the year from surgery patients and cadavers. While the non-random methods of selecting participating organizations and of obtaining tissue specimens within the hospitals may affect the interpretation of national averages and comparisons between different years, the specimen collection procedures do not affect the Comparability Study results.

The individual tissue specimens were sent to MRI for storage until further processing was performed. The composite samples were prepared in the beginning of 1986. GC/ECD analyses were performed in the first half of 1986. HRGC/MS samples in batch 1 were analyzed in April 1987; the remaining batches were analyzed at the end of 1987 and the first half of 1988.

3.2 Sample Design for the Comparability Study

The sample design specified which individual FY84 adipose tissue specimens were to be combined into composite samples and the order of preparation and analysis of the composites, method blanks, and quality control samples.

The sample design specified four types of samples:

- Single-split composite samples, composite samples which were split into two portions, one for GC/ECD analysis and one for HRGC/MS analysis;
- Multisplit composite samples, composite samples which were split into 10 portions, five portions for GC/ECD analysis (four of which were spiked) and five for the HRGC/MS analysis (four of which were spiked);
- Quality control samples to monitor recovery and precision; and
- Blank samples to identify and correct for possible contamination of the samples.

The sample design specified 43 single-split composites, 3 multisplit composites, 10 quality control samples, and 10 blank samples for each method.

The criteria for choosing which specimens to composite were based on demographic factors: geographic region, age group, race, and sex. Composites were constructed from individual specimens within the same age group and census division, allowing estimation of mean concentrations for census division and age group. Within a census division and age group, specimens were combined in a way that permitted estimation of differences between sex and race groups.

Composite samples were created in order to reduce the analytical costs and to achieve the desired sensitivity of the analytical methods. The sensitivity of the HRGC/MS and GC/ECD methods depends on the size of the sample being analyzed. Individual

NHATS tissue specimens are not usually large enough to provide adequate sensitivity for both HRGC/MS and GC/ECD analyses. Therefore, individual specimens were composited to create a larger sample for chemical analysis. The HRGC/MS method required a 20-gram sample while the GC/ECD method required a 5-gram tissue sample. Thus the target wet weight of the single-split composite samples was 25 grams. Multisplit composite samples required 125 grams of tissue.

The samples were grouped into 10 batches for analysis. Each batch was designed to have one quality control (QC) sample spiked with a known concentration of the target compounds, and one blank sample. The multisplit composites were analyzed in three different batches. For each analytical method, the unspiked portion and two of the spiked portions were analyzed in one batch. The remaining two spiked portions were analyzed in two separate batches.

There were two deviations from the sample design. First, one sample for the GC/ECD analysis was lost during preparation, leaving 45 paired samples for the Comparability Study.[3] Second, problems in the preparation of two composite samples from batch 8 and one composite sample from batch 9 resulted in the need to reconstruct these composites. When these samples were reconstructed from stored tissue specimens, an additional blank was prepared for batches 8 and 9. As a result, batches 8 and 9 each had two blank samples.

The term "paired" samples is used in this report to refer to the single-split composite samples and the unspiked portions of the multisplit composite samples. The paired samples were used to compare the GC/ECD and HRGC/MS analysis methods.

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[3]A 47th composite was prepared to replace the lost GC/ECD sample for making national estimates and evaluating trends over time. This sample was not part of the comparability study. In all, 697 individual specimens were combined into 47 composite samples.

A total of 80 samples was analyzed using each method. However, the extracts from the unspiked multisplit GC/ECD samples were analyzed three times, once in each batch containing a spiked multisplit sample. Therefore, there are 2 additional analyses for each of the 3 unspiked multisplit composites, giving 86 total analyses for the GC/ECD method. Table 1 summarizes the number of FY84 samples of different types.

3.3 Sample Preparation

Sample preparation is discussed below, including compositing of the individual tissue specimens, extracting the lipid from the tissue for analysis, and preparing blank and spiked QC samples. A more complete description of the sample processing procedures is presented in Appendix D.

3.3.1 Preparing the composite samples and batches

The sample design specified which individual tissue specimens were to be combined into each composite sample and which composites were in each batch. The tissue weight contributed by each individual specimen to a composite was made as consistent as possible. A method blank sample and all composites within a batch were prepared at the same time.

The target compounds to be measured are associated with the lipid portion of the composite adipose tissue samples. The first processing step extracts the lipid from the tissue. As part of the extraction step, the weight of the lipid as a percentage of the wet weight of the sample is determined. The lipid extracted from the composite sample was split into two portions, one reserved for the GC/ECD analysis and another reserved for the HRGC/MS analysis.

Table 1. Number of Samples for HRGC/MS and GC/ECD Chemical Analysis by Sample Type and Batch

Sample Type	Total	Batch 1	2	3	4	5	6	7	8	9	10
Design Composite Samples											
Single-split Paired	42	2	5	3	5	5	3	5	5	5	4
Unspiked Multisplit Paired	3	1		1			1				
Paired Samples Subtotal	**45**										
Unpaired Composites	1	1									
Design Samples Subtotal	**46**										
Spiked Multisplit Samples											
Low level spike	4	2	1			1					
Mid level spike	4						2		1	1	
High level spike	4			2	1			1			
Blank and QC Samples											
QC samples	10	1	1	1	1	1	1	1	1	1	1
Blank	10	1	1	1	1	1	1	1	1	1	1
Regenerated blanks	2								1	1	
HRGC/MS Samples Total	**80**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**9**	**9**	**6**
GC/ECD Extract Analyses	6		1		1	1		1	1	1	
GC/ECD Samples and Extract Analyses Total	**86**	**8**	**9**	**8**	**9**	**9**	**8**	**9**	**10**	**10**	**6**

Note. Blank entries indicate that no samples with the indicated sample type were analyzed in that batch.

The three multisplit samples were prepared following the procedure for the single-split samples, with the exception that the multisplit samples had a total wet weight of 125 grams. One 25 gram portion of each multisplit composite was removed and handled identically to the single-split samples. The remaining lipid was sent to CSU for spiking and splitting into eight spiked multisplit samples, four for GC/ECD analysis and four for HRGC/MS analysis. The spiking levels for the multisplit composite samples are shown in Table 2. Figure 1 graphically summarizes the sample processing steps.

3.3.2 Method blanks samples

A procedural blank sample, consisting of 100 milliliters of methylene chloride, was prepared for each batch. The blank was passed through all sample processing steps used for the single-split samples. The measurements from these blanks were used to determine if the laboratory background contributed to the target analytes in the samples.

3.3.3 Quality control samples

The QC samples were prepared with known quantities of target compounds. The QC samples are different for the GC/ECD and HRGC/MS analyses. Reference porcine fat samples were obtained from EPA's Las Vegas Laboratory (EPA/EMSL-LV) for the GC/ECD analyses. The porcine samples in batches 1 to 3 had different spiking levels than the samples in batches 4 through 10. The concentration levels in these two groups of porcine fat samples are provided in Table 3. Because the HRGC/MS method was new to CSU and there was a limited source of standard reference adipose material, a sample of spiked dichloromethane[4] was prepared for each batch analyzed using the HRGC/MS method. The dichloromethane samples were spiked with 10 nanograms of all HRGC/MS target compounds.

[4]Dichloromethane is a synonym for methylene chloride.

Table 2. Spiking Levels for the Multisplit Composite Samples

Compound	Spiking levels ug/g wet weight Low	Mid	High
Dieldrin	.20	.40	.60
beta-BHC	.10	.20	.40
Hexachlorobenzene	.05	.08	.11
Heptachlor Epoxide	.10	.20	.30
trans-Nonachlor	.10	.20	.30
Oxychlordane	.10	.15	.20
p,p'-DDE	1.00	3.00	5.00

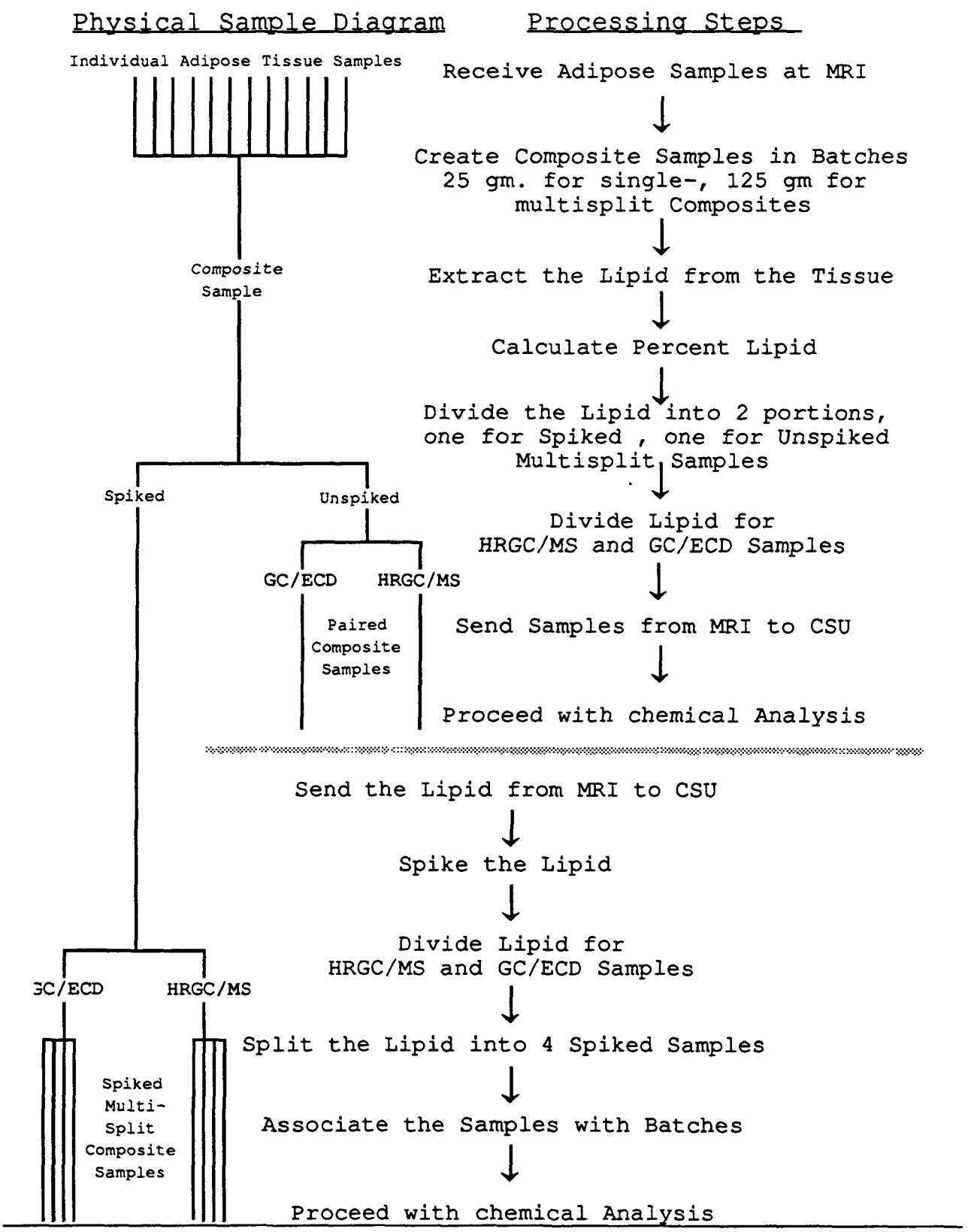

Figure 1. Processing steps for the sample preparation and analysis of composite samples.

Table 3. Concentrations of Target Compounds in GC/ECD Porcine Fat QC Samples, in ug/g wet weight

Compound	Batches 1-3	Batches 4-10
Hexachlorobenzene	.070	.049
beta-BHC	.120	.300
Mirex	.065	.129
Oxychlordane	.060	.112
Heptachlor Epoxide	.046	.075
trans-Nonachlor	.080	.119
Dieldrin	.150	.040
p,p'-DDE	1.620	1.860
p,p'-DDT	.230	.175
PCB (Aroclor 1254)	-	1.000

3.4 Chemical Analysis Methods

Although the specifics of the chemical analysis for the two analysis methods differ, the general steps followed are:

(1) Spiking with surrogate standards for estimating recovery;

(2) Removing the lipid and leaving the target compounds in a solvent solution;

(3) Separating the target compounds into two fractions to be analyzed separately;

(4) Spiking with standard solutions to aid in identification and quantitation and for estimating recovery (for the HRGC/MS method only); and

(5) Identification and quantification of the target compounds.

Once the amounts of the target compounds in each sample have been determined, the concentrations can be calculated by expressing the amounts as a proportion of the original sample wet weight or lipid weight. The concentration in micrograms per gram (ug/g) wet weight is:

$$X_{\text{Wet Weight}} \text{ (ug/g)} = \frac{\text{Weight of the Target Compound (ug)}}{\text{Wet weight (g)}}, \qquad (3.1)$$

where X is the measured concentration. The concentration in micrograms per gram (ug/g) lipid weight is:

$$X_{\text{Lipid Weight}} \text{ (ug/g)} = \frac{\text{Weight of the Target Compound (ug)}}{\text{(Wet Weight (g))(Percent Lipid/100)}}$$

$$= \frac{\text{Weight of the Target Compound (ug)}}{\text{Lipid Weight (g)}} \qquad (3.2)$$

Unless otherwise stated, all concentrations in this report are expressed on a lipid basis.

The following sections discuss the analysis procedures, performed at CSU, for the GC/ECD and HRGC/MS methods. Specifics of each method are presented in Appendix D.

3.4.1 GC/ECD analysis procedures

For the FY84 samples, the GC/ECD method was used to quantify the amount of 20 target compounds.[5] The modified MOG procedure was used for preparation of the samples submitted for GC/ECD analysis. The lipid extracts for the GC/ECD analysis were spiked with one microgram of aldrin. The bulk lipid material was removed by partitioning the lipid sample between hexane and acetonitrile. The target compounds were separated into two fractions using a florisil chromatography column. The target compounds in the final extract were identified and quantified using the packed column gas chromatograph (GC) with electron capture detection (ECD).

Concentrations of compounds were quantitated based on the area of the peak representing the target compound and the aldrin peak. The recovery of aldrin was calculated as a quality assurance check on the entire process. Based on historical information on the recovery at the partitioning step, the concentration of several compounds were reported as measured (referred to as uncorrected) and corrected for recovery. Concentrations for hexachlorobenzene and mirex were computed on both a corrected and an uncorrected basis. Concentrations for p,p' DDT were reported on a corrected and uncorrected basis for the porcine fat tissue samples in batches 4 through 10.[6]

Total PCB concentrations were reported on the following interval scale using letters to designate each interval: V = not

---

[5]Eighteen individual chemicals, one class of chemicals (PCBs), and aldrin which was used to estimate recovery.

[6] The values reported in the space provided on the form for hexachlorobenzene, mirex and p,p' DDT are uncorrected unless the uncorrected values for these compounds are written in at the end of the list, in which case the entry for these compounds is the corrected value.

detected, W = .33 to 1 ug/g wet weight, Y = 1 to 3 ug/g wet weight, and Z = greater than 3 ug/g wet weight.

The following information was reported for each composite sample and target compound:

- Compound name and code;
- "<" if the amount detected was less than the level of quantification (LOQ) but greater than the level of detection (LOD), in which case the amount reported was the LOQ; and
- The concentration reported to 0.01 ug/g wet weight or a letter code for the interval in which the PCB concentration lies.

3.4.2 HRGC/MS analysis procedures

In order to expand the list of chemicals that could be monitored by NHATS, the standard NHATS method for detection and quantification of chemical compounds was changed from GC/ECD method to the HRGC/MS method. For the FY84 samples, the HRGC/MS method was used to quantify the amount of 57 target compounds. The sample was spiked with known amounts of the eleven surrogate compounds. Table 4 identifies the surrogate compounds and the level added to each sample. Gel permeation chromatography (GPC) was used to separate target analytes from the lipid material. The target compounds were separated into two fractions (referred to as Fraction 1 and Fraction 2) using a Florisil chromatography cleanup similar to the MOG method (the Fraction 2 extract was not analyzed in batches 4 through 10). Known amounts of the three internal standards, anthracene-d10, naphthalene-d8, and benzo-(a)-anthracene-d12, were added to each of the final extracts, prior to analysis by high resolution gas chromatography/ mass spectrometry.

The identification and quantification was based on relative retention times and response factors established during calibration. Specific compounds were identified by matching characteris-

Table 4. Spiking Levels of Surrogate Compounds in HRGC/MS Samples

Compound	Spike Level ug
Chrysene-d_{12}	2
1,2,4 -Trichlorobenzene-d_3	2
$^{13}C_6$-1,2,4,5 - Tetrachlorobenzene	2
$^{13}C_6$- Hexachlorobenzene	2
$^{13}C_6$- 4 - Chlorobiphenyl	2
$^{13}C_{12}$-3,3 ,4,4 - Tetrachlorobiphenyl	5
$^{13}C_{12}$-3,3 ,3,3 ,5,5 ,6,6 - Octachlorobiphenyl	8
$^{13}C_{12}$ Decachlorobiphenyl	10
Diethyl Phthalate - 3,4,5,6 -d_4	2
Di-N-Butyl Phthalate - 3,4,5,6 -d_4	2
Butyl Benzyl Phthalate - 3,4,5,6 -d_4	2

tic spectra with reference material. One of the three internal standards was designated as the appropriate internal standard for each target analyte for purposes of identification and quantification.

In order for an analyte to be identified, the following four criteria had to be satisfied:

(1) The primary and secondary masses had to achieve their maximum values within a specified time span;

(2) The retention time of the primary and secondary mass fragments relative to the designated internal standard had to be within 10 seconds of the known relative retention time of the analyte;

(3) The relative abundances of the primary and secondary masses all had to be within 20% of the relative abundances in the reference spectrum of the analyte;

(4) The abundances of the primary and secondary masses all had to exceed 2.5 times the background signal to noise ratio.

Concentrations of analytes were computed from the calculated amounts, the weight of the composite sample, and the percent lipid in the composite sample. No concentrations were corrected for recovery. The recoveries of the surrogate compounds were calculated as a check on method performance. A data qualifier was determined based on the relative magnitude of the noise, peak signal, and the quantity of each target compound in the lowest calibration standard.

The following equation was used to calculate the lipid adjusted concentration of each target compound in each sample:

$$X = \frac{A_s}{1000} \quad \frac{I_{is}}{A_{is}} \quad \frac{1}{RRF} \quad \frac{1}{\text{Lipid Wt}} \qquad (3.3)$$

where:

X = Lipid adjusted concentration of the target compound (ug/g);

A_S = Area of the primary characteristic ion response for the compound being quantified;

I_{is} = Amount of internal standard added to the extract (ng);

A_{is} = Area of the primary characteristic ion response for the corresponding internal standard;

RRF = Relative response factor, determined during calibration of the instrument; and

Lipid Wt = Weight of the lipid in the adipose tissue sample analyzed (g), calculated as the product of the wet weight and the percent lipid divided by 100.

This equation is used later in the development of the statistical model for the data.

The following information was recorded for each sample and target analyte:

- Compound name;
- Data qualifier (either not-detected "ND", trace "TR", or positively quantified "PQ");
- The calculated LOD for trace and not detected measurements;
- The amount of each compound in nanograms (ng.) for positively quantified and trace measurements; and
- Remarks to explain exceptional circumstances.

3.5 Data File Preparation

The measurements reported by the laboratory were entered into computer files at EPA's National Computer Center (NCC) for use in the statistical analysis. After data entry, the data were verified against the original reports and consistency checks were performed to catch possible errors in the data entry or unusual observations.

4 DESCRIPTION OF THE DATA

This chapter describes the general characteristics of the HRGC/MS and GC/ECD concentration measurements in the FY84 samples. Numerical summaries are shown in Appendix A.

4.1 Organization of the Data

Measurements were made on four different types of samples:

(1) Method Blanks;

(2) QC standards: porcine fat samples in the GC/ECD analysis, and spiked dichloromethane samples in the HRGC/MS analysis;

(3) Spiked multisplit composite adipose tissue samples (at three different spiking levels) and the associated extract samples (for the GC/ECD analysis only); and

(4) Paired composite adipose tissue samples.

The compounds measured can be divided into two categories:

(1) Target compounds, those compounds of interest in the NHATS program. These compounds have different levels in different samples; and

(2) Surrogate compounds used for quality control and estimating recovery. These compounds have similar levels in all samples.

The different combinations of compounds and sample types define groups of measurements which are directly comparable. Figure 2 graphically portrays these groups of comparable measurements. Tables A-1 through A-18 in Appendix A summarize the HRGC/MS and GC/ECD measurements within each group using the percent detected, median, and, for the positively quantitated observations, the mean, median and extremes.

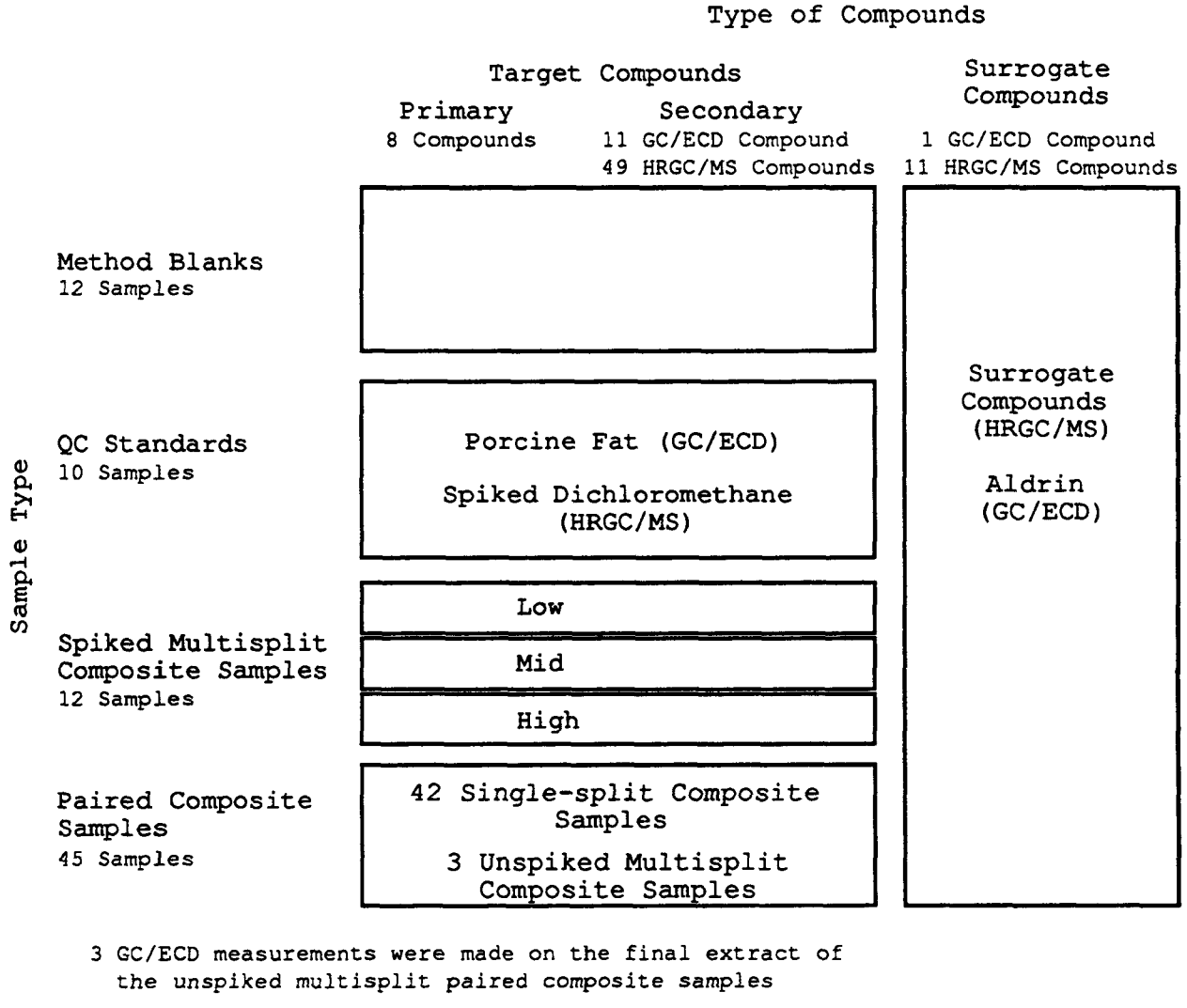

Figure 2. Subsets of the data with comparable measurements by sample type and type of compound.

The target compounds can be further divided into two groups:

- Primary compounds: compounds which are positively quantified in numerous samples using both the GC/ECD and HRGC/MS methods; and
- Secondary compounds which were either measured using only one method or which were not positively quantified using both methods.

Only nine compounds were positively quantified in the paired samples using both the GC/ECD and HRGC/MS methods. These compounds are: p,p'-DDT, p,p'-DDE, beta-BHC, dieldrin, heptachlor epoxide, oxychlordane, trans-nonachlor, hexachlorobenzene and PCBs. These compounds are referred to as the primary compounds for the Comparability Study. In all cases except dieldrin and PCBs, the number of positively quantified observations which can be used to compare the two measurement methods is 37 or greater. Dieldrin, in fraction 2, was only analyzed using the HRGC/MS method in batches 1, 2, and 3, and therefore has few HRGC/MS measurements. Only five samples had positively quantified dieldrin concentrations reported for both the HRGC/MS and GC/ECD methods. PCB measurements from the GC/ECD method were reported on an interval scale, as described in Section 3.4.1, rather than as a finite continuous value. The PCB results are discussed separately in Chapter 9. Because the results for dieldrin were limited, the comparison of the two analytical methods is based primarily on the measurements of: p,p'-DDT; p,p'-DDE; beta-BHC; heptachlor epoxide; oxychlordane; trans-nonachlor; hexachlorobenzene; and PCBs.

4.2 Preliminary Review of the Data

The initial step in any data analysis is called exploratory data analysis. This step usually involves plotting the data in various ways to help identify important characteristics of the measurements.

The percent lipid in the composite samples ranged from 41.5 to 99.3 percent, with an average of 77 percent, providing adequate

lipid in each composite for measuring the concentrations of the target compounds.

Initial plots of the data suggested that the distribution of the data was skewed and that the standard deviation of the measurements increased as the magnitude of the measurement increased. There were no obvious outliers or extremely unusual observations which might make subsequent preliminary work suspect. Plots using the log of the measurements were consistent with the assumption that the data can be described by a lognormal distribution. Plots of the HRGC/MS surrogate compound measurements suggested that there were systematic differences between batches.

Little laboratory background contamination was noted. There were no quantifiable measurements in method blank samples using the GC/ECD method. Positively quantified measurements in the method blank samples were observed for 4 of 50 compounds[7] using the HRGC/MS method. With the exception of Di-n-Butyl Phthalate, the measured quantities were close to the level of detection. Only one measurement for a primary compound, p,p'-DDE, was positively quantified.

From the tables in Appendix A, the coefficient of variation can be calculated using the following equation:

$$\text{coefficient of variation (cv)} = \frac{\text{standard deviation}}{\text{mean}} \qquad (4.1)$$

The coefficient of variation is one measure of the variability of the data. For many chemical measurements, the coefficient of variation provides a stable measure of variability across a range of concentrations. For samples with the same actual concentration, such as spiked multisplit samples from the same composite or QC samples, the coefficient of variation measures the precision of the analytical technique. For the paired composite samples,

[7]p,p'-DDE, 1,2,4-trichlorobenzene, di-n-butyl phthalate, and di-n-octyl phthalate

the coefficient of variation measures the variability resulting from both differences between composite samples and measurement variation.

The coefficient of variation of the positively quantified measurements was calculated for the compounds and groups of data listed in Appendix A. It was found to be roughly similar for all compounds within the two groups: paired samples and all other samples. The average coefficient of variation across target compounds measured in paired composite samples was 68% for GC/ECD measurements and 72% for HRGC/MS measurements. For the remaining samples, the average coefficient of variation across all compounds was 11% for the GC/ECD method and 29% for the HRGC/MS method, suggesting that the laboratory measurement error is greater for the HRGC/MS method than for the GC/ECD method. The coefficients of variation for measurements on the paired samples are substantially greater than that for the spiked QC samples due to the additional variation in the contaminant concentrations among composites. Because the reported GC/ECD concentrations were rounded to 0.01 ug/g wet weight, the variance estimates for the GC/ECD method will tend to underestimate the true variance. However, the effect of rounding on the results is expected to be small.

The distribution of a set of concentration values which have a coefficient of variation greater than 50% is often skewed to the right. Investigation of the data confirms that, as a general rule, the measurements on the paired samples are skewed to the right. Measurements within a group of samples with the same actual concentrations, such as the QC samples, have a smaller coefficient of variation and a distribution which is roughly symmetric.

Figures 3 and 4 show histograms of the p,p'-DDE measurements on paired samples using the HRGC/MS and GC/ECD methods. Note that the plots have different concentration scales and that the GC/ECD

measurements are larger than the HRGC/MS measurements. Both figures show a skewed distribution. Measurements on the composite samples estimate the average of the concentrations in the individual samples which were composited. Since averaging decreases the coefficient of variation, measurements in individual tissues specimens can be expected to be more skewed than those from the composites.

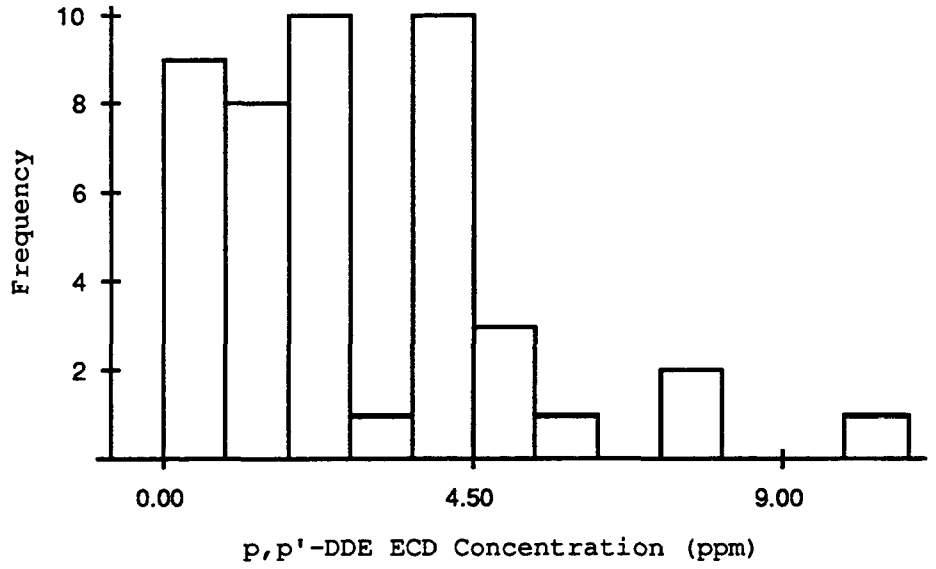

Figure 3. Histogram of GC/ECD measurements of p,p'-DDE on paired composite adipose tissue samples.

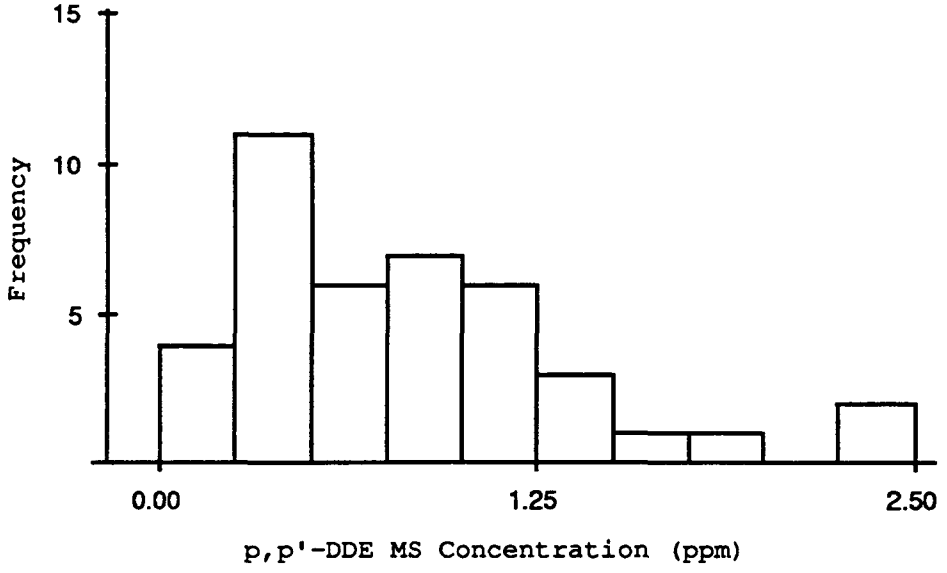

Figure 4. Histogram of HRGC/MS measurements of p,p'-DDE on paired composite adipose tissue samples.

5 OVERVIEW OF THE STATISTICAL ANALYSIS

Different subsets of the data were used to achieve each of the study objectives. The precision of the measurements was determined from the QC samples, the spiked multisplit samples, and the surrogate compounds which were common to all samples. The recovery was estimated from the QC samples and the spiked multisplit samples. The HRGC/MS and GC/ECD methods were compared directly using the primary compounds in the paired samples. Figure 5 summarizes graphically the different subsets of the data and how the data in each subset were used in the Comparability Study.

In order to achieve the objectives, some common procedures for analyzing the data were established and a model for the data, a mathematical description of the structure and important relationships in the data, was developed. This chapter discusses the model for the data and assumptions behind the analysis.

5.1 Correction for Blanks

The method blanks consist of solvent samples that are processed as single-split composite samples. If there is background contamination during the sample processing, this contamination will be observed in the blank samples. Assuming the background is the same for all samples within a batch, the measured quantity in the blank can be subtracted from the measured quantity in the sample to correct for the contamination. Note however, when the quantity in the blank is below the detection limit, the appropriate contamination correction is some unknown concentration between zero and the lowest quantifiable quantity.

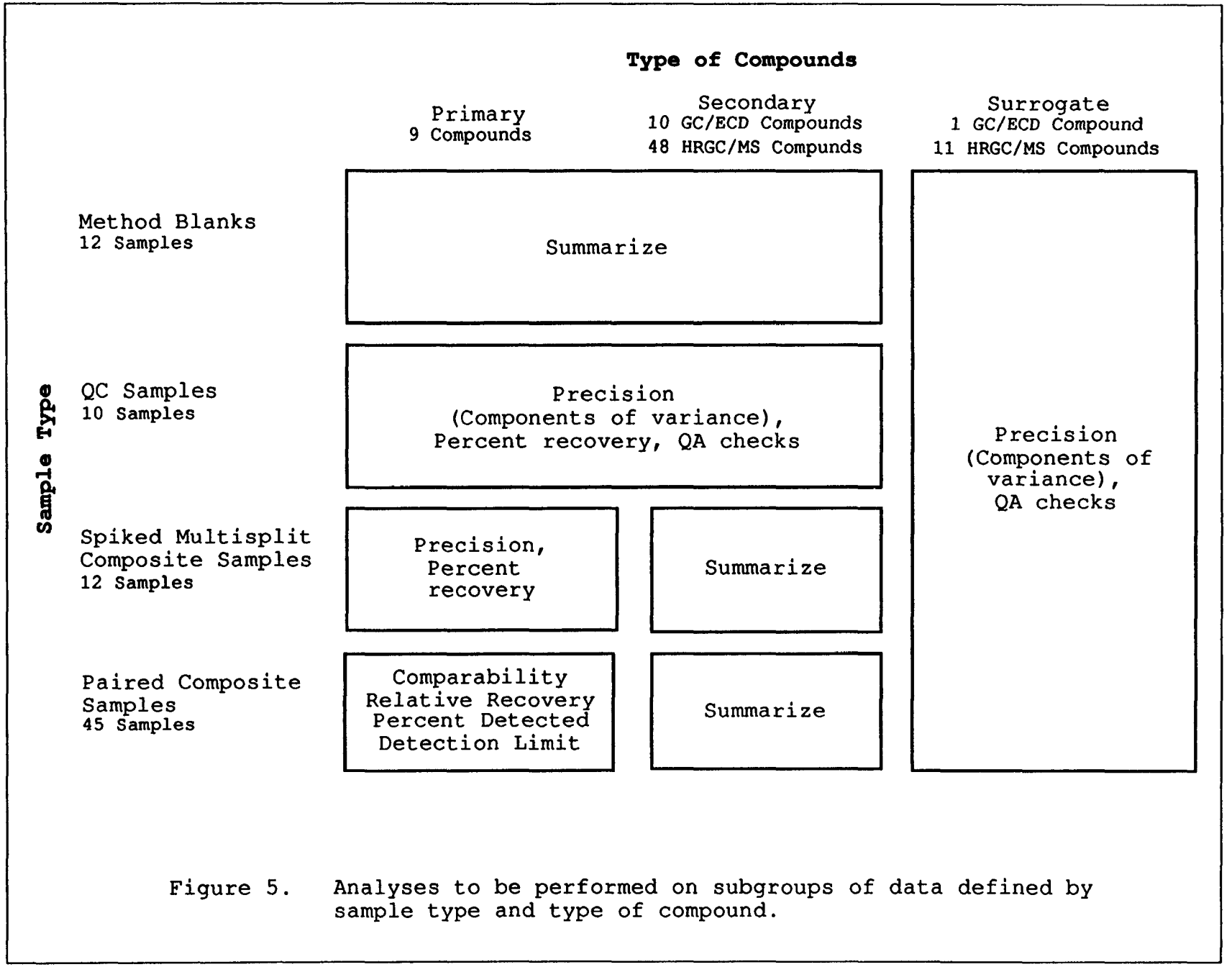

Figure 5. Analyses to be performed on subgroups of data defined by sample type and type of compound.

For the GC/ECD blank samples, no compounds were positively quantified. In the HRGC/MS blank samples, four compounds were positively quantified (p,p'-DDE, 1,2,4-Trichlorobenzene, Di-n-Butyl Phthalate, and Di-n-Octyl Phthalate) of which only one, p,p'-DDE, is used in the comparability analysis. The compound p,p'-DDE was detected in only 1 of 12 blank samples, and then at a level only 10% greater than the limit of detection.

It was decided to use all measurements without a blank correction rather than to correct a few measurements while ignoring the unknown correction for most of the measurements. This decision is expected to have little effect on the statistical analysis.

5.2 Outliers and the Use of Remarks

Outliers are observations which appear to be unusual compared to the bulk of observations. A preliminary analysis of the data indicated that there were no particularly unusual observations which might significantly affect the statistical analyses. As a result, no formal outlier analysis was performed before analyzing the data. However, as different analyses are discussed in the report, any values which might affect the statistical conclusions are discussed.

The comment fields on the HRGC/MS data sheets sometimes contained information on the quality of the reported quantities. If a comment was supplied for a positively quantified measurement, the comment generally indicated that the actual quantity was judged to be either 1) equal to or possibly less than or 2) equal to or possibly greater than the reported measurement. Since the size of any bias in these measurements could not be assessed, they were used as reported.

5.3 A Model for the Data

The model for the data is a mathematical description of the relationships within the data which result from the process which generated the data. Statistical procedures are used to estimate parameters in the model and to check if the assumptions behind the model are consistent with the data. The model is based on 1) the sample design, i.e., the way in which the data are collected; 2) the objectives of the study; and 3) the characteristics of the data. The statistical analysis procedures are chosen to be consistent with the model.

The model used to describe the NHATS data is developed below. This particular model was selected only after data analysis determined that the model was consistent with the data.

The model assumes the measurement errors have a lognormal distribution, and therefore that the standard deviation of the measurement error is proportional to the magnitude of the measured concentration. Environmental measurements and measurements made on a scale from zero to infinity, such as chemical concentrations, often have a skewed distribution such as a lognormal distribution.

A log transformation converts data which have a lognormal distribution to data with a normal distribution. While the variance or standard deviation of the original measurements depends on the concentration being measured, the variance of the log transformed measurements is constant. Since many standard statistical techniques are based on the assumption that the errors have a normal distribution with constant variance, the log transformed data are often easier to handle statistically than data in the original scale.

The equation for quantifying the HRGC/MS concentration X can be used as a starting point to develop the model for the data[8]:

$$X = \frac{A_S}{1000} \quad \frac{I_{is}}{A_{is}} \quad \frac{1}{RRF} \quad \frac{1}{\text{Lipid Wt}} \tag{5.1}$$

Using the log of the measured concentration provides some insight into an appropriate model for the log transformed data. The equation for the log of the measurements is:

$$\ln(X) = \ln\left(\frac{A_S}{1000}\right) + \ln\left(\frac{I_{is}}{A_{is}}\right) + \ln\left(\frac{1}{RRF}\right) + \ln\left(\frac{1}{\text{Lipid Wt}}\right) \tag{5.2}$$

where:

$\ln\left(\frac{A_S}{1000}\right)$ depends on the actual concentration of the measured compound. This term might also be written as:

$$\ln\left(\frac{A_S}{1000}\right) = f(C) + \varepsilon \tag{5.3}$$

where $f(C)$ is a function relating the actual concentration to the measured concentration and ε is the measurement error not associated with other terms.

$\ln\left(\frac{I_{is}}{A_{is}}\right)$ depends on how much internal standard was added to the sample and how much was detected by the equipment. This term can be written as δ_i.

$\ln\left(\frac{1}{RRF}\right)$ depends on the calibration of the equipment. This term can be written as δ_j.

$\ln\left(\frac{1}{\text{Lipid Wt}}\right)$ depends on the characteristics of the sample, in particular the measurements of wet weight and percent lipid. This term can be written as δ_s.

[8]This equation is the same as equation (3.3).

After making the substitutions above, the model for one HRGC/MS measurement might be written:

$$\ln(X) = f(C) + \delta_j + \delta_s + \delta_i + \varepsilon \qquad (5.4)$$

The equivalent model in the original concentration units would be:

$$X = e^{f(C)} e^{(\delta_j + \delta_s + \delta_i + \varepsilon)} \qquad (5.5)$$

Of these two models, equation (5.4), based on the log of the measurements, is easier to fit using standard statistical techniques than equation (5.5) because 1) the distribution of the residuals is expected to be closer to normal; 2) the standard deviation of the residuals are expected to be roughly constant; and 3) the terms are additive.

Equation (5.4) provides an incomplete model in that it ignores some characteristics of the data and the function f(C) is not defined. Under ideal situations the HRGC/MS method would measure the actual concentration in the sample, not counting some variation in the measurements around the actual value. Because of losses in the chemical preparation steps, the measured quantity is usually less than the actual quantity of a compound in the sample. Thus, the model for the measured concentration X might be:

$$X = CR \qquad (5.6)$$

where R is the percent recovery expressed as a fraction.

For spiked samples, an additional quantity of the compound being measured is added to the sample, resulting in an increase in concentration of the compound of S. With this addition, the model for the log of the data might be:

$$\ln(X) = \ln(C+S) + \ln(R). \qquad (5.7)$$

The recovery may be different for the HRGC/MS and GC/ECD measurements due to differences in the sample preparation. There is also the possibility that the recovery depends on the characteristics of the sample material. In other words, the recovery may be different for QC samples, blank samples, and adipose tissue samples.

In the final model for the relationship between the actual and measured concentration, it is assumed that:

$$X = (C+S)^{\beta} * R \tag{5.8}$$

where β is added to cover the case in which the relationship between the actual and measured concentrations is not linear. In the log transformed units, equation (5.8) is:

$$\ln(X) = \beta \ln(C+S) + \ln(R). \tag{5.9}$$

Because we have limited information on when the instruments were calibrated, the effect associated with calibration, δ_j, is difficult to estimate. The calibration term may be different for each internal standard. According to the Quality Assurance Project Plan (QAPjP) for the comparability analysis (USEPA 1986), the instrument's calibration was checked every day and calibrated as necessary. Since batches were usually processed in one or two days, the term for calibration effects is assumed to be confounded with batch effects (i.e., cannot be estimated separately, based on the data).

After the addition of some terms for random effects associated with batch preparation, calibration, measurement of sample characteristics, sample preparation and injection, and measurement of the internal standard and target responses, the complete model for the HRGC/MS and GC/ECD measurements is:

$$\ln(X_{mcbsi}) = \beta_{mc} \ln(C_{cs}+S_{cs}) + \ln(R_{mct}) + \delta_{mb} + \delta_{mbi} + \delta_{Mbic} + \delta_{s} + \delta_{ms} + \delta_{msi} + \varepsilon_{mcbsi} \tag{5.10}$$

The terms in the model are explained in Table 5 and Appendix E.

Converted back to the original measurement units, equation (5.10) becomes:

$$X_{mcbsi}=(C_{cs}+S_{cs})^{\beta_{mc}} * R_{mct} * \exp(\delta_{mb}+\delta_{mbi}+\delta_{Mbic}+\delta_{s}+\delta_{ms}+\delta_{msi}+\varepsilon_{mcbsi}) \quad (5.11)$$

For the analysis of most of the data, the model shown in Table 5 can be significantly simplified. When discussing specific analyses in the following sections, the appropriate simplified version of the model will be presented. The simplification usually comes from combining terms which are confounded. In this case, the combined term will be indicated by a change in the form of the subscripts. When equations are provided in later chapters, terms are defined only if they have not been previously defined.

5.4 Basis for Analyzing the Log Transformed Concentrations

Transforming the data has implications for the model to use in the analysis, the variance of the residuals (i.e., the estimated magnitude of the measurement error), the distribution of the residuals, and the interpretation of the results. Assuming the data have a lognormal distribution, the following statements can be made:

- For the original measurements:
  - The standard deviation of the measurement error is linearly related to concentration (the coefficient of variation of the original data is constant); and
  - The measurement errors have a skewed distribution.
- For the log of the original data:
  - The transformed data have a constant variance; and
  - The transformed data will have a normal distribution.

Table 5. Model for the GC/ECD and HRGC/MS Measurements with an Explanation of Each Term

$$\ln(X_{mcbsi}) = \beta_{mc}\ln(C_{cs}+S_{cs})+\ln(R_{mct})+\delta_{mb}+\delta_{mbi}+\delta_{Mbic}+\delta_{s}+\delta_{ms}+\delta_{msi}+\varepsilon_{mcbsi}$$

Where:

m = an index for the analytical method used to measure the concentration, the two analytical methods are GC/ECD and HRGC/MS (subscripts E and M respectively when specified methods are used).

c = an index for the compound being quantitated. Note that some compounds were quantitated using only one analytical method.

b = an index for the batch in which the samples were processed. There were 10 batches of samples for each analytical method.

s = an index for the sample being analyzed. There were a total of 80 samples (not counting extracts for the GC/ECD method) which are uniquely identified by the EPA ID number, the batch in which the samples were analyzed and the "Fldindic" number which indicates if the sample was a spiked multisplit sample.

i = an index for the internal standard used to quantitate the data. Three different internal standards were used to quantitate compounds in the HRGC/MS analysis.

t = an index for the sample type. There are three different sample types, Blank, QC samples, and Adipose tissue samples.

X_{mcbsi} = the measured concentration using analytical procedure m for compound c measured in batch b, sample s, and quantitated using internal standard i.

C_{cs} = the actual concentration of compound c in sample s before addition of any spike.

S_{cs} = the increase in concentration of compound c due to a spike being added to sample s.

β_{mc} = the slope coefficient for the relationship between $\ln(X_{cs})$ and $\ln(C_{cs} + S_{cs})$. This relationship may depend on the analytical method m. In situations where there is not enough data to estimate this term, it is assumed to be 1.0.

Table 5. (Continued)

R_{mct} = a constant. If $\beta_{mc} = 1.0$ this constant can be interpreted as the recovery for chemical c using analytical method m on a sample with type t.

δ_{mb} = a random effect associated with batch b using analytical method m, assumed to be normally distributed with a mean zero and standard deviation of σ_{mb}, depending only on m.

δ_{mbi} = a random effect associated with internal standard i in batch b using HRGC/MS method, assumed to be normally distributed with a mean zero and standard deviation of σ_{mbi}. This term is confounded with δ_{mb} in the GC/ECD measurements.

δ_{Mbic} = a random effect associated with calibration for compound c quantitated by internal standard i in batch b using HRGC/MS method, assumed to be normally distributed with a mean zero and standard deviation of σ_{Mbic}.

δ_s = a random effect associated with the measurement of wet weight and percent lipid in sample s, assumed to be normally distributed with a mean zero and standard deviation σ_s. This term is identical for both the GC/ECD and HRGC/MS paired samples.

δ_{ms} = a random effect associated with sample s using analytical method m, assumed to be normally distributed with a mean zero and standard deviation σ_{ms}, depending only on m.

δ_{msi} = a random effect associated with internal standard i in sample s measured with the HRGC/MS method, assumed to be normally distributed with a mean zero and standard deviation σ_{msi}, depending only on m. This term is confounded with δ_{ms} in the GC/ECD measurements.

ε_{mcbsi} = a random measurement error for compound c measured using analytical method m, in batch b, in sample s, and quantitated using internal standard i, assumed to be normally distributed with a mean zero and standard deviation σ_m.

The characteristics of the data have been checked by looking at the relationship between the measurement error and the concentration. This relationship is discussed in Chapter 10. Although the relationship between the standard deviation of the data to the measured concentration is not very precise, it is consistent with the use of a log transformation.

Use of a log transformation may be justified if the residuals based on the logged data appear to have a normal distribution. Several analyses were performed using both the original data and the logged data. Based on histograms of the residuals, the residuals from the log transformation are more normally distributed. However, for many analyses, the differences are small.

We have assumed that the measurement error can be described by a lognormal distribution for the following reasons:

- On theoretical grounds, the data can be expected to have a distribution similar to a lognormal distribution;
- The residuals from the statistical analyses of the original data have a skewed distribution. The residuals from the statistical analyses of the log transformed data have a roughly symmetric normal distribution; and
- The standard deviation of the residuals from the original data increase roughly linearly with increasing concentration.

When fitting models to the log transformed data, the estimated error variance is for the transformed data. It may be desirable to convert the variance in the log scale to a coefficient of variation in the original scale. The following formula relates the variance of the log data, s^2, to the coefficient of variation of the original measurements, cv:

$$cv = \sqrt{\exp(s^2)-1} \qquad (5.12)$$

For reference, Table B-1 in Appendix B tabulates the coefficient of variation for selected values of s. In the discussion of

measurement errors, the results are presented in terms of both the variance of the log transformed values and the coefficient of variation of the untransformed measurements.

6 ANALYSIS OF DETECTION LIMITS AND PERCENT RECOVERY

This chapter describes and compares the detection limits, the recoveries, and the proportion of samples with measured concentrations above the detection limit (i.e., percent detected), for the paired NHATS samples. The detection limits and recoveries are discussed first. The percent detected is affected by both the magnitude of the detection limit and the recovery and is therefore discussed after these other two topics.

6.1 Comparison Detection Limits

The measurement which is very unlikely to be exceeded in the analysis of a blank sample is referred to as the detection limit or limit of detection (LOD). The detection limit is defined differently for the HRGC/MS and GC/ECD methods. The GC/ECD detection limit is based on historical experience with the method. The detection limit using the HRGC/MS method is based on the lowest calibration standard and the mass spectrometer signal to noise ratio.

The lowest reported concentration for the GC/ECD method, the limit of quantification (LOQ), has been established from years of experience with the method. The limit of detection is defined as LOQ/3. Measurements between the LOD and the LOQ are reported as less than the LOQ using the symbol "<" on the report forms. The label "Trace" was assigned in these cases in the data set. A final concentration of zero was reported for samples with quantitated concentrations less than the limit of detection. The average limit of detection for the GC/ECD target compounds is displayed in Table 6 in ug/g lipid weight. Because the GC/ECD detection limits are defined on a wet weight basis, the LOD based on wet weight has been converted to an LOD based on lipid weight to determine the average detection limit.

Table 6. Detection Limits (ug/g) Using the HRGC/MS and GC/ECD Methods, for all Compounds Reported on the GC/ECD Forms

Compound	GC/ECD Average LOD for all samples (ug/g)[a]	HRGC/MS Average reported LOD (ug/g)[b]
p,p'-DDT	.009	.049
o,p'-DDT	.009	.013
p,p'-DDE	.004	.352
o,p'-DDE	.009	.013
p,p'-DDD	.009	.013
o,p'-DDD	.009	.127
alpha-BHC	.004	.020
beta-BHC	.009	.212
gamma-BHC (Lindane)	.004	.019
delta-BHC	.004	.018
Aldrin	.004	.013
Dieldrin	.004	.020
Endrin	.009	.041
Heptachlor	.004	.018
Heptachlor Epoxide	.004	.016
PCB	.433	.011
Oxychlordane	.009	.016
Mirex	.043	.013
trans-Nonachlor	.004	.014
Uncorrected Hexachlorobenzene	.004	.015

[a] Conversion from ug/g wet weight to lipid weight assumes percent lipid equals 77%. The percent lipid in paired samples ranged from 41.5% to 99.3%.

[b] Based on the LOD for trace and nondetect measurements.

For the HRGC/MS method, the detection limit is the lowest reported concentration and is based on the lowest calibration standard used to calibrate the instrument and characteristics of the signal and noise. For signals meeting the quality criteria (see Section 3.4.2), the detection limit is the maximum of the concentration based on 1) the lower calibration standard and 2) 2.5 times the noise level. For signals not meeting the quality criteria, the LOD is the maximum of the concentration based on 1) the lower calibration standard and 2) the observed signal. LODs are reported for both nondetect and trace measurements. Because the detection limit depends on several factors, it can vary considerably between samples; however, it will always be equal to or greater than the lipid adjusted concentration based on the lower calibration standard. The average reported detection limits for nondetect and trace HRGC/MS measurements in paired samples are shown in Table 6. For p,p'-DDT, p,p'-DDE, and gamma-BHC, the average HRGC/MS detection limits are based on five or fewer reported detection limits, some of which were much larger than that based on the lowest calibration limit.

For all compounds except PCBs and Mirex, the average GC/ECD detection limit is less than the average HRGC/MS detection limit. In addition, the average GC/ECD detection limits are less than the minimum HRGC/MS detection limits based on the lowest calibration standard. The average GC/ECD detection limits are smaller than the average HRGC/MS detection limits by a factor of at least 10 for p,p'-DDE, beta-BHC, and o,p-DDD. Figure 6 compares the HRGC/MS and GC/ECD detection limits for the primary compounds.

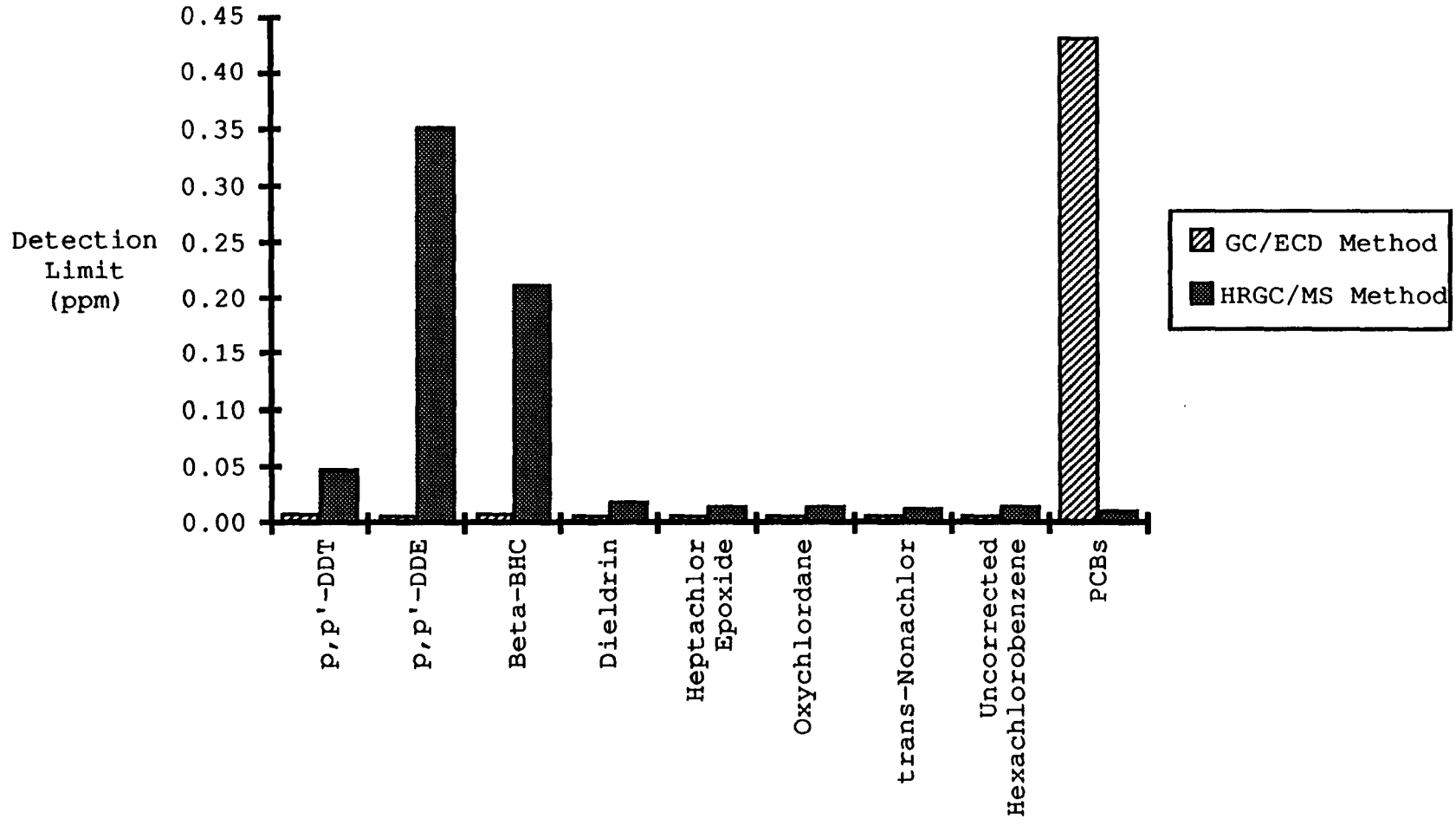

Figure 6. Average HRGC/MS and GC/ECD detection limits for primary compounds.

6.2 Calculating Recovery

Due to losses in the sample processing steps, not all of the target material in the original adipose tissue sample appears in the final extract for measurement. As a result, the quantity of compound measured by the GC/ECD or HRGC/MS equipment is less than that in the original sample. The ratio of the quantity measured to the quantity in the original sample is the recovery, usually expressed as a percentage.

The percent recovery can be estimated using two approaches:

- Comparing measured concentrations with known concentrations in quality control samples; or
- Determining changes in the measured concentration as a result of spiking a tissue sample with a known amount of compound, as in the multisplit samples.

Sections 6.2.1 through 6.2.3 discuss the calculation of recovery using HRGC/MS and GC/ECD procedures for the QC samples and spiked multisplit composite samples.

The model for the data, equation (5.10), allows for different recoveries at different concentration levels. Because the QC samples and multisplit samples were tested at three or fewer concentrations, testing of the hypothesis to determine whether the recovery is constant is not possible because there are insufficient degrees of freedom. Therefore, the calculation of recovery assumes that the recovery is a constant, independent of the actual concentration in the sample.

6.2.1 Calculating recovery using spiked multisplit samples

For the multisplit samples, the average recovery and its standard error are based on separate recovery estimates for the three spiking levels. The calculations assume that the recovery is the same for all concentrations. The recovery estimate at each

spiking level is based on measurements in one unspiked multisplit sample and four spiked multisplit samples.

Using equations for the measured concentration in the unspiked and spiked portions of the multisplit samples (see equation 5.10), the following formula can be derived:

$$R_{mc} = \frac{X_{mcs}^{(s)} - X_{mcs}^{(u)}}{S_{cs}} + \tilde{\varepsilon}_{mcs} \qquad (6.1)$$

Where:

$X_{mcs}^{(s)}$ = Measured concentration of compound c in the spiked split of the sample;

$X_{mcs}^{(u)}$ = Measured concentration of compound c in the unspiked split of the sample; and

$\tilde{\varepsilon}_{mcs}$ = random measurement error for the fraction R_{mc}, roughly lognormally distributed.

Equation (6.1) can be used to calculate recovery in the multisplit samples. With measurements on four spiked multisplit samples four estimates of recovery for each spiking level can be calculated. Because the HRGC/MS measurements in the unspiked portions were sometimes below the detection limit or missing[9], an adjustment for the unspiked concentration required some judgment. For the adjustments, the missing observations were replaced by zero and the nondetect observations were replaced by the LOD/2.

A weighted average of the estimates from the four spiked sample portions was used to determine the recovery for each spiking level. The weights for calculation of the weighted average are usually based on the measurement variance. Because of possible differences between batches, the variance of the recovery

[9]No concentration or detection limit was provided for two trans-Nonachlor measurements in the mid level unspiked samples. The footnote stated "compound is present but cannot be quantitated." As a result of substituting zero for the missing unspiked concentration, the calculated recovery will tend to overestimate of the actual recovery.

estimates based on spiked samples in the same batch as the unspiked sample are likely to be less than that for spiked samples in different batches. The measurement variances could be estimated using the components of variance which are provided in Chapter 10. However, assumptions must be made concerning the best estimate of each variance component. For simplicity, the recovery estimates from each spiked sample were given the same weight (equivalent to assuming no batch effects) in order to calculate the recovery at each spike level. An analysis showed that the final results are affected very little by the weights chosen.

The recovery estimates across the three spiking levels are independent and will have similar variances. Therefore, a confidence interval for the mean recovery across all spiking levels was calculated using a t-statistic. Because the t-statistic for each compound had at most two degrees of freedom, a pooled estimate of variance (pooled across all spiked compounds) was used to calculate the confidence intervals for the recoveries. Calculation of the t-statistic assumes that the recovery estimates have a normal distribution. For the Comparability Study data, the bias introduced by assuming normal errors was judged to be acceptably small, relative to the standard error of the estimates.

In the GC/ECD analyses, portions of the final extract from the unspiked samples were analyzed with each batch which included a spiked sample. Although these measurements can be used in the calculation of recovery, there was some concern that changes in the extract samples over time as a result of storage might significantly affect the estimated recoveries. Therefore, the results summarized in Section 6.2.3 are not based on the extract measurements; however, these results are summarized in Appendix C. As shown in Appendix C, the recoveries based on the extracts are similar to those calculated without using the extracts, except that the recovery estimates based on the extract have slightly smaller variances.

6.2.2 Calculating recovery using quality control samples

For the quality control samples, average recovery and its standard error were determined from the spiked QC samples in each of the 10 independent batches. The calculations assume that the recovery is the same for all concentrations. The recovery for each sample is estimated from the measured quantity of each compound and the known actual quantity in the sample.

The following equation was used to estimate recovery for the quality control samples:

$$R_{mc} = \frac{X_{mcb}}{S_c} + \tilde{\varepsilon}_{mcb} \qquad (6.2)$$

This equation can be derived from equation (6.1) where the concentration in the unspiked portion is zero.

Confidence intervals on the average recoveries were based on a t-statistic assuming normally distributed measurement errors. However, if the measurements have a lognormal distribution, the errors in equation (6.2) will also have a lognormal distribution. For the Comparability Study data, the bias introduced by assuming a normal distribution was small relative to the standard error of the estimates. Equation (6.2) provides estimates which can be directly compared to the results from the spiked multisplit samples using equation (6.1). Equation (6.2) was used to estimate recovery for the HRGC/MS analyses on dichloromethane spiked samples and the GC/ECD analyses on porcine fat samples.

Table 7. Average Recovery, with 95% Confidence Intervals, for the GC/ECD Measurements on Spiked Multisplit and Quality Control Samples

Compound	Spiked multi-split samples[a]	Porcine fat samples batches 1-3 [a]	Porcine fat samples batches 4-10
p,p'-DDT	b	67%±14%	98%±10%
p,p'-DDE	82%±19%	73%±14%	109%±15%
Beta-BHC	89%±19%	78%±14%	98%±15%
Dieldrin	90%±19%	60%±14%	104%±26%
Heptachlor Epoxide	83%±19%	65%±14%	97%±36%
Oxychlordane	73%±19%	67%±14%	96%±27%
Mirex	b	b	106%±18%
trans-Nonachlor	73%±19%	75%±14%	98%±23%
Corrected Hexachlorobenzene	77%±19%	52%±14%	99%±7%
Uncorrected Hexachlorobenzene	53%±19%	33%±14%	61% [c]
PCBs	b	b	78%±7% [d]

[a] 95% Confidence intervals are based on a pooled variance due to the small number of measurements for each compound
[b] This compound was not spiked into the samples
[c] All measurements were identical
[d] See Chapter 9.

6.2.3 Comparison of HRGC/MS and GC/ECD recovery

Recovery estimates for GC/ECD measurements are shown in Table 7. Different samples of porcine material, with different spiking levels, were used for batches 1 to 3 and 4 through 10. Therefore, recoveries for these two groups of samples are presented separately. In general, recovery for the compounds tested using the GC/ECD procedure was between 60% and 109%, with the exception of uncorrected hexachlorobenzene which has a lower recovery. The recovery based on the spiked multisplit samples was between 73% and 90% for all compounds except uncorrected hexachlorobenzene with a recovery of 53%. For batches 1 to 3, the recovery was between 60% and 78% with the exception of hexachlorobenzene with a recovery of 33% for the uncorrected measurements and 53% for the corrected measurements. Recovery based on the porcine adipose tissue samples from batches 4 through 10 were between 96% and 109% with the exception of uncorrected hexachlorobenzene and PCBs, with recoveries of 61% and 78% respectively.

For each compound except trans-Nonachlor, the GC/ECD recovery estimates for the porcine fat samples from batches 1 to 3 were less than for spiked multisplit samples which are in turn less than that for the porcine samples in batches 4 through 10. This pattern is statistically significant[10]. The differences between the recovery in the three sets of samples may be due to differences in the sample material, deviations in the spiking solutions from the nominal levels, or differences in the recovery in batches 1 to 3 versus in batches 4 through 10. In either case, the estimated recovery depended on the set of samples being analyzed.

Recovery estimates for HRGC/MS measurements are shown in Table 8. The average HRGC/MS recovery estimates for spiked compounds in the dichloromethane samples measured were between 69%

[10]A two-way analysis of variance (assuming the errors in the recovery estimates are independent) suggested that differences among compounds were not significant and that differences among types of sample material were very significant ($p<.0001$).

and 81% for, with the exception of hexachlorobenzene with an estimated recovery of 51%. The recovery estimates based on the HRGC/MS spiked multisplit samples are very variable and not very accurate. One compound, beta-BHC, contributes substantially to the pooled error estimate. The precise estimate of recovery for beta-BHC depended greatly on the weights used to average the recovery in the four multisplit spiked portions. Removing beta-BHC from the calculations gave a pooled error estimate of ±23% (±28% for dieldrin). The recovery estimates for compounds other than beta-BHC were between 26% and 56%.

The recoveries for all samples using the GC/ECD method were similar to those from the spiked dichloromethane samples using the HRGC/MS method; however, they were roughly twice those based on the multisplit samples using the HRGC/MS method. The recovery estimates differed depending on the sample matrix. Therefore, any corrections for recovery should be based on recovery estimates based on the same sample matrix. Considering only the recoveries from lipid material, the GC/ECD recovery was greater than the HRGC/MS recovery except for beta-BHC for which the recovery estimates were similar.

6.3 Comparison of Percent Detected

The number of paired samples in which each compound was detected, i.e., has a data qualifier of "Trace" or "Positively Quantified", is shown in Tables A-3 and A-11 in Appendix A. The nine primary compounds were detected using both the GC/ECD and HRGC/MS methods. These compounds were used to compare the percent detected for the two analysis methods. Table 9 shows the number of measurements and percent detected for each method. Figure 7 shows the percentage of HRGC/MS and GC/ECD paired samples with detectable quantities of each compound. The HRGC/MS dieldrin measurements were determined in only 3 of the 10 batches, resulting in fewer HRGC/MS measurements than GC/ECD measurements for

Table 8. Average Recovery, with 95% Confidence Intervals, for the HRGC/MS Measurements on Spiked Multisplit and Quality Control Samples

Compound	Spiked multisplit samples [a]	Dichloromethane spiked samples
p,p'-DDT	[b]	76%±13%
p,p'-DDE	26%±50%	80%±21%
Beta-BHC [c]	99%±50%	74%±15%
Dieldrin [d]	37%±61%	77%±75%
Heptachlor Epoxide	50%±50%	81%±9%
Oxychlordane	42%±50%	71%±6%
trans-Nonachlor	56%±50% [e]	73%±8%
Hexachlorobenzene	41%±50%	51%±5%
PCBs [f]	[b]	69%±6%

[a] ±95% Confidence intervals based on pooled variance

[b] This compound was not spiked into the samples

[c] The estimate of recovery for the lowest spike level of beta-BHC in the multisplit samples is quite high, resulting in a high estimate of recovery and contributing to the large pooled variance.

[d] Dieldrin, in Fraction 2, was analyzed in only 3 batches, thus has fewer measurements and a larger confidence interval.

[e] As a result of substituting zero for the missing unspiked concentration, the calculated recovery will tend to overestimate of the actual recovery.

[f] See Chapter 9.

Table 9. Number of GC/ECD and HRGC/MS Measurements on Paired Composite Samples and Percent Detected, by Data Qualifier and Analytical Method

Compound	ECD				MS				Positively quantified using both methods
	PQ	TR	ND	Percent Detected	PQ	TR	ND	Percent Detected	
pp'-DDT	45	0	0	100%	39	0	5	89%	39
pp'-DDE	45	0	0	100%	41	0	2	95%	41
ß-BHC	42	3	0	100%	40	0	5	89%	39
Dieldrin	45	0	0	100%	5	0	7	42%	5
Heptachlor Epoxide	45	0	0	100%	37	0	8	82%	37
Oxychlordane	41	4	0	100%	37	0	8	82%	37
trans-Nonachlor	44	0	0	100%	42	0	2	95%	41
Hexachlorobenzene	45	0	0	100%	37	0	8	82%	37
PCB	1	44	0	100%	44	0	1	98%	1

PQ = positively Quantified, TR = Trace, ND = Not detected.

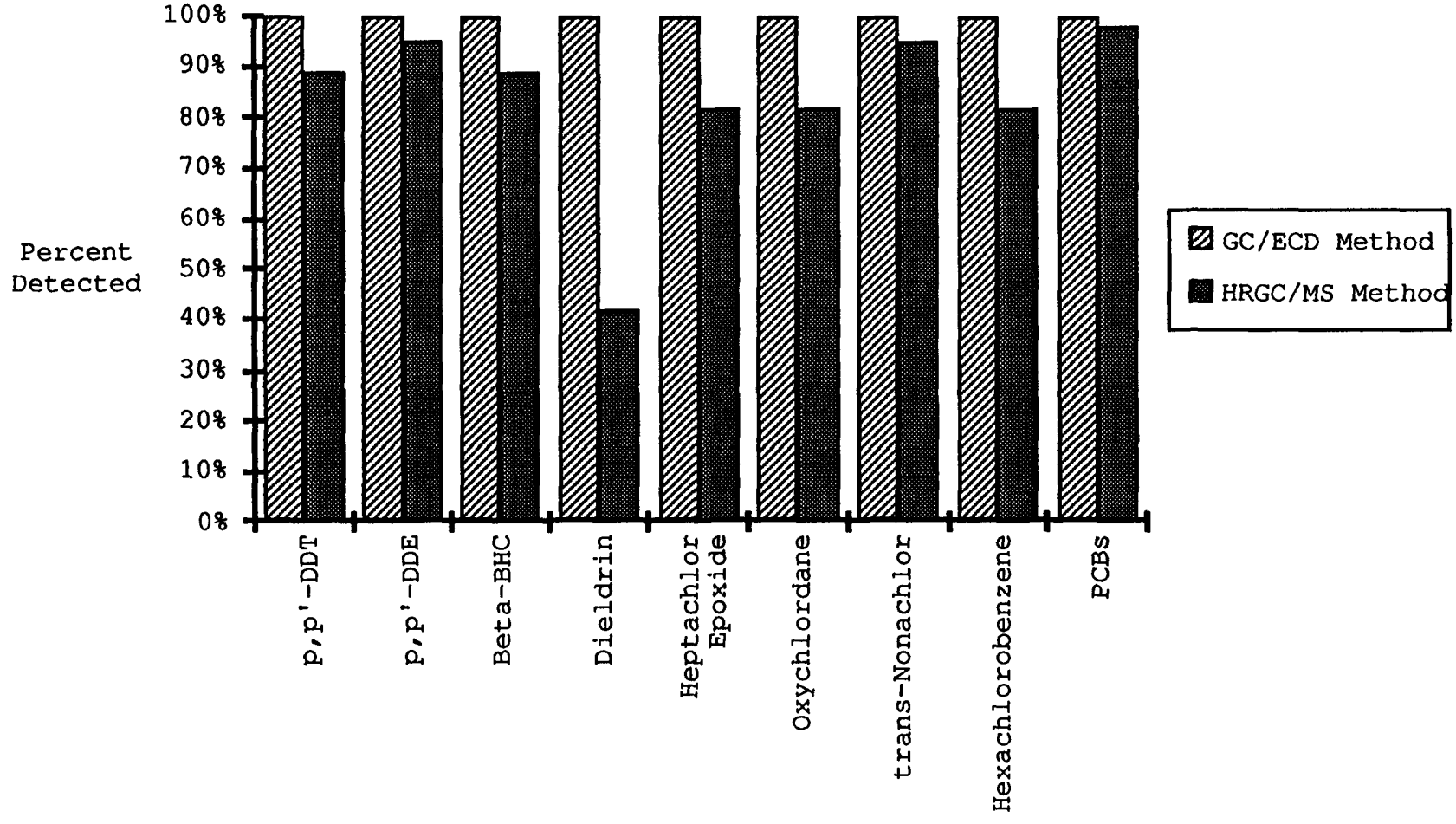

Figure 7. Percent of paired samples with detected concentrations using the HRGC/MS and GC/ECD methods.

this compound. For PCBs the GC/ECD concentrations were coded into concentration ranges. The coded concentrations were reported on the analysis report forms.

The percentage of samples with detected quantities depends on the detection limits, the recovery, and the concentration level in the samples. Low concentrations are more likely to be detected using methods with lower detection limits. The detection limits apply to the concentration after recovery losses. Therefore, given two methods with the same detection limit, the method with lower recovery will have lower measured concentrations and possibly more samples with concentrations below the detection limit. Note that the recovery and detection limits make no difference to the percent detected if all concentrations are high enough to be positively quantitated in all samples.

In general, the analysis shows that the recovery for the HRGC/MS method is similar to or lower than that for the GC/ECD method and the detection limits for the HRGC/MS method are higher than those for the GC/ECD method. Both of these factors would indicate that there would generally be a higher percent detected in the GC/ECD than the HRGC/MS paired samples. With the exception of PCBs, this conclusion is consistent with the results shown in Table 9. The GC/ECD detection limit for PCBs was much higher than the HRGC/MS detection limit. However, the percent detected is similar because the concentrations in most samples were high enough that PCBs were detected in all GC/ECD samples.

7 RELATIONSHIP BETWEEN THE GC/ECD AND HRGC/MS METHODS

This chapter presents the statistical procedures and results comparing the GC/ECD and HRGC/MS measurements in the paired samples. After a summary of the results, the relationship between the GC/ECD and HRGC/MS measurements for each compound are discussed, followed by scatter plots of the measurements on the paired samples.

7.1 Modeling the Relationship Between the GC/ECD and HRGC/MS Methods

Determining the relationship between the GC/ECD and HRGC/MS[11] measurements is desirable in order to determine factors that affect the relationship and to predict the measurements which would be obtained using one analytical method based on the observed measurements from the other method. The relationship between the measurements can be expressed by a mathematical formula such as:

$$ECD = f(MS) + error \qquad (7.1)$$

where f is a function to be determined. This equation can be solved for the HRGC/MS measurements to give:

$$MS = g(ECD) + error \qquad (7.2)$$

where g is the inverse function of f.

Differences between the HRGC/MS and GC/ECD measurements may be due to many factors, including differences in recovery (extraction efficiency) and calibration. The following discussion assumes that, due to the calibration process, the HRGC/MS and GC/ECD measurements are unbiased estimates of the concentrations

[11]HRGC/MS and GC/ECD are abbreviated as MS and ECD, respectively, in the equations.

in the final extract. Assuming the two portions of the split sample have the same initial concentration, consistent differences between HRGC/MS and GC/ECD measurements will be due to differences in recovery. Therefore, the expected relationship between the GC/ECD and HRGC/MS measurements is:

$$MS = R' \, ECD \tag{7.3}$$

where R' is the ratio of the recovery using the HRGC/MS method to the recovery using the GC/ECD method. Note that R' can also be described as the ratio of the HRGC/MS to GC/ECD measurement. According to equation (7.3), a doubling of either the GC/ECD or HRGC/MS measurement should be accompanied by a doubling of the other measurement.

Questions which might be asked about the applicability of equation (7.3) as a model for the observed data are:

- Is the ratio of the HRGC/MS to GC/ECD measurements really constant?
- If the ratio is constant, is it different from 1.0? and
- Does the ratio of the HRGC/MS to GC/ECD measurements depend on the batch?

Statistical analysis is used to determine if equation (7.3) provides an adequate description of the data. For this analysis, both the HRGC/MS and GC/ECD measurement errors are assumed to have a lognormal distribution. The analysis of measurement error variances to support this assumption is discussed in Chapter 10. For data with a lognormal distribution, 1) the measurement error increases as the concentration increases such that the coefficient of variation is constant, and 2) the log transformed data has constant measurement error variance, independent of concentration.

To make the data consistent with the assumptions behind the statistical analysis (i.e., that the error variance is constant), the log transformed data are used. Taking the logarithm of the

HRGC/MS and GC/ECD measurements in equation (7.3) gives the following equation:

$$\ln(MS) = \ln(R') + \ln(ECD) \qquad (7.4)$$

The correlation of ln(MS) and ln(ECD) provides a descriptive measure of the linear relationship between the HRGC/MS and GC/ECD measurements. The correlation can be used to test if there is a significant linear relationship between the HRGC/MS and GC/ECD measurements.

The appropriateness of equation (7.4) for describing the data can be tested by fitting additional terms to describe other factors which might also affect the HRGC/MS-GC/ECD relationship. Two additional terms were used: terms for differences between batches and for nonconstant recovery ratio, i.e., a recovery ratio which depends on the concentration in the sample. If these additional terms are statistically significant, there is evidence that equation (7.3) and (7.4) do not adequately describe the HRGC/MS-GC/ECD relationship. With these additional terms added, the model which was fit to the data is shown below in equation (7.5). This model can also be obtained from the model (equation 5.10) by equating the true concentrations in the equations for the HRGC/MS and GC/ECD measurements and combining terms.

$$\ln(X_{Mcbs}) = R_c + \beta_c * \ln(X_{Ecbs}) + \delta_{bc} + \varepsilon_{cbs} \qquad (7.5)$$

Where:

$\ln(X_{Mcbs})$ = the HRGC/MS measurements for compound c in sample s analyzed in batch b;

$\ln(X_{Ecbs})$ = the GC/ECD measurements for compound c in sample s analyzed in batch b;

β_c = a constant for each compound. This term will be 1.0 if the recovery ratio for the two methods is constant, i.e. independent of concentration. Testing if the recovery

ratio is not constant is achieved by testing if β_c is significantly different from 1.0;

R_c = a constant for each compound. If $\beta_c = 1$, this constant can be interpreted as the log of the ratio of the HRGC/MS recovery to the GC/ECD recovery;

δ_{bc} = a random effect associated with batch b and compound c, assumed to be normally distributed with a mean zero and standard deviation of σ_{bc}. This term combines the batch effect terms in the models for both methods.

ε_{cbs} = a random error for compound c in sample s analyzed in batch b, assumed to be normally distributed with a mean zero and standard deviation σ. This error is the combined result of within sample errors and measurement errors in both the HRGC/MS and GC/ECD measurements.

Equation (7.5) can be obtained from the model (equation (5.10)) by equating the true concentration (C_{cs}) in equations for the HRGC/MS and GC/ECD measurements and combining confounded terms.

Standard regression procedures can be used to estimate the parameters R_c, β_c, and δ_{bc} in equation (7.5). However, regression procedures assume that only the dependent variable (HRGC/MS in this case) has measurement error. When both the dependent and the independent variable are measured with error, the regression estimates of the parameters are biased. Fitting equation (7.5) to the data using regression provides a <u>functional</u> equation. This equation is optimal in the sense that it minimizes the squared prediction error for the data on which the equation is based. However, the functional equation may provide particularly poor predictions of HRGC/MS measurements from GC/ECD measurements when both measurements have error and the equation is extrapolated beyond the range of the original data.

Statistical models which account for measurement error in both the independent and dependent variable are called <u>structural</u> models. The slope for the functional model, estimated using regression, is a biased estimate of the slope for the structural

model. Under either one of the following conditions the bias is minimized and the regression slope provides a good approximation to the slope for both the functional and structural model:

- Measurement errors in the independent variable are small relative to the range in the independent variable; or
- The slope of the relationship, β_c, is close to zero.

Although the slope estimate may be biased, a test of the hypothesis that the slope is significantly different from zero is relatively unaffected by errors in the independent variable.[12] Therefore, regression results can be used to test if the slope for either the functional or structural model is significantly different from zero.

The slope in equation (7.5) is expected to be 1.0 if the recovery ratio is constant. Therefore, in order to test if the recovery ratio is constant, the slope from the structural model must be compared to 1.0. Unfortunately, with errors in the independent variable, regression, the primary tool for fitting equation (7.5), may perform poorly unless the slope is close to zero. An alternate approach is to transform the data by rotating the coordinate axes so that a statistical test comparing the regression slope to zero is used to test if the slope in the functional model is different from 1.0.

Both the HRGC/MS and GC/ECD data from the Comparability Study have measurement errors which cannot be ignored. As a result, standard regression procedures must be modified to model the structural relationship between the two measurements and to satisfy the assumptions behind the regression procedures. To meet these objectives, the following five steps were used to model the relationship between the HRGC/MS and GC/ECD measurements:

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[12]Errors in the independent variable will reduce the power of the test, however the probability level for the test will be correct.

(1) Use log transformed data, equalizing the measurement error variance across concentrations within each analysis method;

(2) Scale the data to make the measurement error for the scaled GC/ECD and the scaled HRGC/MS data equal;

(3) Rotate the coordinate axes so that the regression slope will be zero if the recovery ratio is constant;

(4) Use analysis of covariance on the transformed scaled and rotated data to estimate parameters and test hypotheses; and

(5) Transform the parameters back to the original units for reporting and plotting.

The scaling of the data to equalize the HRGC/MS and GC/ECD measurement errors is based on the analysis of variance components discussed in Chapter 10. The measurement errors for the HRGC/MS method were found to be roughly three times those for the GC/ECD method. For estimating the structural models, a measurement error ratio of 3 was used for all compounds.

The result of the scaling, transformation, and rotation is equivalent to fitting the following equation to the data for each compound:

$$\ln(Y) = a + b * \ln(Z) + \delta_b + \text{error} \qquad (7.6)$$

Where:

Y = the ratio of the HRGC/MS and GC/ECD measurements;

Z = the transformed product of the HRGC/MS and GC/ECD measurements: $MS^{\cdot 33}\ ECD^{3}$;

b = slope; if the slope b is equal to zero, the ratio of the HRGC/MS and GC/ECD measurements is constant;

a = intercept; and

δ_b = a batch effect; if the batch effect is significant, then there are differences in the ratio of the HRGC/MS and GC/ECD measurements between batches.

Equation (7.6) was fit to all positively quantified measurements, ignoring pairs of measurements in which one member of the pair was either a trace or nondetect measurement. Except for dieldrin, both methods had positively quantifiable concentrations for most (at least 37 out of 45) samples, thus any bias caused by ignoring the below-detection results is expected to be small. Because most of the trace and nondetect measurements were obtained using the HRGC/MS method, the slope will tend to be biased toward the low side. An analysis was performed to assess the affect of trace and nondetect measurements. The results of the hypothesis tests (significant versus nonsignificant) were unaffected by replacing trace and nondetect observations by the corresponding LOQ or LOD and including these samples into the model fit.

The full model for the comparability analysis (equation 7.6) was fit to data for the following seven compounds: p,p'-DDT, p,p'-DDE, beta-BHC, heptachlor epoxide, oxychlordane, trans-nonachlor, and hexachlorobenzene. For hexachlorobenzene the relationship between the HRGC/MS measurements and both the corrected and uncorrected GC/ECD measurements were modeled. Due to the small number of paired observations for dieldrin, the results for dieldrin are approximate and differences between batches could not be tested.

The results of fitting equation (7.6) to the data include parameter estimates for the slope and tests of the hypotheses that there are no batch effects and that the ratio of the HRGC/MS to GC/ECD measurements (i.e., the recovery ratio) is constant. If the regression slope is significantly different from zero, then the hypothesis that the recovery ratio is constant is rejected. The equation which fits the data has the form:

$$MS = R' * ECD^{d} \qquad (7.7)$$

where R' and d are constants. The exponent d is determined by transforming the slope in equation (7.6) back to the original scale, involving an adjustment to correct for measurement error

and to reverse the rotation, scaling, and log transformations. Equation (7.7) for the HRGC/MS-GC/ECD relationship is referred to as the "best fit" equation. The constant, R', in equation (7.7), depends on the measurement units. For the analysis, all concentrations are in micrograms per gram of extracted lipid (ug/g). If different measurement units are used (for example, nanograms per gram) the exponent in equation (7.7) remains the same, however the constant must be adjusted for the new measurement units. No adjustment is necessary if d = 1.0.

The scaling constant R' is calculated so that the best fit line passes through the average of the HRGC/MS and GC/ECD measurements. The method for calculating the averages depends on assumptions about the significance of a batch effect. If there are significant differences between measurements in different batches, an estimate of R' is obtained by averaging the measurements within a batch and then averaging the batch averages to obtain the overall average, as in equation (7.8):

$$\ln(R') = \sum_{b=1}^{10} \frac{\sum_{s}^{m} \ln\left(\frac{MS}{ECD^d}\right)}{10\ m} \qquad (7.8)$$

where m is the number of paired samples with measurements within batch b.

If there are no differences between batches, then an estimate of R' is obtained by averaging across all samples as in equation (7.9):

$$\ln(R') = \sum_{s}^{n} \frac{\ln\left(\frac{MS}{ECD^d}\right)}{n} \qquad (7.9)$$

where n is the number of paired samples with measurements.

The two equations for estimating R' are equivalent if the number of measurements in each batch is the same. Because (1) the number of samples per batch vary from 1 to 5, (2) there are statistically significant batch effects for some, if not all, compounds, and (3) batch effects are significant based on the components of variance, equation (7.8) was used in all cases to estimate R'.

7.2 Comparison of HRGC/MS and GC/ECD Measurements

Statistical hypothesis tests were used to test the following overall hypotheses: (1) there is no linear relationship between the GC/ECD and the HRGC/MS measurements, (2) there are no differences between batches, (3) the recovery ratio is constant, and (4) the average recovery ratio is 1.0. Each of these overall hypotheses were tested at the 95 percent confidence level. There were multiple chances to test each hypothesis, using data from each of the primary compounds. To limit the probability of incorrectly rejecting these overall hypotheses due to the use of hypothesis tests on multiple individual compounds, the following procedure, based on the Bonferroni inequality, was used. An overall hypothesis was rejected at the 5 percent level if the probability level for the hypothesis test using any one compound was less than 0.71 percent[13]. If the overall hypothesis was rejected at the 5 percent level then hypothesis tests for each compound, testing at the 5 percent level, were used to identify individual compounds for which the data were inconsistent with the hypothesis. Using this procedure, the probability of rejecting an overall hypothesis using all of the compounds is less than 5 percent.

For the first overall hypothesis, the correlations of the log transformed HRGC/MS and GC/ECD measurements are shown in Table 10,

[13]There are 7 compounds with data which are both independent and numerous enough to test the hypotheses (corrected and uncorrected Hexachlorobenzene are considered to be one compound and dieldrin is not counted). The conservative formula for the Bonferroni limit would set alpha (i.e., 1 - the confidence level) for the overall hypothesis test to 0.05/7 = .0071 or 0.71 percent.

along with the results of a test of the hypothesis that the true correlation is zero (i.e., no linear relationship). The significance levels are only approximate, since possible batch effects have not been considered. The overall hypothesis of no linear relationship is rejected at the 5 percent level, indicating that there is a statistically significant linear relationship between the measurements from the HRGC/MS and the GC/ECD methods. As can be seen from Table 10, the hypothesis of no linear relationship is rejected at the 5 percent level for all compounds except dieldrin. Significant correlations are indicated by using bold type.

For testing the second and third overall hypotheses, equation (7.6) was fit to the GC/ECD and HRGC/MS data for the primary compounds. Table 11 shows the results of the statistical tests to answer the following questions:

- Were there consistent differences in the HRGC/MS-GC/ECD relationship <u>between</u> batches (i.e., are there batch effects)? and
- For samples <u>within</u> batches, does the ratio of the HRGC/MS to GC/ECD measurement depend on the concentration (i.e., is there non-constant recovery)?

Both the overall hypotheses of no batch effects and constant recovery are rejected, indicating that, at least for some compounds, the recovery ratio differs among batches or that the recovery depends on the concentration. As can be seen from Table 11, the hypothesis of no batch effects is rejected at the 5 percent level for p,p'-DDE, beta-BHC, oxychlordane, and trans-nonachlor. The hypothesis of constant recovery is rejected at the 5 percent level for p,p'-DDE, beta-BHC, and hexachlorobenzene (both corrected and uncorrected).

Table 10. Correlation Between Log Transformed HRGC/MS and GC/ECD Measurements

Compound	Number of paired samples	Correlation[a]	Significance test for correlation different from zero
p,p'-DDT	39	**0.86**	p < .0001
p,p'-DDE	41	**0.84**	p < .0001
Beta-BHC	39	**0.52**	p = .0008
Dieldrin	5	0.52	p = .68
Heptachlor Epoxide	37	**0.57**	p = .0002
Oxychlordane	37	**0.57**	p = .0002
trans-Nonachlor	41	**0.84**	p < .0001
Uncorrected Hexachloro-benzene	37	**0.63**	p < .0001
Corrected Hexachloro-benzene	37	**0.71**	p < .0001

[a]The overall hypothesis of no linear relationship between the HRGC/MS and the GC/ECD measurements was rejected at the 5 percent level using the Bonferroni approach. Values in Bold text identify individual compounds for which the hypothesis is rejected.

Table 11. Summary of the Statistical Tests for Batch Effects and Nonconstant Recovery

Compound	Test for Batch Effects[a]	Test for a nonconstant HRGC/MS-GC/ECD measurement ratio[a]	Comments
p,p'-DDT	p = .37	p = .16	
p,p'-DDE	**p = .014**	**p < .0001**	Batch effects are not significant after removing batch 3
Beta-BHC	**p = .0001**	**p = .014**	Data in batches 1, 2, and 3 are significantly different than the other batches
Dieldrin		p = .99	Not enough data to fit a batch effect
Heptachlor Epoxide	p = .13	p = .44	There is one extreme observation
Oxychlordane	**p = .024**	p = .065	
trans-Nonachlor	**p = .0089**	p = .86	Data in batches 1, and 2 are significantly different than the other batches
Uncorrected Hexachloro-benzene	p = .13	**p < .0001**	
Corrected Hexachloro-benzene	p = .25	**p = .0006**	

[a]The overall hypotheses of no batch effects and constant recovery were rejected at the 5 percent level using the Bonferroni approach. Values in Bold text identify individual compounds for which the hypothesis is rejected.

On the assumption that the recovery ratio is constant (i.e., d = 1.0), the fourth overall hypothesis was tested using the ratio of the GC/ECD to the HRGC/MS measurements in equation (7.8). The results are presented in Table 12 and Figure 8. The confidence intervals were calculated using a t-statistic and the standard error of ln(R'), assuming the batch means are independent.

Based on the confidence intervals, the overall hypothesis that the recovery ratio is equal to 1.0 is rejected at the 5 percent level, indicating that, for at least some compounds, recoveries for the HRGC/MS and the GC/ECD methods are different. As can be seen from Table 12, the hypothesis that the recovery ratio is equal to 1.0 is rejected at the 5 percent level for all compounds except beta-BHC and dieldrin. For compounds for which the test for nonconstant recovery ratio is significant, the calculated ratio (and confidence interval) represents an average ratio for composite samples in the 1984 NHATS survey.

Table 12 also shows the ratio of the GC/ECD to HRGC/MS recoveries estimated from the multisplit spiked samples. Although this ratio estimate is not very precise, the correlation of the recovery ratio calculated from the multisplit samples and the ratio of the GC/ECD to HRGC/MS measurements is statistically significant at the 5 percent level.

The estimated GC/ECD to HRGC/MS ratios were greater than 1.0 for all compounds, indicating that the recovery for the GC/ECD method is typically greater than for the HRGC/MS method. However, the 95% confidence intervals for beta-BHC and dieldrin include 1.0. For all other compounds tested, the GC/ECD recovery was significantly greater than that for the HRGC/MS method.

Table 12. Geometric Mean Ratio of GC/ECD to HRGC/MS Measurements

Compound	Geometric Mean Ratio of GC/ECD to HRGC/MS Measurements[a]	95% Confidence interval	Ratio of GC/ECD to HRGC/MS Recoveries in Multisplit Samples	p-value
p,p'-DDT	**3.88**	**3.38-4.47**	[b]	<.05
p,p'-DDE[c]	**2.67**	**1.99-3.59**	3.15	<.05
Beta-BHC[c]	1.25	0.78-2.02	0.90	
Dieldrin	2.63	0.50-14.0	2.43	
Heptachlor Epoxide	**2.76**	**2.13-3.57**	1.66	<.05
Oxychlordane	**2.04**	**1.52-2.75**	1.74	<.05
trans-Nonachlor	**2.31**	**1.90-2.80**	1.30	<.05
Uncorrected Hexachloro-benzene[c]	**1.41**	**1.19-1.68**	1.29	<.05
Corrected Hexachloro-benzene[c]	**2.11**	**1.85-2.41**	1.88	<.05

[a]The overall hypothesis that the recovery ratio is 1.0 was rejected at the 5 percent level using the Bonferroni approach. Values in Bold text identify individual compounds for which the hypothesis is rejected.

[b]p,p'-DDT was not spiked into the multisplit samples.

[c]The data suggests that the recovery ratio is not constant for these compounds.

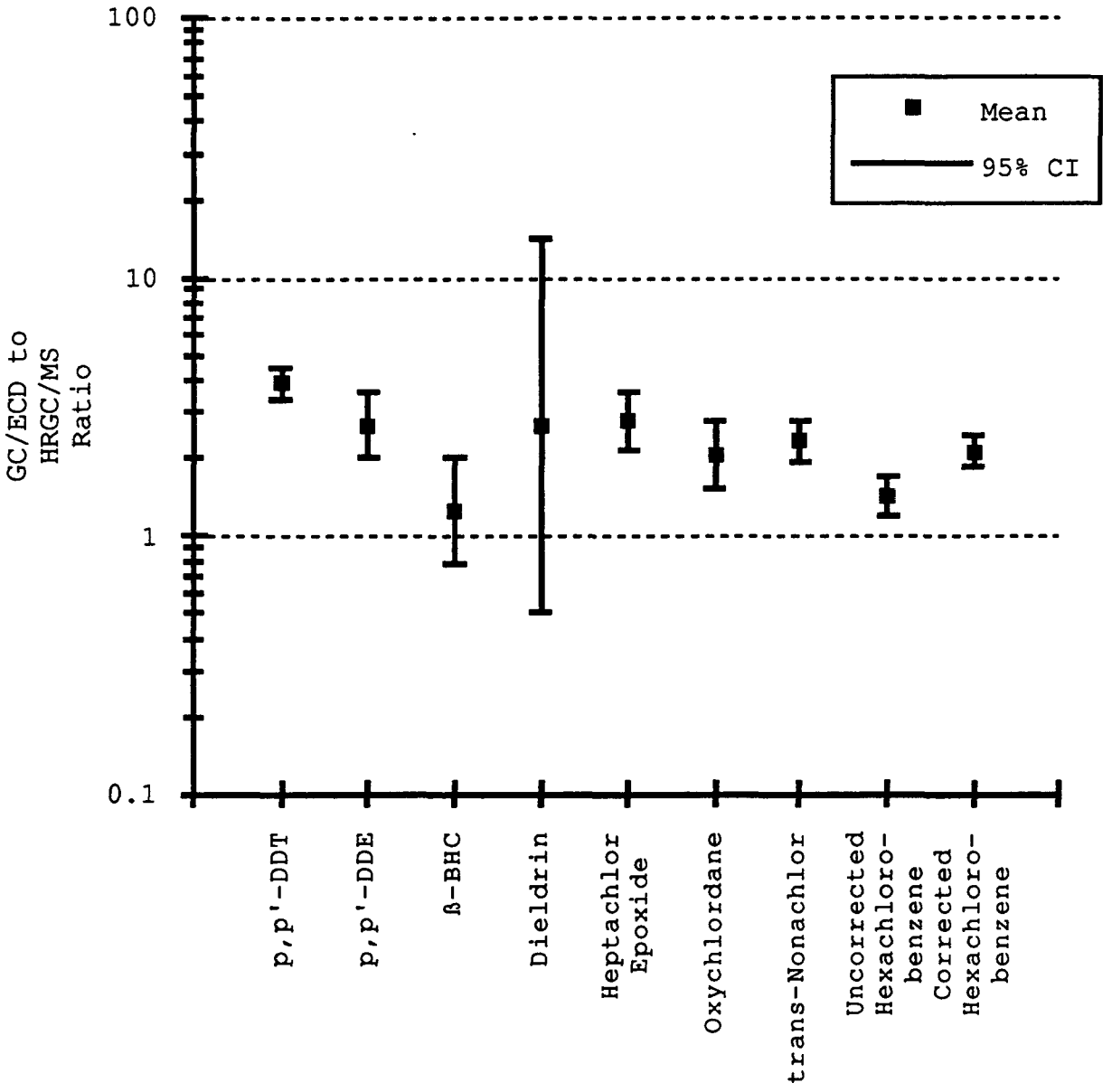

Figure 8 Geometric mean ratio of the GC/ECD and HRGC/MS measurements for primary compounds, with 95% confidence intervals.

7.3 Comparisons for Each Compound

The results for each compound are discussed below along with an equation describing the relationship between the HRGC/MS and the GC/ECD measurements assuming constant recovery ratio. If the assumption of constant recovery ratio is rejected based on the statistical tests, both the best fit equation and a simpler equation based on the assumption of constant recovery ratio are presented. Although the best fit equation is likely to apply over a wider range of concentrations, the simpler equation is easier to use and describes the ratio of the geometric mean HRGC/MS and geometric mean GC/ECD measurements for the 1984 NHATS survey.

p,p'-DDT

Neither the test for batch effects nor the test for nonconstant recovery ratio was significant. The equation which best approximates the relationship between the HRGC/MS p,p'-DDT and GC/ECD p,p'-DDT measurements in the 1984 NHATS survey is:

$$MS = \frac{ECD}{3.88} = 0.26 * ECD \qquad (7.10)$$

Differences between the geometric mean HRGC/MS and GC/ECD measurements were significant, indicating that the recoveries for the two methods are different.

p,p'-DDE

The test for nonconstant recovery ratio was highly significant ($p < .0001$) and the test for batch effects was significant ($p = .0136$). The data for batch 3 are noticeably different from the other batches. After removing the data for batch 3, the batch effect is no longer significant and the test for nonconstant recovery ratio is still highly significant ($p < .0001$). Because the slope estimates are almost identical with and without batch 3,

all of the data are used for the summary statistics presented below.

The equation which best approximates the relationship between the HRGC/MS p,p'-DDE and GC/ECD p,p'-DDE measurements is:

$$MS = 0.47 * ECD^{0.64} \qquad (7.11)$$

The following equation relates the HRGC/MS p,p'-DDE and GC/ECD p,p'-DDE measurements in the 1984 NHATS survey, and can be used for extrapolation if the recovery ratio can be assumed to be constant:

$$MS = \frac{ECD}{2.67} = 0.37 * ECD \qquad (7.12)$$

Differences between the geometric mean HRGC/MS and GC/ECD measurements were significant, indicating that the recoveries for the two methods are different.

Beta-BHC

The test for nonconstant recovery ratio was significant ($p = .0138$) and the test for batch effects was highly significant ($p = .0001$). Although the batch differences follow a general trend, with higher measurement ratios in batches 1, 2, and 3 than in later batches, no observations or batches are obviously unusual. Excluding the first three batches makes little difference in the results.

The equation which best approximates the relationship between the HRGC/MS beta-BHC and GC/ECD beta-BHC measurements is:

$$MS = 0.38 * ECD^{0.64} \qquad (7.13)$$

The following equation relates the HRGC/MS beta-BHC and GC/ECD beta-BHC measurements in the 1984 NHATS survey, and can be

used for extrapolation if the recovery ratio can be assumed to be constant:

$$MS = \frac{ECD}{1.25} = 0.80 * ECD \qquad (7.14)$$

Differences between the geometric mean HRGC/MS and GC/ECD measurements were not statistically significant.

Dieldrin

There were only five paired samples with positively quantified measurements for dieldrin. Although the full model could not be fit to so few data points, the data were used to test for nonconstant recovery ratio. No significant relationships were found.

The equation which best approximates the relationship between the HRGC/MS dieldrin and GC/ECD dieldrin measurements in the 1984 NHATS survey is:

$$MS = \frac{ECD}{2.63} = 0.38 * ECD \qquad (7.15)$$

Differences between the geometric mean HRGC/MS and GC/ECD measurements were not statistically significant.

Heptachlor Epoxide

Neither the test for batch effects nor the test for nonconstant recovery ratio was significant. The lowest observation was particularly influential on the regression fit. However, with this observation removed, the conclusions are unchanged.

The equation which best approximates the relationship between the HRGC/MS heptachlor epoxide and GC/ECD heptachlor epoxide measurements in the 1984 NHATS survey is:

$$MS = \frac{ECD}{2.76} = 0.36 * ECD \qquad (7.16)$$

Differences between the geometric mean HRGC/MS and GC/ECD measurements were significant, indicating that the recoveries for the two methods are different.

Oxychlordane

The test for nonconstant recovery ratio was not significant; however, the recovery ratios for the GC/ECD and HRGC/MS oxychlordane measurements differed significantly between batches ($p = .0243$).

The equation which best approximates the relationship between the HRGC/MS oxychlordane and GC/ECD oxychlordane measurements in the 1984 NHATS survey is:

$$MS = \frac{ECD}{2.04} = 0.49 * ECD \qquad (7.17)$$

Differences between the geometric mean HRGC/MS and GC/ECD measurements were significant, indicating that the recoveries for the two methods are different.

trans-Nonachlor

The test for nonconstant recovery ratio was not significant, however, the ratio of the GC/ECD and HRGC/MS recoveries varied significantly between batches ($p = .0089$). The ratio of the GC/ECD to HRGC/MS measurement was smaller in batches 1 and 2 than for the other batches.

The equation which best approximates the relationship between the HRGC/MS trans-nonachlor and GC/ECD trans-nonachlor measurements in the 1984 NHATS survey is:

$$MS = \frac{ECD}{2.31} = 0.43 * ECD \qquad (7.18)$$

Differences between the geometric mean HRGC/MS and GC/ECD measurements were significant, indicating that the recoveries for the two methods are different.

Uncorrected Hexachlorobenzene

The test for nonconstant recovery ratio of uncorrected hexachlorobenzene was highly significant ($p < .0001$) and the test for batch effects was not significant. Inspection of the plot of the data and the residuals from the fit suggested that there were five observations which might be judged to be unusual and were influential on the estimated slope. If these five observations were removed from the model, the test for nonconstant recovery ratio is still significant ($p = .0332$). The five unusual observations are associated with the lowest three and highest two GC/ECD measurements. The estimated slope depends on which observations are removed from the data set. Because none of the observations are clearly in error, the results summarized below are based on all data points.[14]

The equation which best approximates the relationship between the HRGC/MS hexachlorobenzene and GC/ECD uncorrected hexachlorobenzene measurements is:

$$MS = 0.12 * ECD^{0.47} \qquad (7.19)$$

The following equation relates the HRGC/MS hexachlorobenzene and GC/ECD uncorrected hexachlorobenzene measurement in the 1984 NHATS survey, and can be used for extrapolation if the recovery ratio can be assumed to be constant:

[14]If the two most influential points are removed, the results change very little and the conclusions do not change.

$$MS = \frac{ECD}{1.41} = 0.41 * ECD \qquad (7.20)$$

Differences between the geometric mean HRGC/MS and GC/ECD measurements were significant, indicating that the recoveries for the two methods are different.

Corrected Hexachlorobenzene

The test for nonconstant recovery ratio for Hexachlorobenzene was very significant ($p = .0006$) and the test for batch effects was not significant. As with the uncorrected hexachlorobenzene, several points are influential in determining the slope; however, removing these points does not change the conclusion that there is a nonconstant recovery ratio.

The equation which best approximates the relationship between the HRGC/MS hexachlorobenzene and GC/ECD corrected hexachlorobenzene measurements is:

$$MS = 0.13 * ECD^{0.56} \qquad (7.21)$$

The following equation relates the HRGC/MS hexachlorobenzene and GC/ECD corrected hexachlorobenzene measurement in the paired 1984 NHATS samples, and can be used for extrapolation if the recovery ratio can be assumed to be constant:

$$MS = \frac{ECD}{2.11} = 0.47 * ECD \qquad (7.22)$$

Differences between the geometric mean HRGC/MS and GC/ECD measurements were significant, indicating that the recoveries for the two methods are different. Note that the corrected GC/ECD hexachlorobenzene measurements were adjusted by the laboratory to correct for low recovery, increasing the ratio of the GC/ECD to HRGC/MS measurements from 1.41 to 2.11.

7.4 Plots of HRGC/MS versus GC/ECD Measurements

Plots of the HRGC/MS versus GC/ECD concentration measurements of primary compounds in paired composite human adipose tissue samples are shown in Figures 9 through 17. Figures 18 through 26 show the same data after the transformation, scaling, and rotation required for the statistical tests. Each figure shows:

- An open diamond (◇), which indicates the paired HRGC/MS and GC/ECD measurements used to compare the two methods;
- A dotted line (- - -), which serves as a reference line showing where the HRGC/MS and GC/ECD measurements are equal;
- A dashed line (— — —), which indicates the best fit relationship between the HRGC/MS and GC/ECD measurements under the assumption that the HRGC/MS measurements are proportional to the GC/ECD measurements (i.e., the recovery ratio is constant, independent of concentration); and
- A solid line (———), which indicates the best fit relationship between the HRGC/MS and GC/ECD measurements using equation 7.7.

In addition, Figures 9 through 17 also show:

- A closed diamond (◆), indicates paired HRGC/MS and GC/ECD measurements where at least one of the measurements was trace or not detected. The plotted value is the LOD for non-detect measurements and the measured amount for trace measurements.

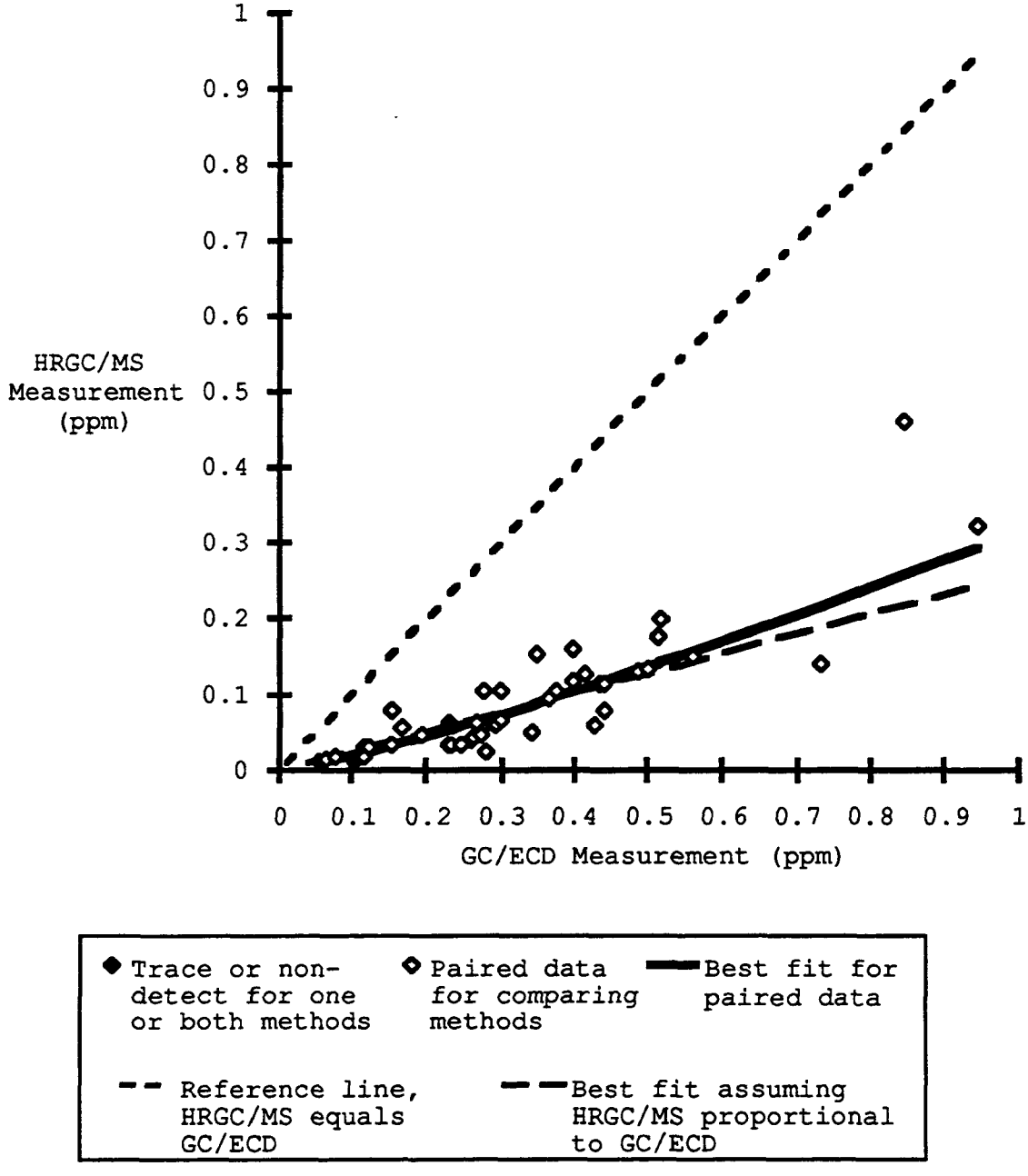

Figure 9. HRGC/MS versus GC/ECD concentration measurements for p,p'-DDT in paired composite human adipose tissue samples.

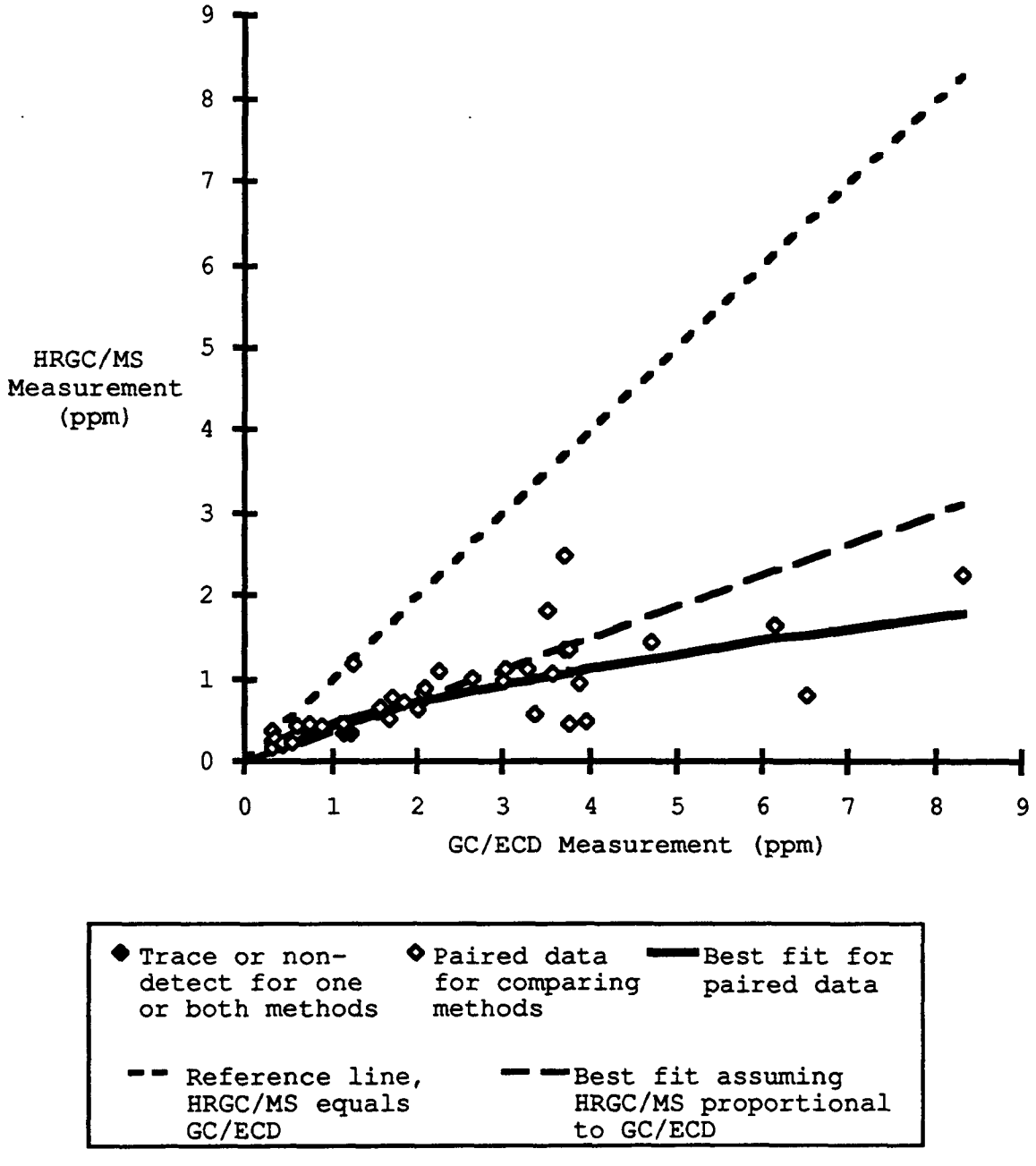

Figure 10. HRGC/MS versus GC/ECD concentration measurements for p,p'-DDE in paired composite human adipose tissue samples.

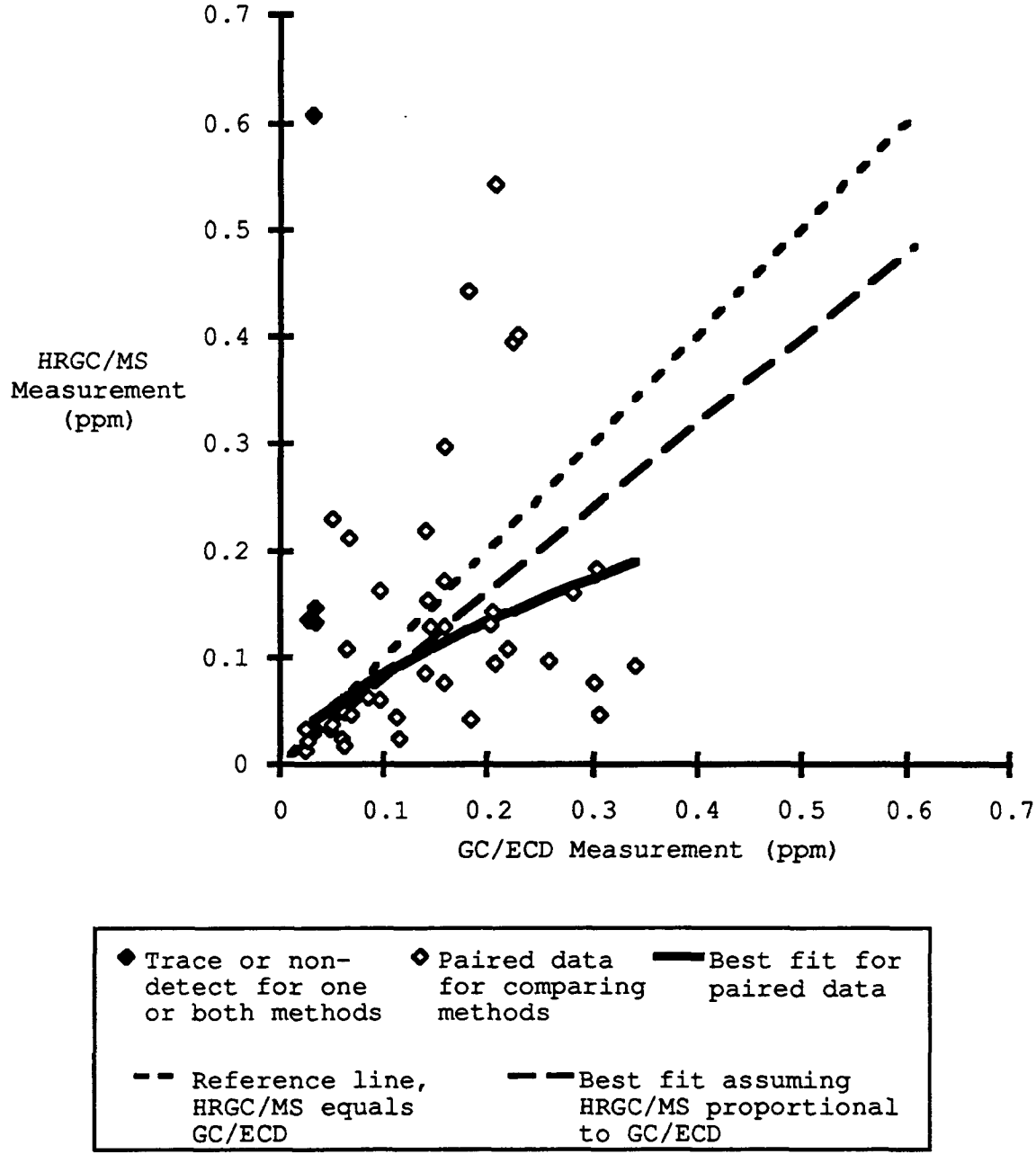

Figure 11. HRGC/MS versus GC/ECD concentration measurements for beta-BHC in paired composite human adipose tissue samples.

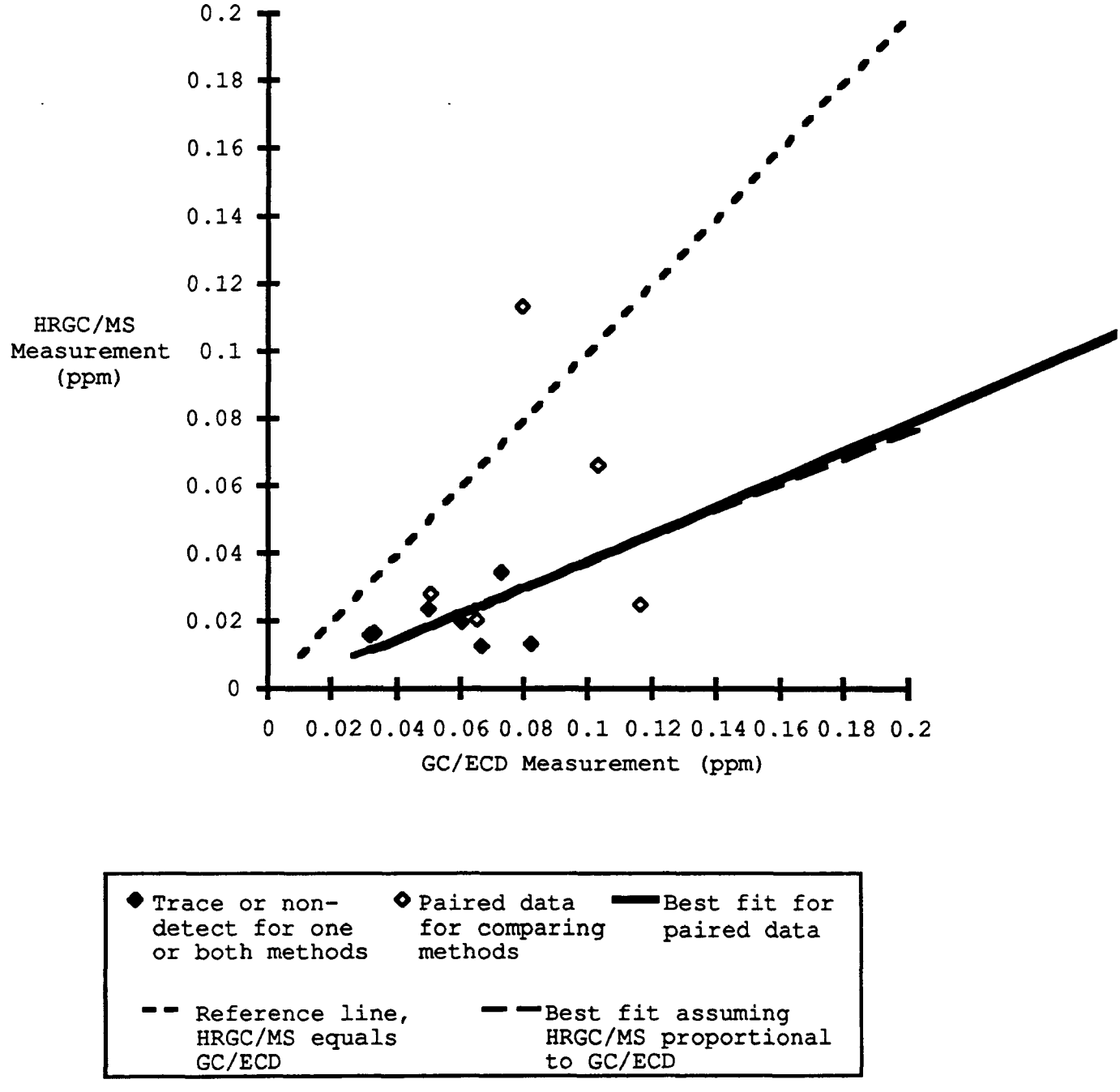

Figure 12. HRGC/MS versus GC/ECD concentration measurements for dieldrin in paired composite human adipose tissue samples.

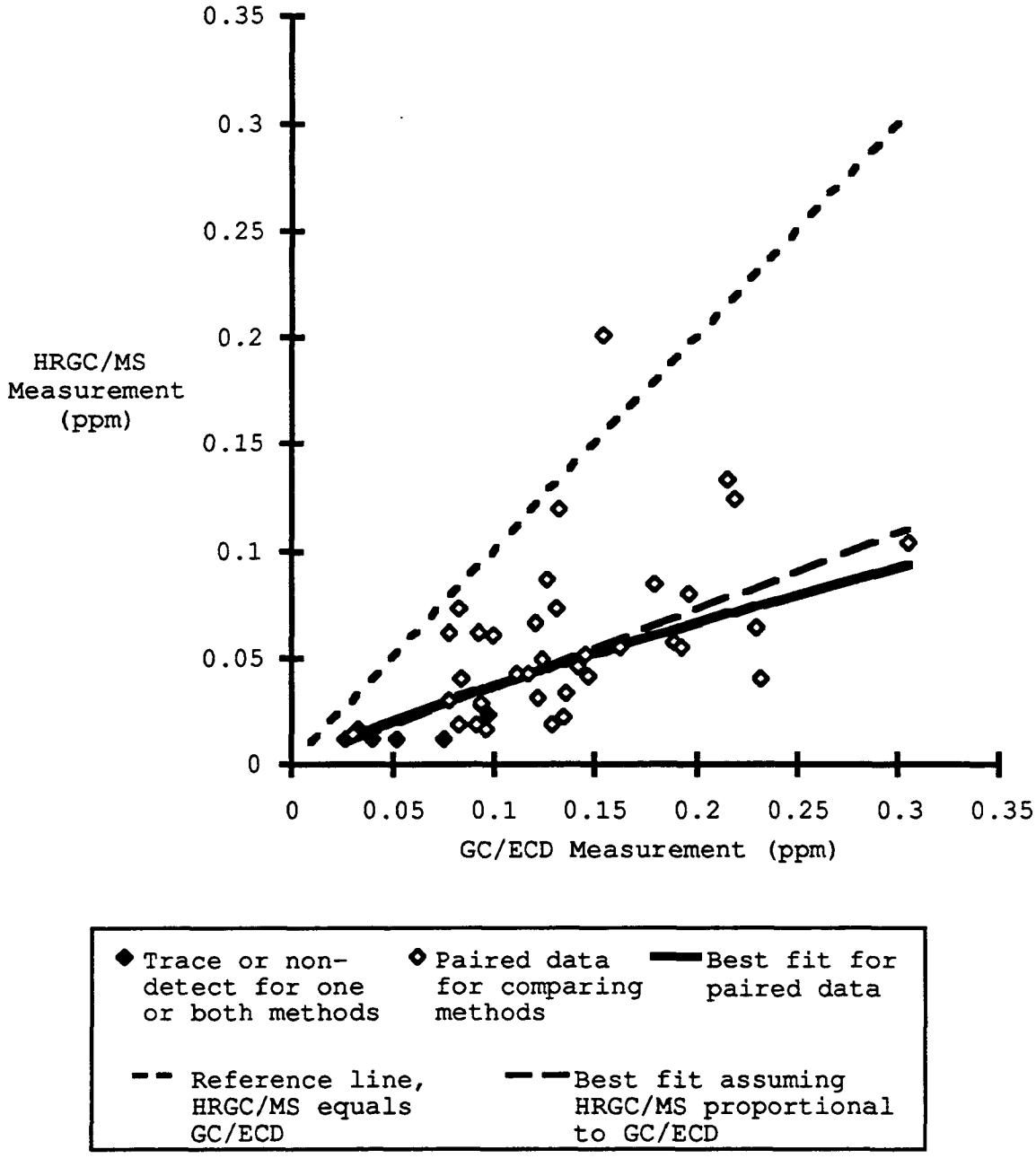

Figure 13. HRGC/MS versus GC/ECD concentration measurements for heptachlor epoxide in paired composite human adipose tissue samples.

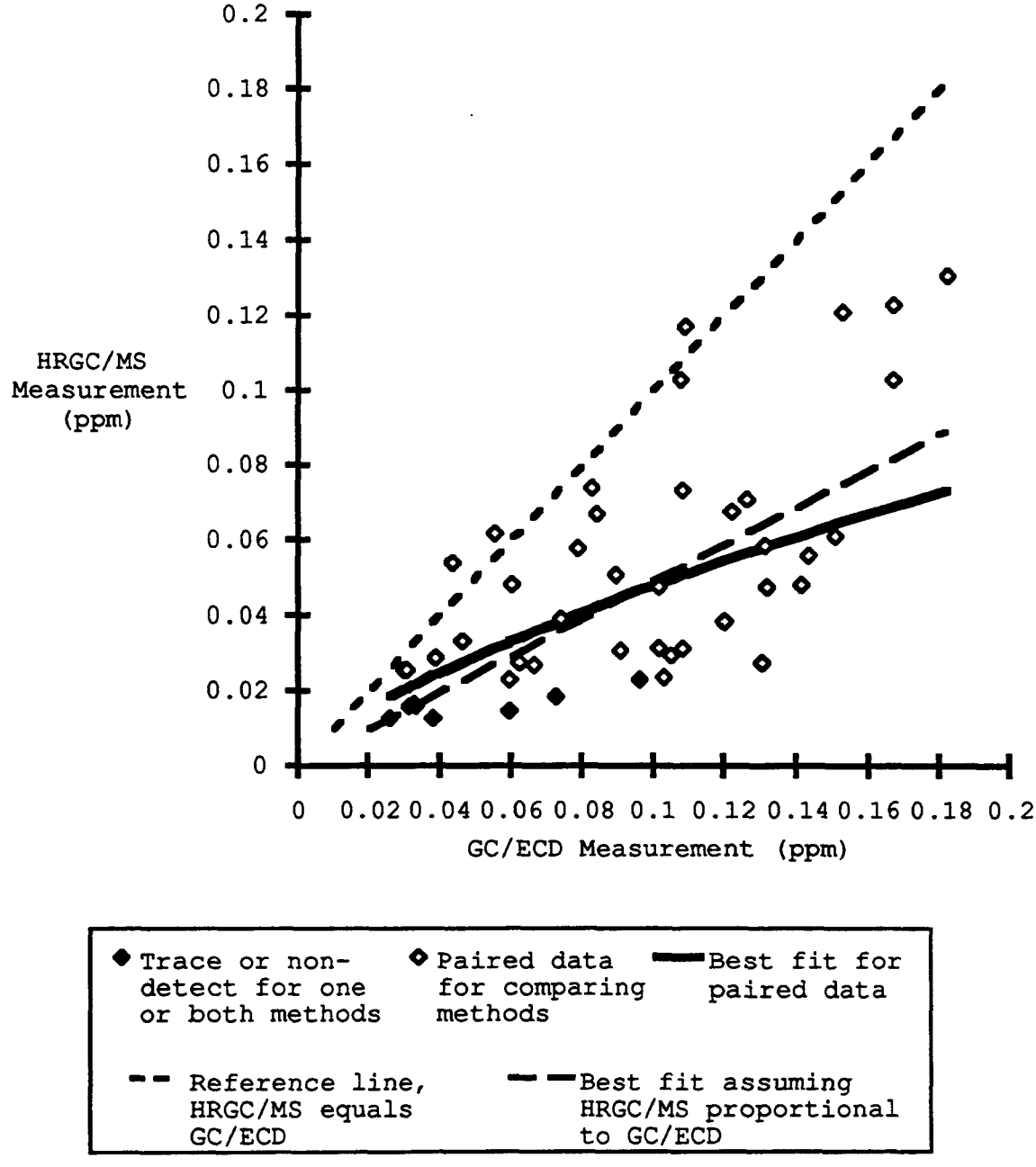

Figure 14. HRGC/MS versus GC/ECD concentration measurements for oxychlordane in paired composite human adipose tissue samples.

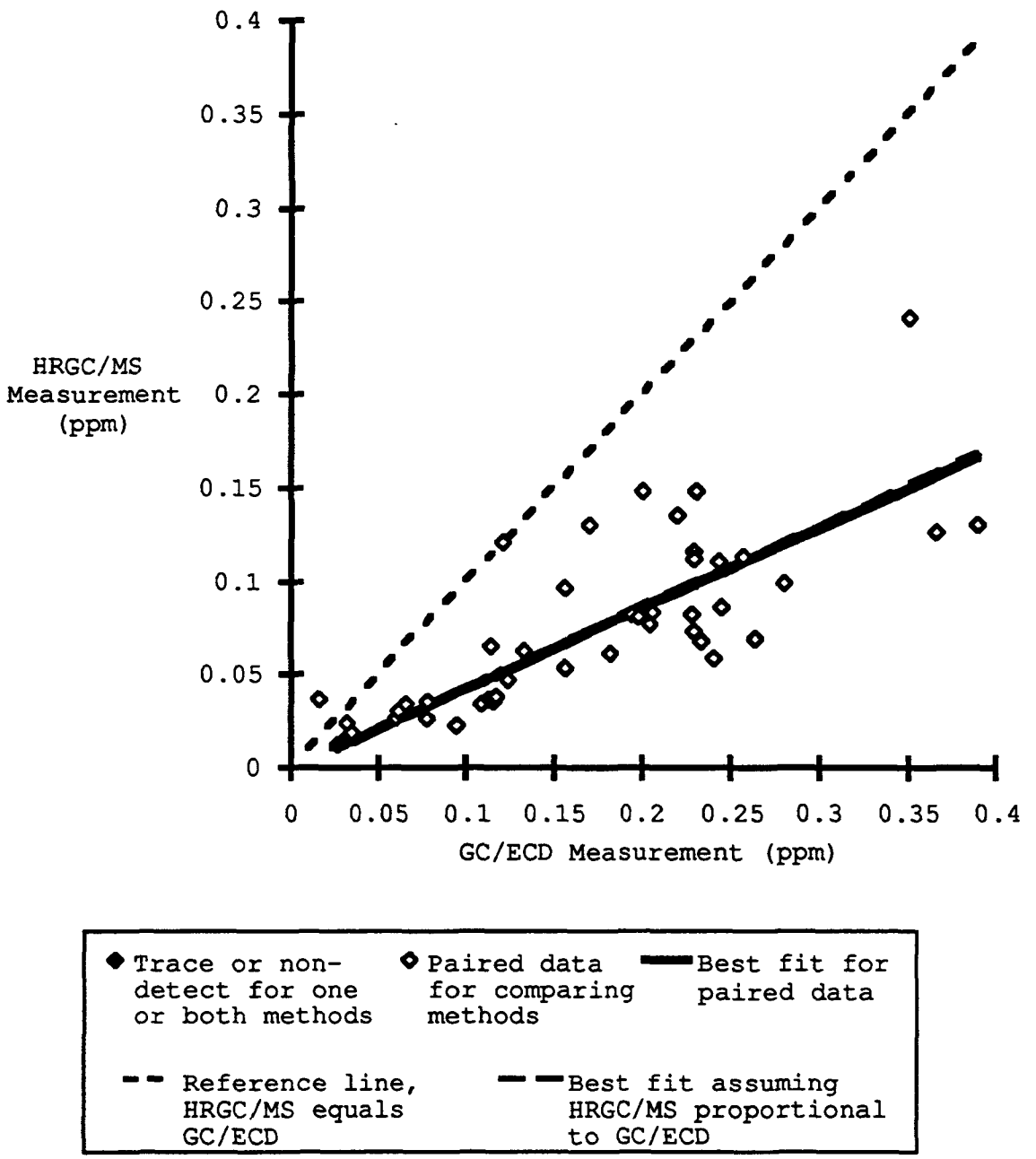

Figure 15. HRGC/MS versus GC/ECD concentration measurements for trans-nonachlor in paired composite human adipose tissue samples.

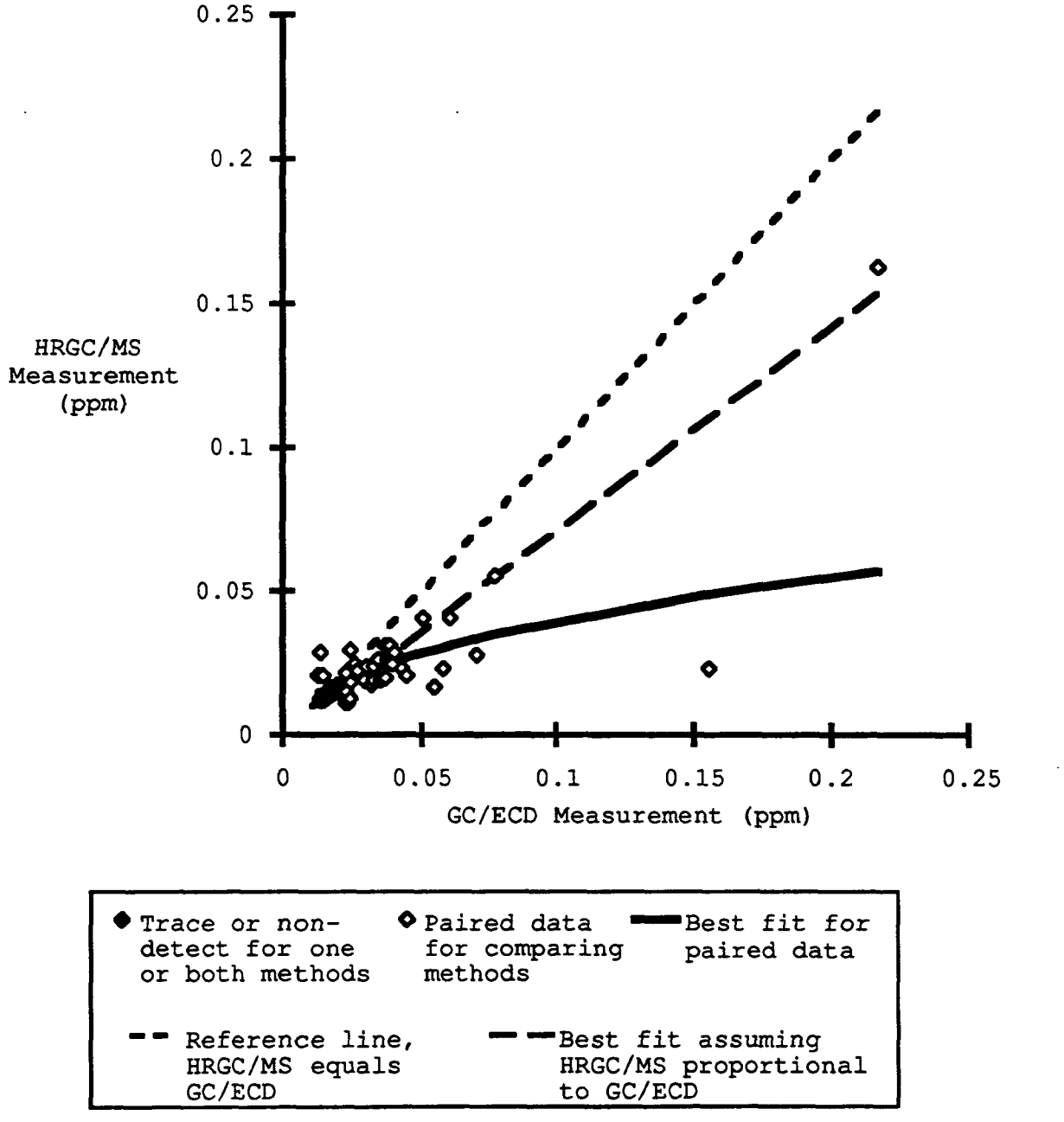

Figure 16. HRGC/MS versus GC/ECD concentration measurements for uncorrected hexachlorobenzene in paired composite human adipose tissue samples.

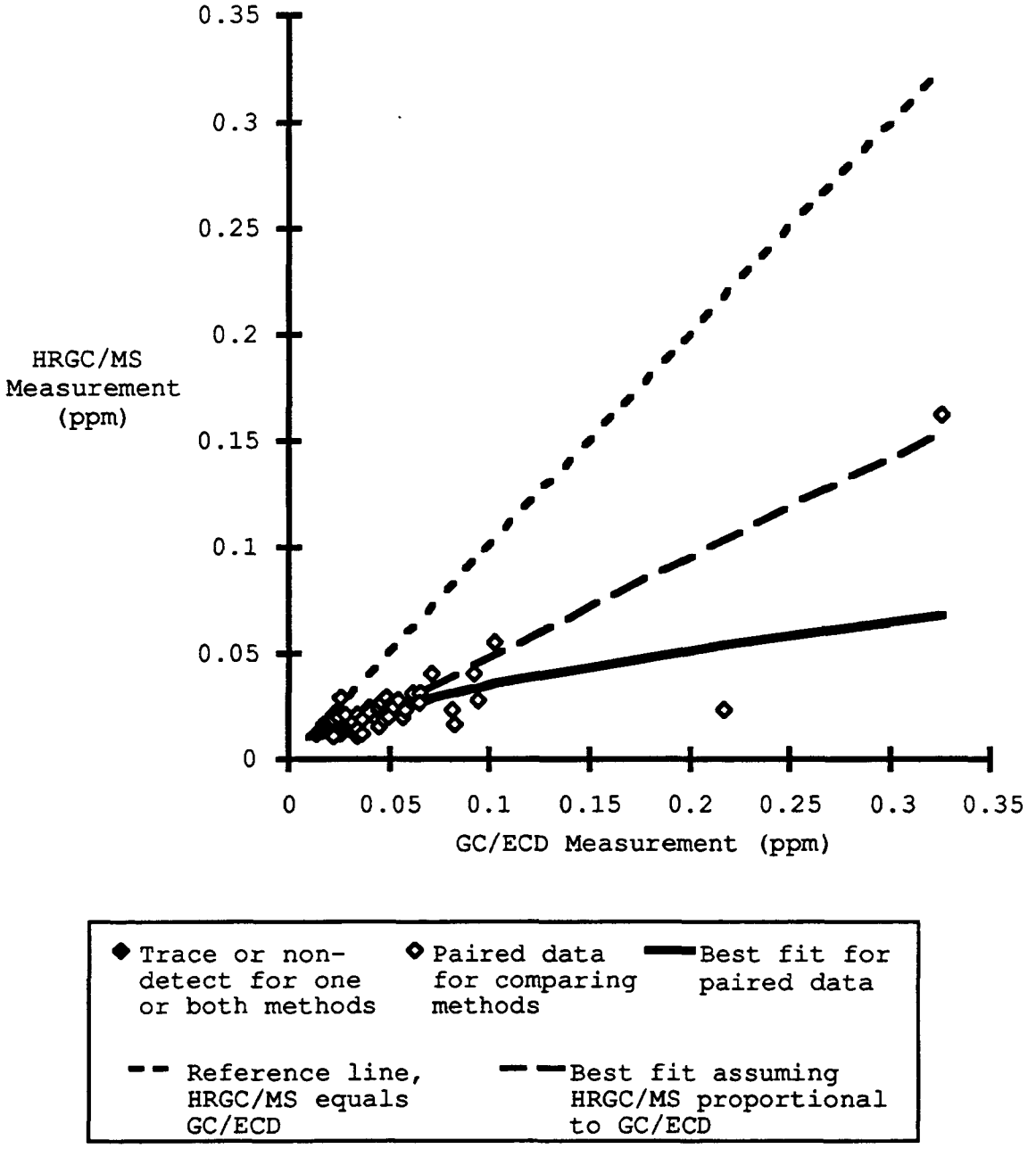

Figure 17. HRGC/MS versus GC/ECD concentration measurements for corrected hexachlorobenzene for recovery in paired composite human adipose tissue samples.

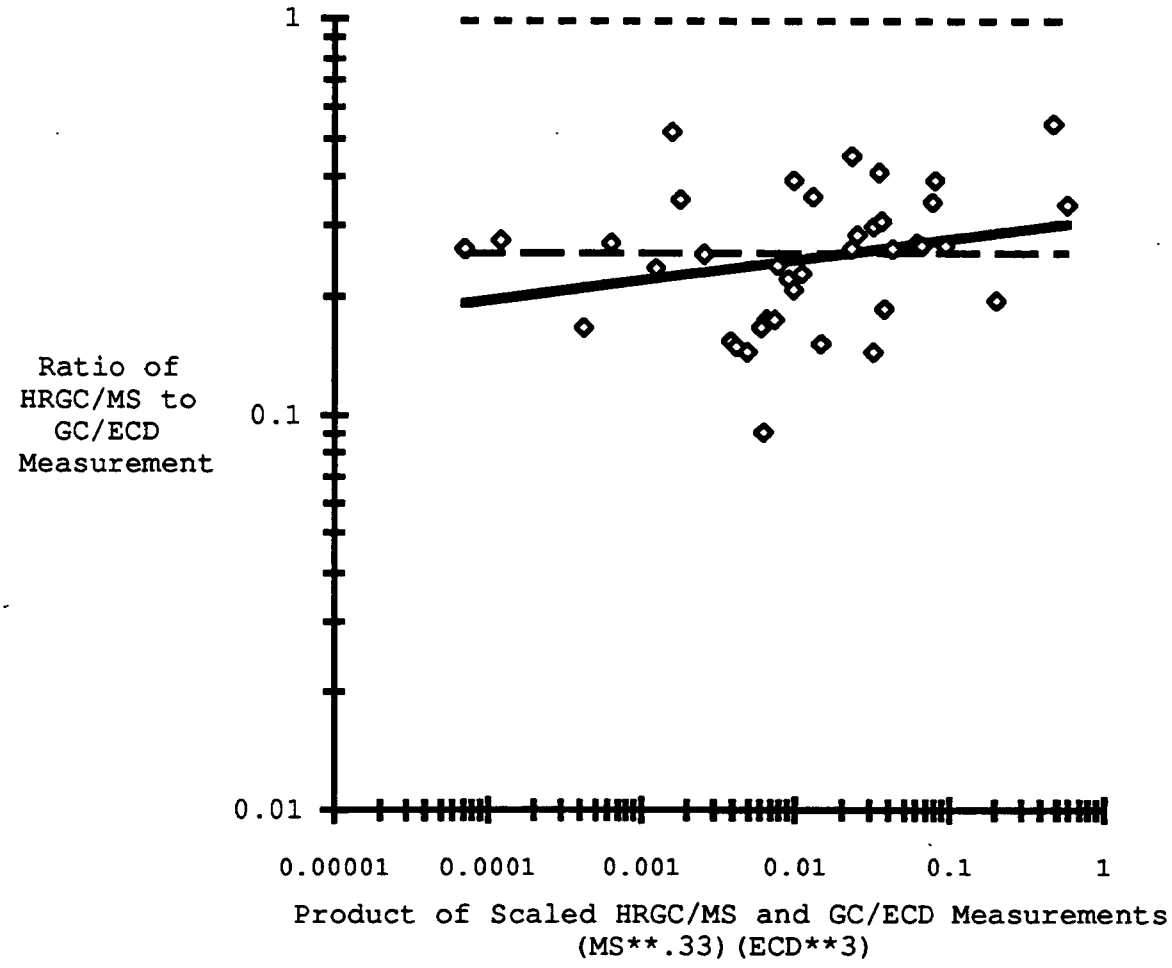

Figure 18. Transformed HRGC/MS versus GC/ECD p,p'-DDT measurements used for statistical tests.

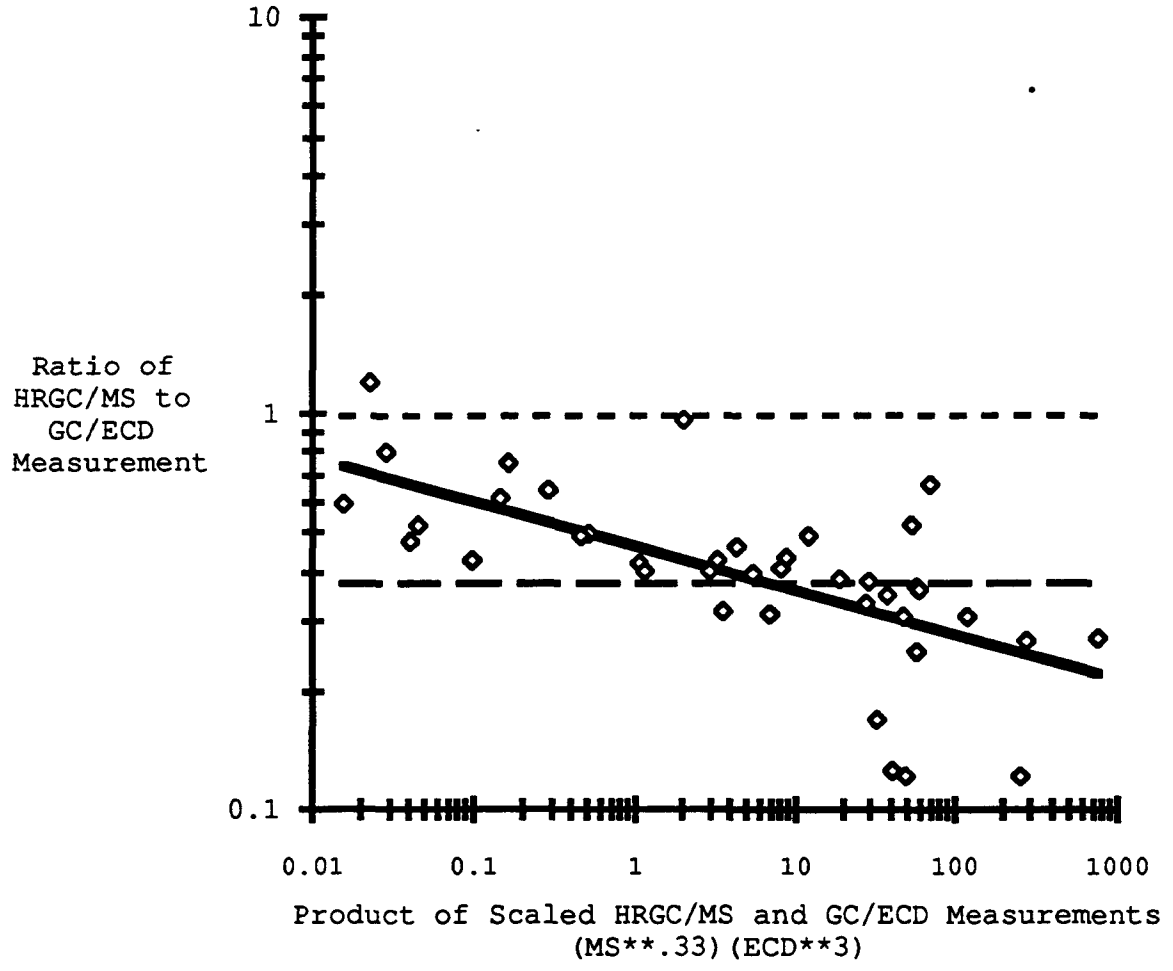

Figure 19. Transformed HRGC/MS versus GC/ECD p,p'-DDE measurements used for statistical tests.

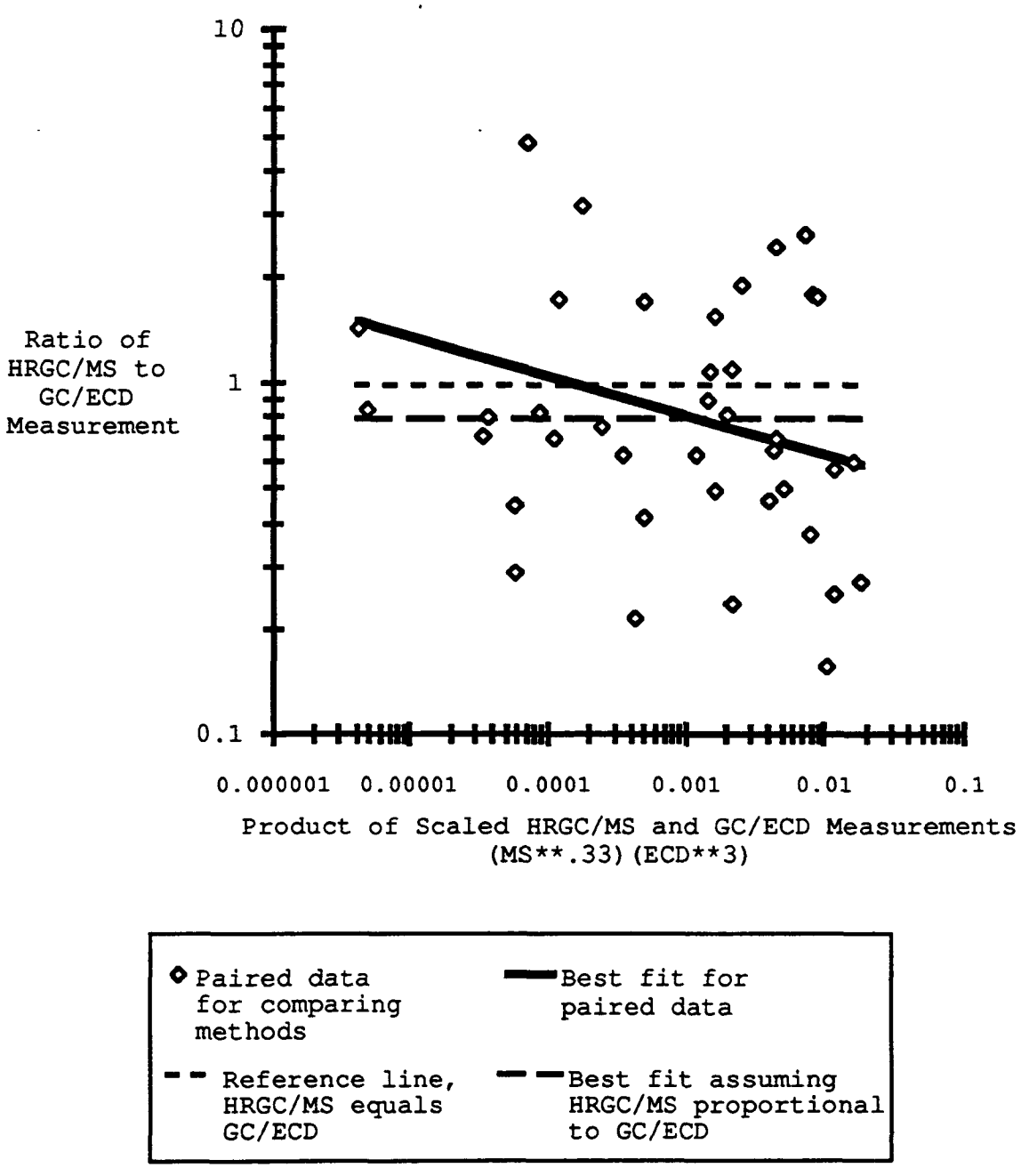

Figure 20. Transformed HRGC/MS versus GC/ECD beta-BHC measurements used for statistical tests.

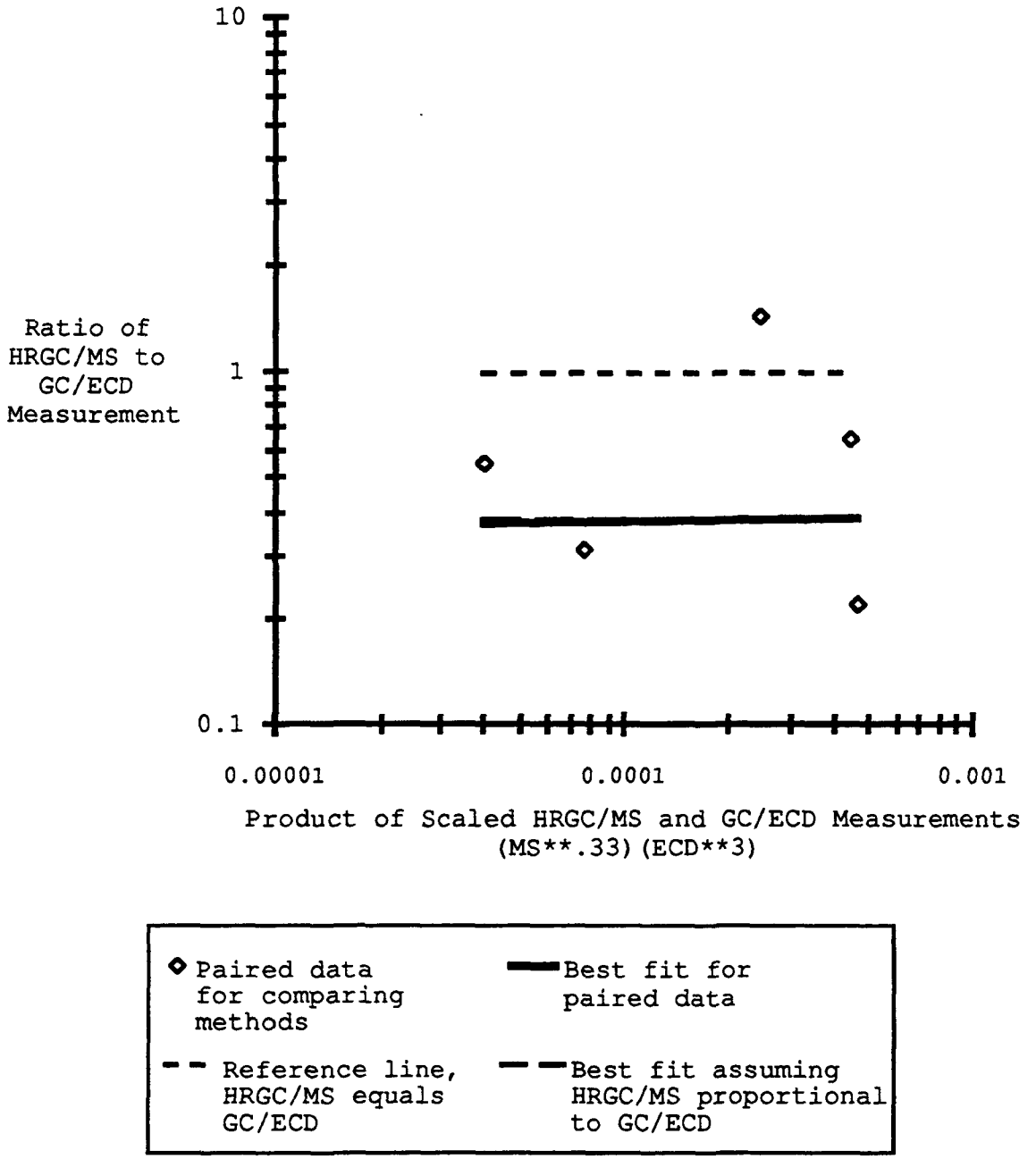

Figure 21. Transformed HRGC/MS versus GC/ECD dieldrin measurements used for statistical tests.

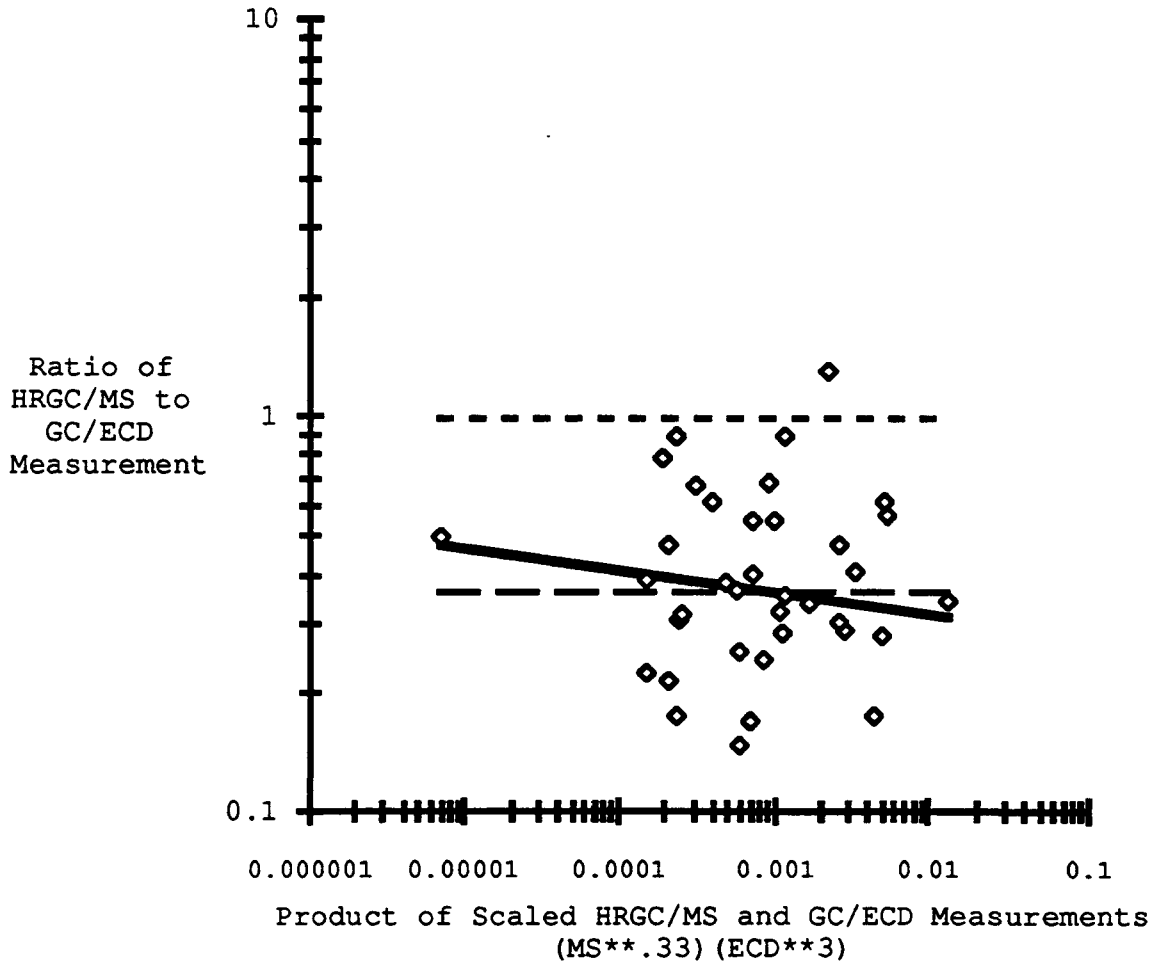

Figure 22. Transformed HRGC/MS versus GC/ECD heptachlor epoxide measurements used for statistical tests.

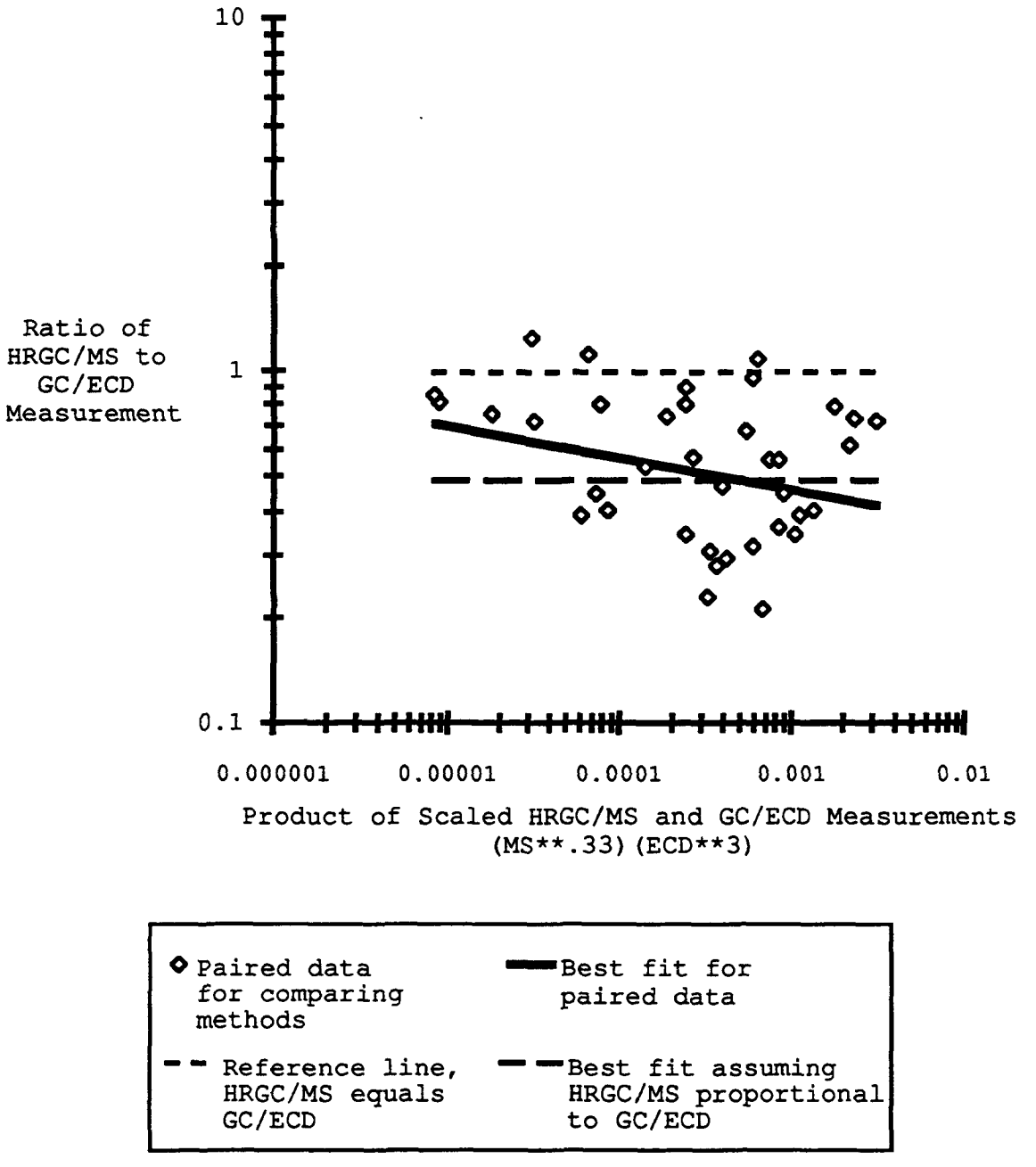

Figure 23. Transformed HRGC/MS versus GC/ECD oxychlordane measurements used for statistical tests.

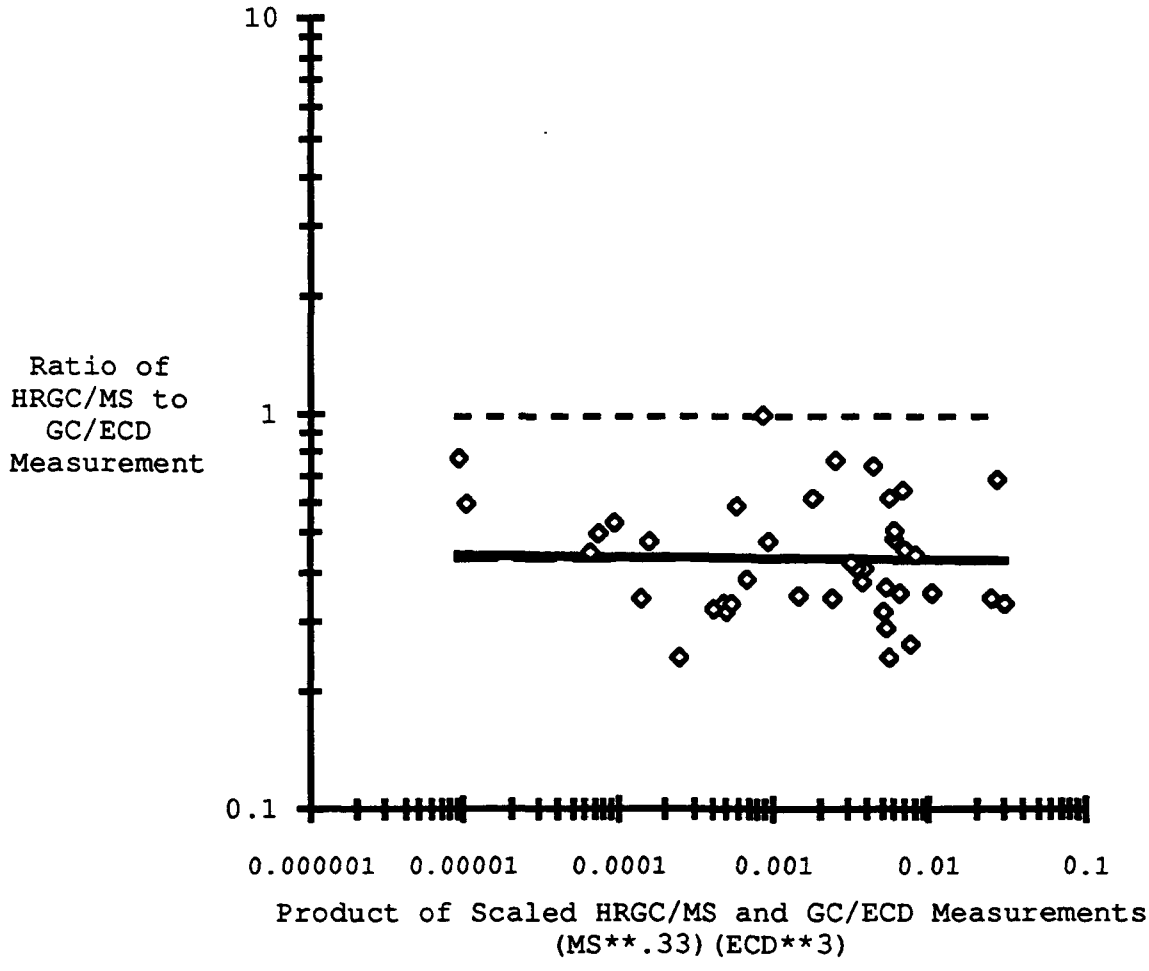

Figure 24. Transformed HRGC/MS versus GC/ECD trans-nonachlor measurements used for statistical tests.

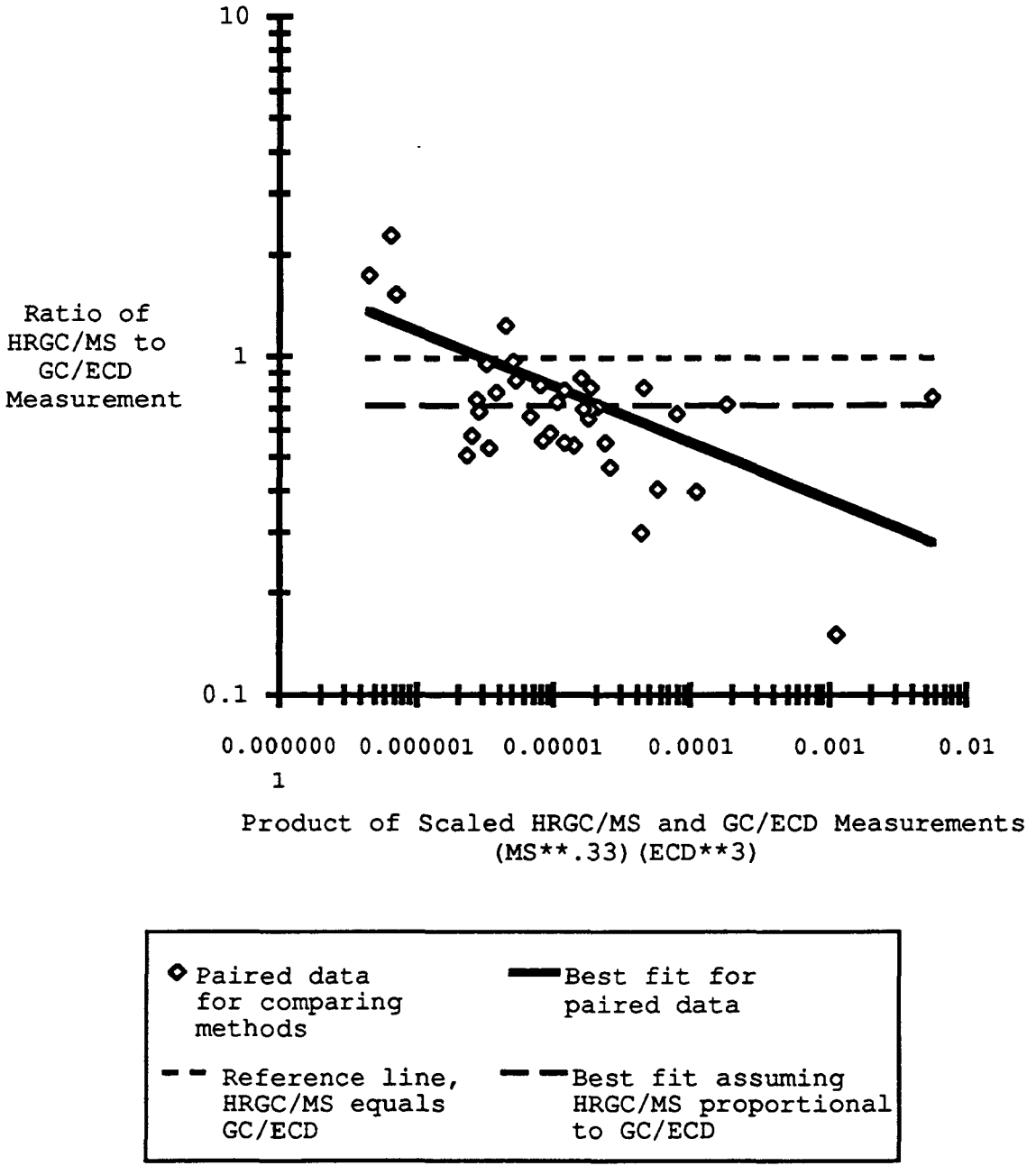

Figure 25. Transformed HRGC/MS versus GC/ECD uncorrected hexachlorobenzene measurements used for statistical tests.

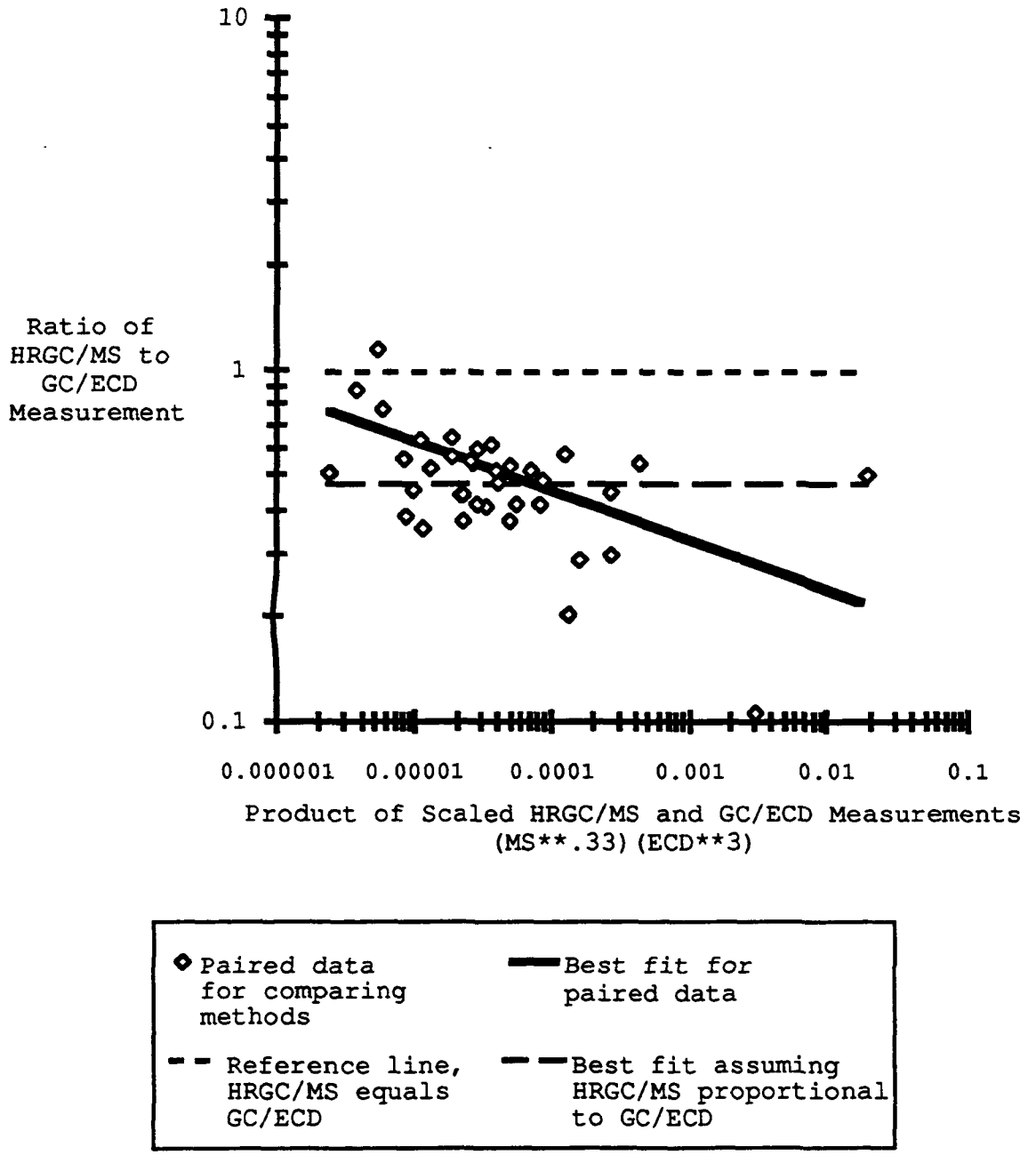

Figure 26. Transformed HRGC/MS versus GC/ECD corrected hexachlorobenzene for recovery measurements used for statistical tests.

8 COMPARISON OF GC/ECD AND HRGC/MS MEASUREMENTS ACROSS YEARS

One objective of the Comparability Study is to assess whether the relationship between the HRGC/MS and GC/ECD measurements in 1984 is useful for comparing trends in concentrations measured by different methods in different years. The analysis in this chapter uses the results from the comparability analysis to adjust the 1982 HRGC/MS measurements for comparison to the GC/ECD measurements using plots of the data.

An important consideration in the decision as to whether measurements from different years and methods can be compared is the extent to which any relationship between the GC/ECD and HRGC/MS results from 1984 might apply to other years. The statistical procedures discussed in Chapter 7 can be used to find the "best" relationship to predict the 1984 HRGC/MS measurements from the 1984 GC/ECD measurements. However, this relationship may not be best for data collected in another year, data analyzed by another laboratory, or for samples with different concentration levels than those for which the equation was developed. These issues are discussed at the end of this chapter.

8.1 Plots of Measurements Over Time

Figures 27 through 34 show the arithmetic average GC/ECD and HRGC/MS measurements for the NHATS design samples[15] from 1970 through 1984. The GC/ECD method was used in 1970 through 1981 and in 1983 and 1984. The HRGC/MS method was used in 1982 and 1984. For these two years, both the HRGC/MS average and the adjusted HRGC/MS average are shown. The averages in the figures are weighted by age group and therefore approximate national averages.

[15]The design samples are those samples which are within the quota for the hospital. Some hospitals collect more samples than required under their quota.

The adjusted HRGC/MS average approximates the arithmetic average concentration which would have been obtained from the GC/ECD method. It was calculated by multiplying the HRGC/MS average for a compound by the corresponding geometric mean ratio of the GC/ECD to HRGC/MS measurements in Table 12, referred to here as the adjustment ratio. The calculation of the adjusted HRGC/MS average assumes that the ratio of the GC/ECD to HRGC/MS measurements observed in the FY84 samples is constant across years. The adjusted HRGC/MS average for 1982 can be compared to the trend in the GC/ECD data in the years around 1982 to assess if this procedure for adjusting the HRGC/MS data provides a reasonable approximation to the averages made using the GC/ECD method.

In calculating the average for the GC/ECD method, zero was used for nondetect measurements and the approximate LOQ[16] was used for trace measurements. For the HRGC/MS average, the value LOD/2 was used for the nondetect measurements and the measured amount for trace measurements. Differences in how the trace and nondetect measurements were handled result in only small differences between the GC/ECD and adjusted HRGC/MS averages. The adjusted HRGC/MS averages for 1984 and corresponding GC/ECD averages for 1984 are not exactly equal due to a combination of 1) different procedures for handling nondetect and trace measurements, 2) exclusion from the averages of specimens from one hospital[17], 3) inclusion of measurements from the unpaired samples, and 4) use of only paired samples to calculate the adjustment ratio. Note that for dieldrin, only five paired measurements were used to calculate the adjustment ratio. However, the GC/ECD average for dieldrin is based on 42 measurements and the HRGC/MS average is based on 11 measurements.

---

[16]The procedures for recording the data have changed slightly over time. The majority of trace measurements use the LOQ. Of the remaining cases, some use the LOQ/2, some use a value higher than the LOQ.

[17]During the course of the Comparability Study, multiple specimens from one hospital were suspected of coming from the same donor. It was decided to exclude specimens from this hospital from the comparisons across years. For the years in which composite samples were analyzed, 4 composite samples were excluded from each of the 1982 and 1984 averages.

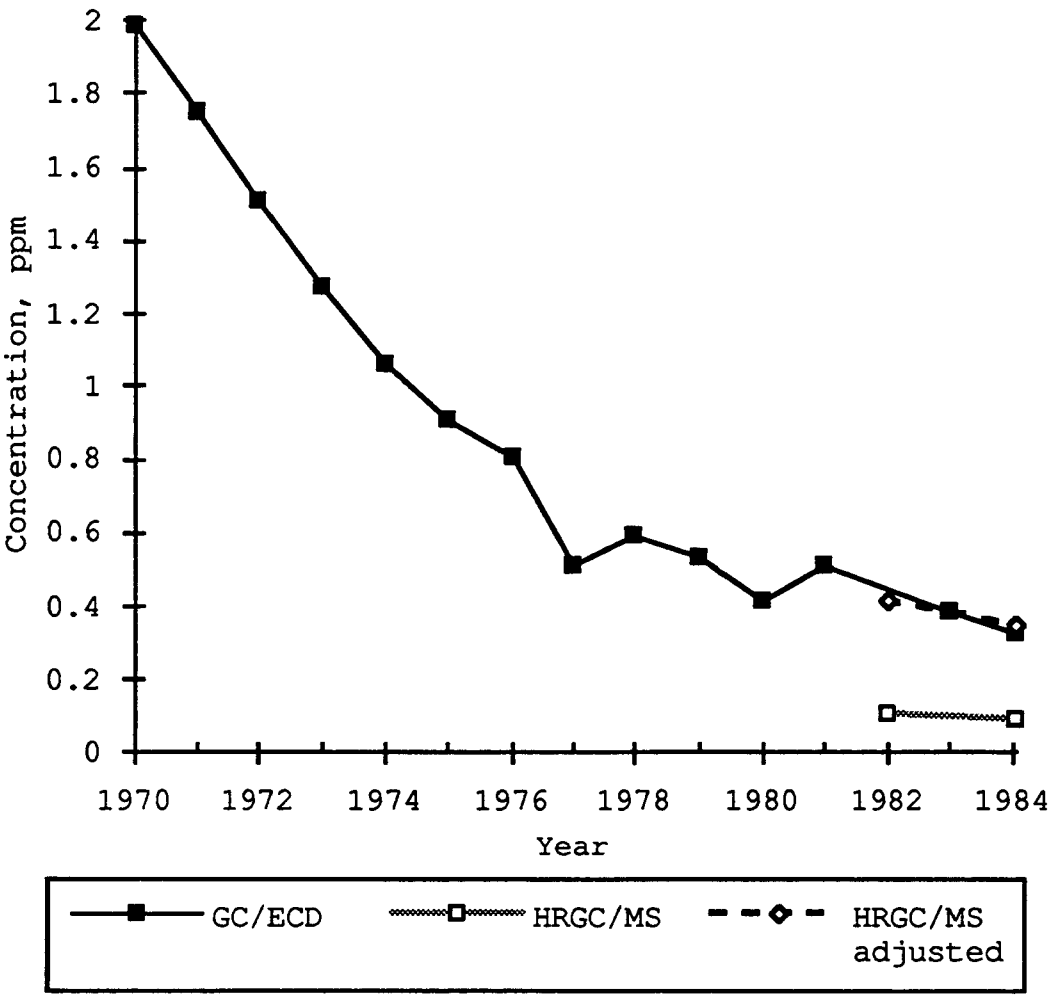

Figure 27. Weighted average GC/ECD, HRGC/MS, and HRGC/MS adjusted p,p'-DDT concentrations for design samples from 1970 through 1984.

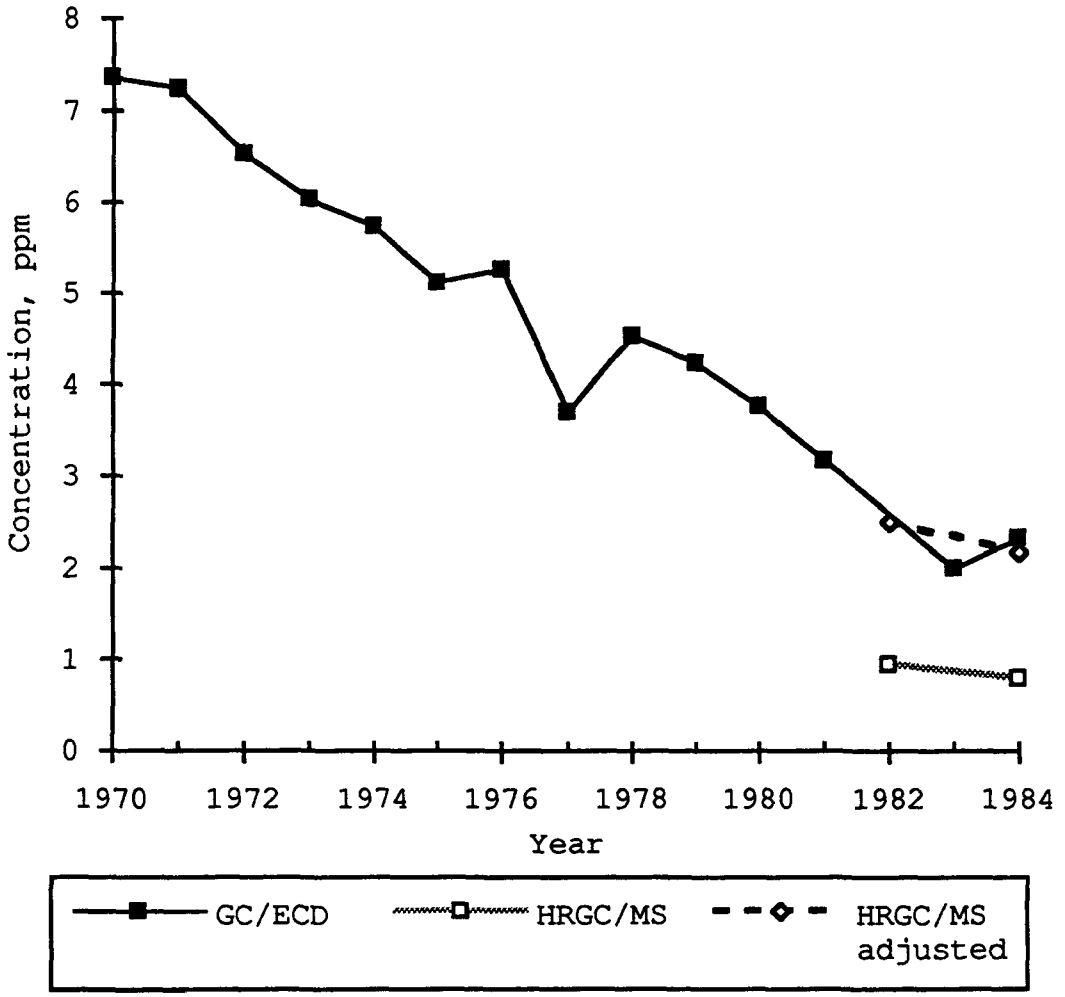

Figure 28. Weighted average GC/ECD, HRGC/MS, and HRGC/MS adjusted p,p'-DDE concentrations for design samples from 1970 through 1984.

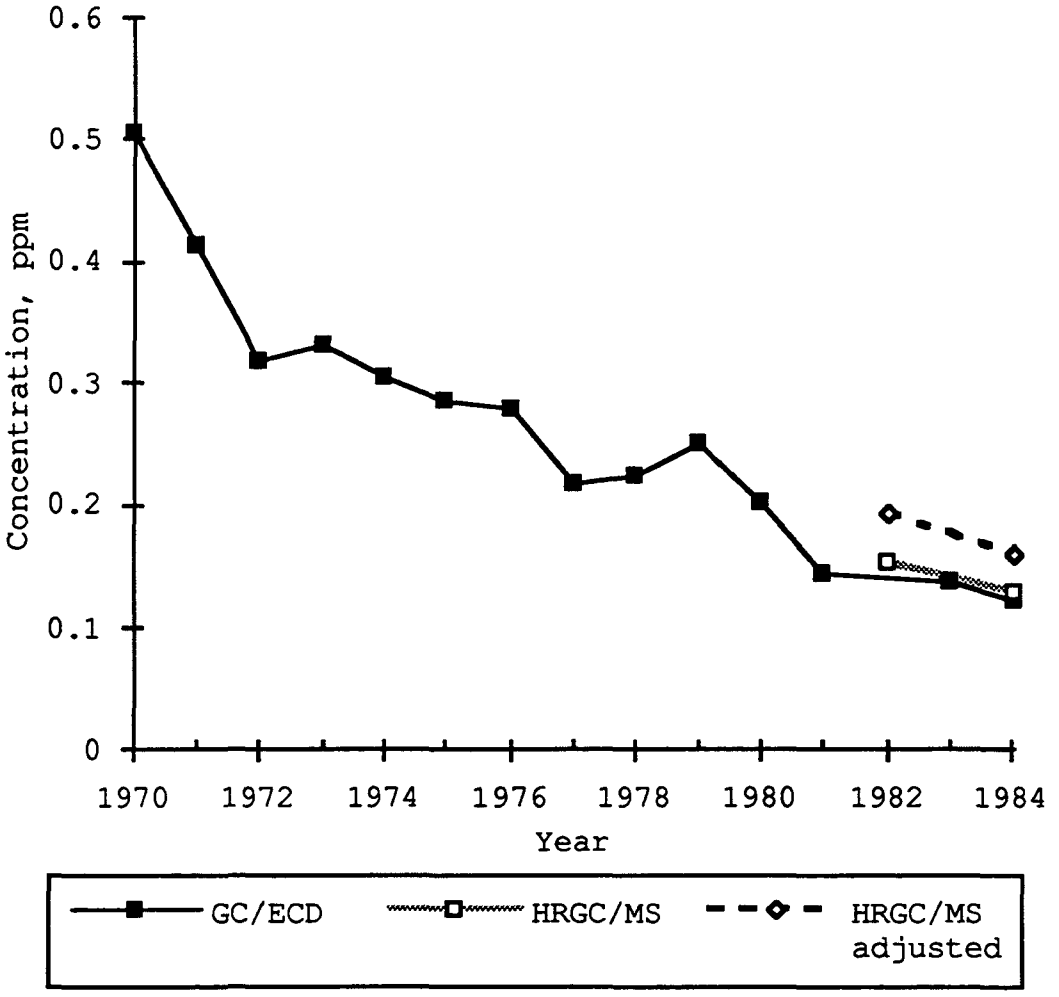

Figure 29. Weighted average GC/ECD, HRGC/MS, and HRGC/MS adjusted beta-BHC concentrations for design samples from 1970 through 1984.

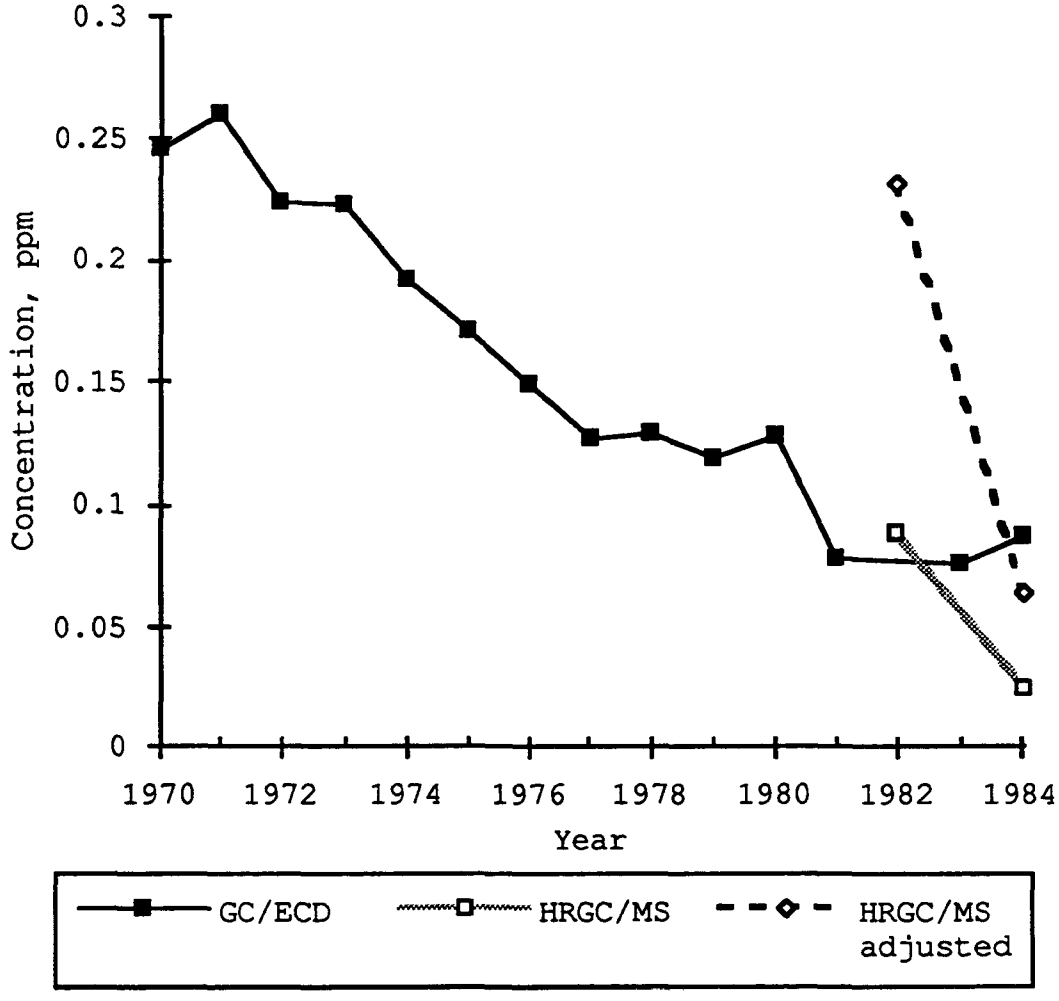

Figure 30. Weighted average GC/ECD, HRGC/MS, and HRGC/MS adjusted dieldrin concentrations for design samples from 1970 through 1984.

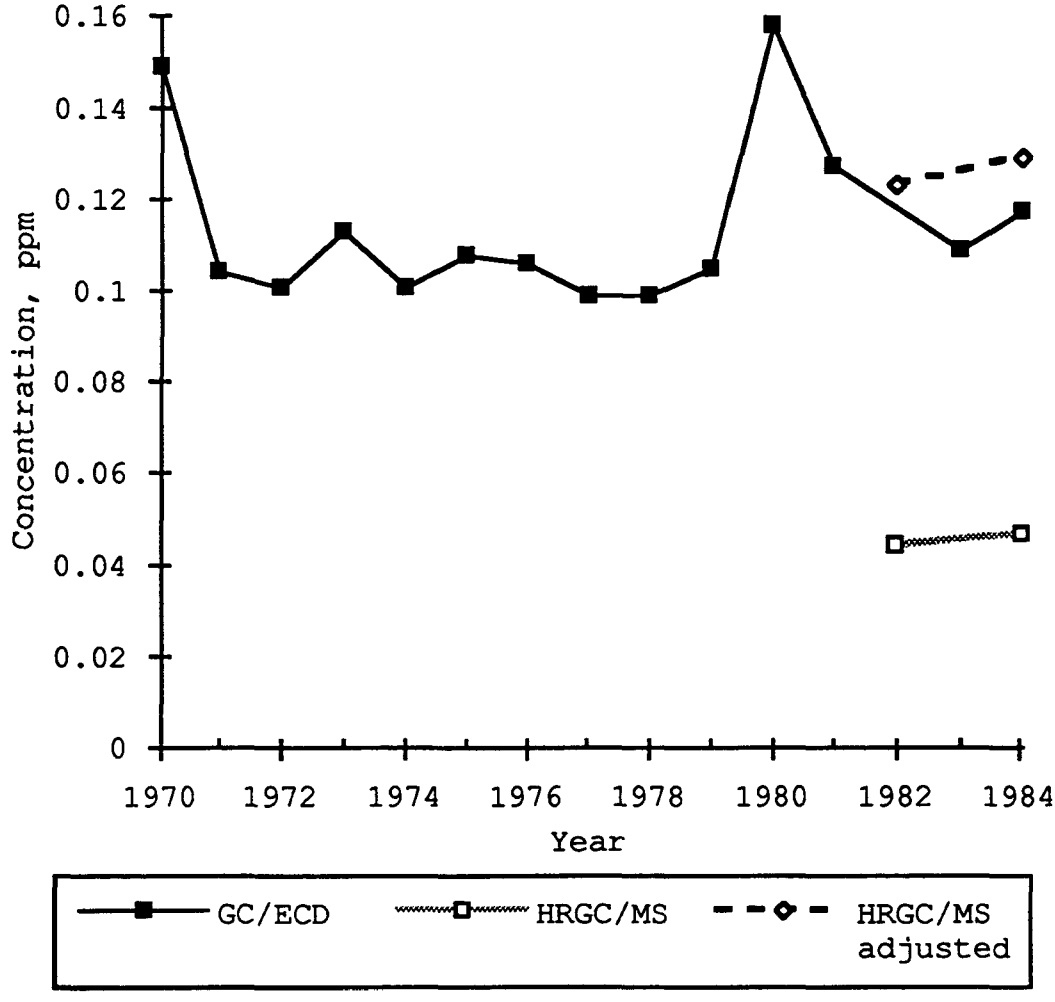

Figure 31. Weighted average GC/ECD, HRGC/MS, and HRGC/MS adjusted heptachlor epoxide concentrations for design samples from 1970 through 1984.

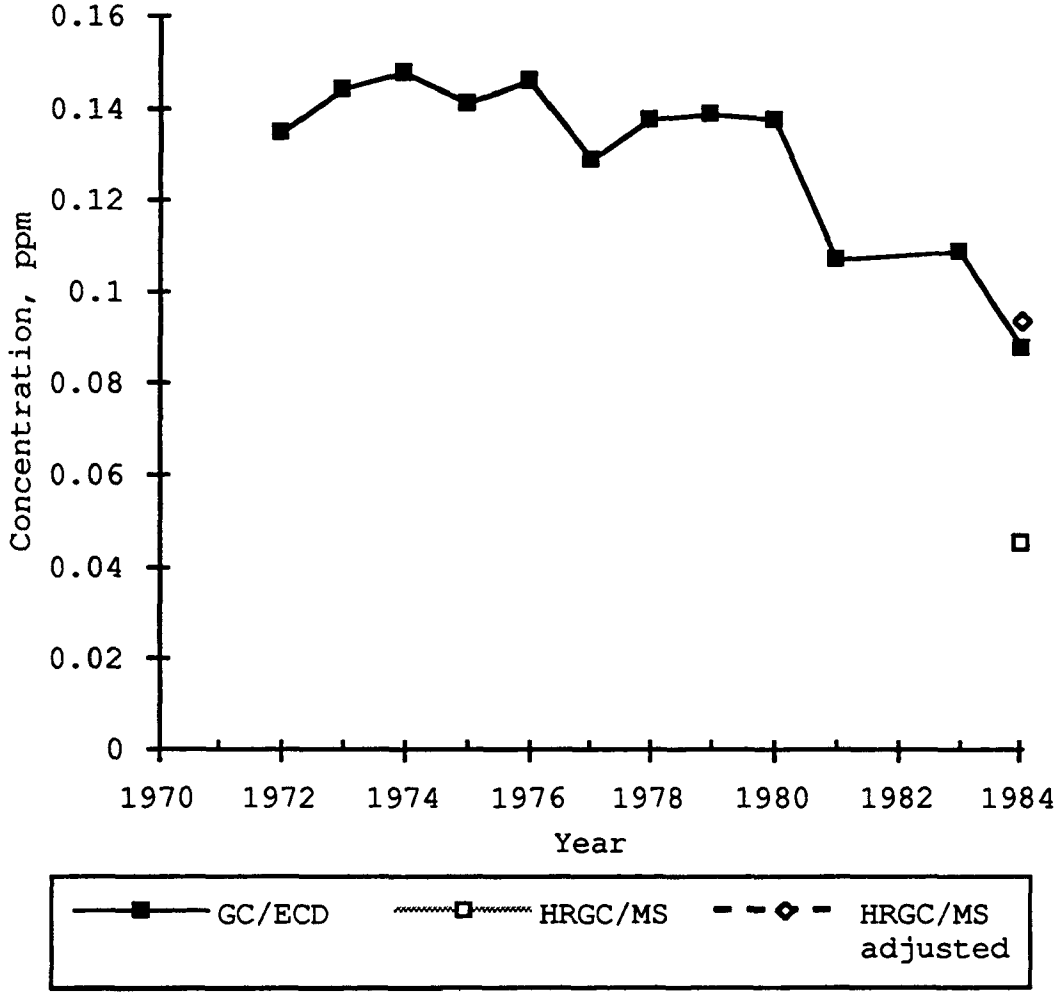

Figure 32. Weighted average GC/ECD, HRGC/MS, and HRGC/MS adjusted oxychlordane concentrations for design samples from 1972 through 1984.

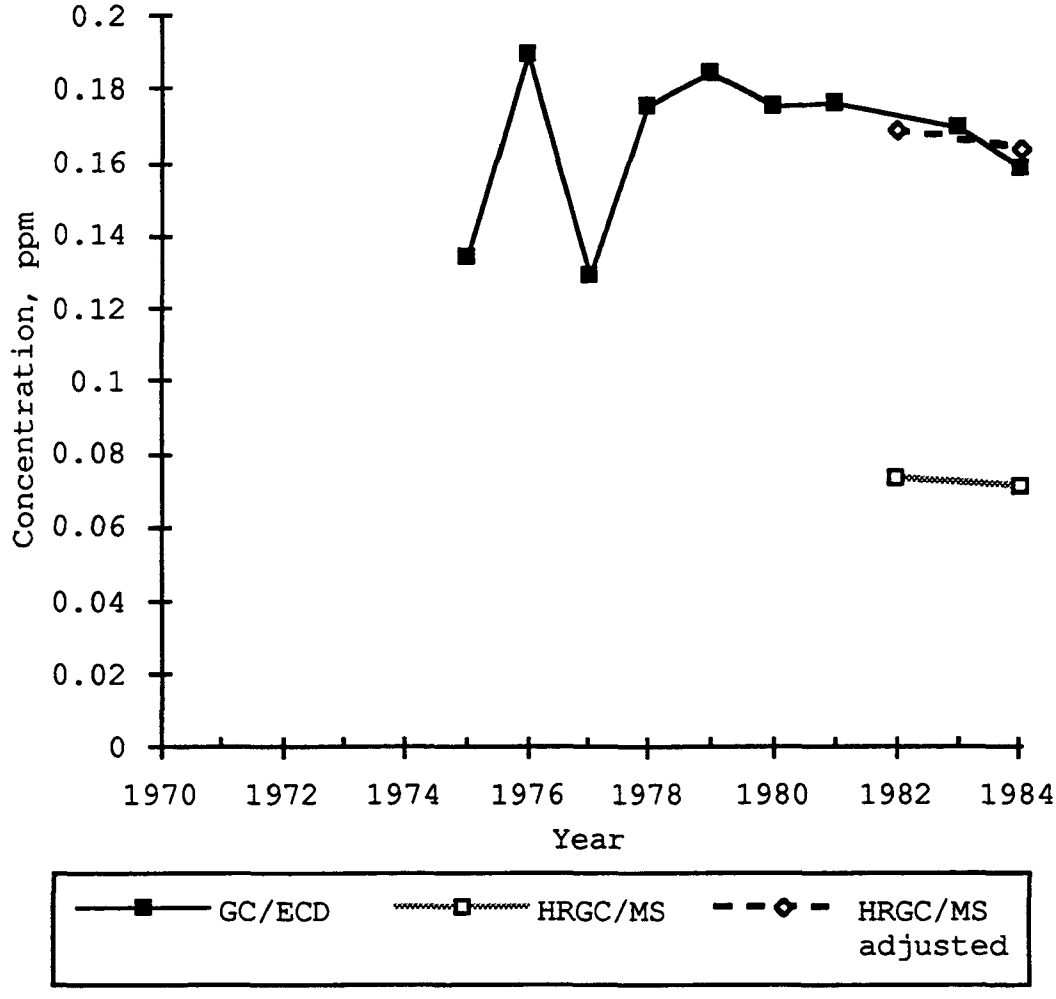

Figure 33. Weighted average GC/ECD, HRGC/MS, and HRGC/MS adjusted trans-nonachlor concentrations for design samples from 1975 through 1984.

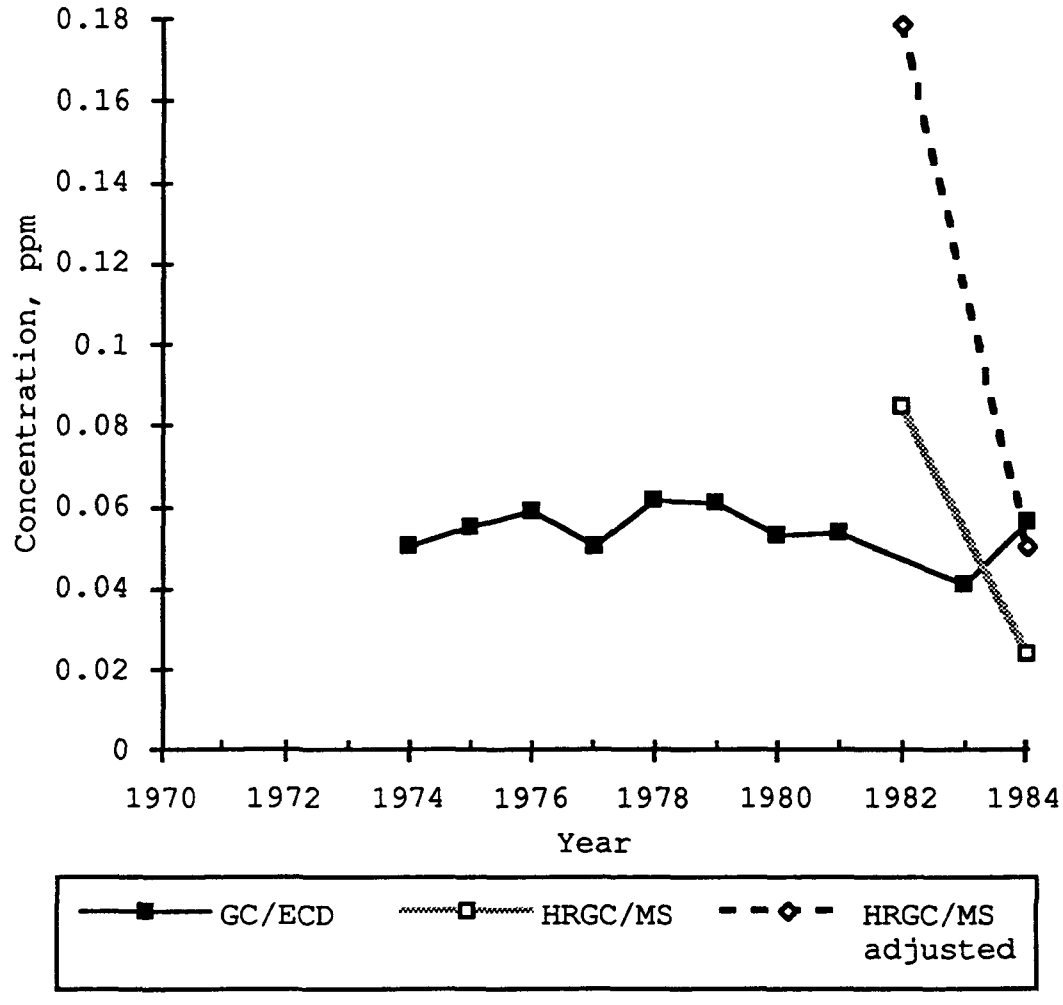

Figure 34. Weighted average GC/ECD, HRGC/MS, and HRGC/MS adjusted corrected hexachlorobenzene concentrations for design samples from 1974 through 1984.

8.2 Assessment of the Results

As can be seen from Figures 27 through 34, the 1982 adjusted HRGC/MS average is closer to the trend in the GC/ECD averages than is the unadjusted average for four compounds, p,p'-DDT, p,p'-DDE, heptachlor epoxide, and trans-nonachlor. Although the unadjusted beta-BHC HRGC/MS average approximates the trend in the GC/ECD averages more closely than the adjusted value, both the adjusted and unadjusted beta-BHC values are close to the GC/ECD trend. For oxychlordane there were no 1982 measurements for comparison.

For two compounds, dieldrin and corrected hexachlorobenzene, the adjusted 1982 averages are considerably higher than the neighboring GC/ECD averages. The 1982 hexachlorobenzene measurements are known to have two very high observations, which might be considered to be outliers. These values contribute to both the high average concentration and to a high standard error of the average. The difference between the HRGC/MS adjusted average and the GC/ECD trend may be due to the presence of the outliers. With the two outliers removed, the adjusted HRGC/MS average reasonably follows the GC/ECD trend, as can be seen in Figure 35.

For dieldrin, the adjusted HRGC/MS average does not agree well with the trend in the GC/ECD averages; however, the differences may be explained by variation in the estimates of the HRGC/MS average and the adjustment ratio. The adjustment ratio from Table 12 has a wide 95% confidence interval due to the small number of paired measurements in 1984. In addition, the estimate of the 1984 HRGC/MS average is imprecise due to the small number of fraction 2 extracts analyzed using the HRGC/MS method. Although the adjustment ratio results in poor agreement between the HRGC/MS adjusted averages and the GC/ECD trend, there are values within the 95% confidence intervals that would provide good agreement. Additional paired samples would be needed to provide a more precise ratio estimate with which to evaluate the adjustment ratio for dieldrin.

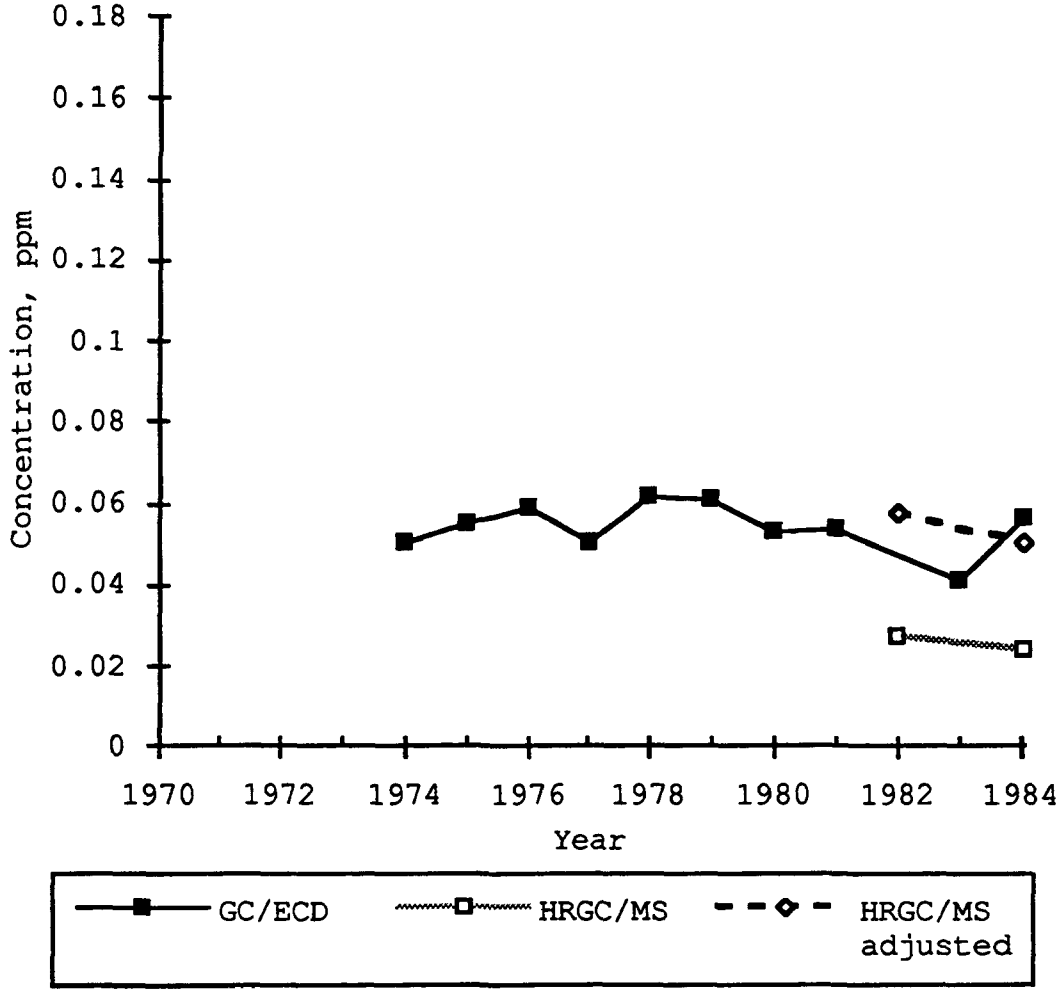

Figure 35. Weighted average GC/ECD, HRGC/MS, and HRGC/MS adjusted corrected hexachlorobenzene concentrations for design samples from 1970 through 1984, with two outliers removed from the calculation of the 1982 HRGC/MS and HRGC/MS adjusted average.

For all compounds tested, given the likely errors in estimation of the yearly averages and the adjustment ratios, the procedure of using the adjustment ratio from the 1984 data (Table 12) and the average HRGC/MS concentration from 1982 to approximate the 1982 GC/ECD averages cannot be rejected based on the data. For five compounds, the adjusted HRGC/MS average was close to the GC/ECD trend. For one other compound, the adjusted HRGC/MS average was close to the GC/ECD trend after removing two outliers from the HRGC/MS data. For one additional compound, the data and the ratio estimates were too variable to attribute the observed differences in the GC/ECD and HRGC/MS adjusted averages to the adjustment procedure. For one compound, oxychlordane, no 1982 measurements are available for comparison.

Extending this procedure to other years requires making the assumption that the ratio of the GC/ECD measurements to the HRGC/MS measurements is constant for all years. The relationship between the GC/ECD and HRGC/MS measurements might be different than in 1984 if the data were from another year, analyzed by another laboratory, or for samples with different concentration levels than those from which the relationship was developed. For example, for the 1984 data, statistically significant differences between batches were found for four compounds: p,p'-DDE, beta-BHC, oxychlordane, and trans-nonachlor. If differences in the sample processing among batches within a year can be significant, there may also be differences in the sample processing between years which would affect the ratio of the HRGC/MS to GC/ECD measurements.

The comparability results for p,p'-DDE, beta-BHC, and hexachlorobenzene suggest that in some circumstances the recovery may not be constant and independent of concentration. In this situation, the ratio correction factor will depend on concentration. Extrapolation to other concentration levels may provide quite inaccurate results when the concentrations substantially change from those observed in 1984.

An alternate procedure for comparing the HRGC/MS and GC/ECD data is to correct all measurements for recovery. This would require good estimates of recovery. Measurements on different sample matrices provide different estimates of recovery. Therefore, care must be taken in selecting the samples on which the recovery estimates are based. The recovery correction can be done on a sample by sample basis, perhaps based on surrogate compounds, or by adjusting all concentrations using a common recovery value. If the same recovery ratio is used to adjust all measurements for a selected compound, an error in the recovery ratio will result in a similar error in the average or median. The results from this Comparability Study can be used to determine the number of samples required to achieve a desired precision in the estimated recoveries and the corresponding averages.

9 PCB MEASUREMENTS

This chapter summarizes the PCB measurements obtained using the HRGC/MS and GC/ECD methods. The PCB recovery measurements are discussed first, followed by a comparison of the PCB measurements on paired NHATS samples using the HRGC/MS and GC/ECD methods. Unlike the primary compounds discussed in Chapter 8, the procedures for reporting PCBs using the HRGC/MS and GC/ECD methods were quite different. Therefore, although the concentration levels can be compared, it is not possible to determine a mathematical relationship between the GC/ECD and HRGC/MS measurements.

9.1 Comparison of PCB Reporting and Measurement Procedures

PCB reporting procedures for the two analytical methods differed considerably. In the GC/ECD method, the limit of quantification for PCBs was 1 ug/g and the PCB concentrations, on a wet weight basis, were reported on the following interval scale:

V = Not detected;

W = Detected, with a concentration between the LOD and the LOQ, i.e., between .33 and 1 ug/g (Equivalent to a trace measurement);

Y = Detected, with a concentration of 1 to 3 ug/g; and

Z = Detected with a concentration > 3 ug/g.

The porcine adipose tissue samples in batches 4 through 10, used for quality control in the GC/ECD method, had known PCB concentrations of 1 ug/g. Only for these GC/ECD samples were the PCB concentrations reported in both the interval categories listed above and in ug/g wet weight[18].

[18]For these samples the measured concentration, rather than the limit of quantification (LOQ), was reported, even though the concentrations were below the LOQ.

Although the GC/ECD method measures PCB concentrations as if "PCB" is one compound, PCBs comprise many different compounds (or congeners). PCBs can be grouped into 10 classes called homologs. Each homolog class includes the chlorinated biphenyl compounds with the same number of chlorine atoms (from 1 to 10). In the HRGC/MS method, the PCB concentration within each homolog class was reported. The average detection limit for each homolog concentration ranged from .01 to .03 ug/g lipid weight. Thus the HRGC/MS measurements were much more sensitive than those from the GC/ECD method for which the detection limit was 0.33 ug/g wet weight.

The GC/ECD concentrations for the paired samples, reported on the interval scale, could not be converted from a wet weight basis to a lipid basis. Therefore, for the analysis in this chapter, PCB concentrations from both methods are expressed as micrograms per gram wet weight. Note that this is different than for the discussion in Chapter 7, which used concentrations on an extractable lipid basis.

Interpretation of differences between the HRGC/MS and GC/ECD measurements is complicated by differences in the reporting procedures and significant differences in the data reduction procedures for the two techniques. The GC/ECD analyses of PCB's is based on comparison of a limited number of major peaks associated with the PCB response. The HRGC/MS analysis of PCB's is generally based on the area sums of the peaks for all congeners at each level of chlorination.

9.2 PCB Recovery Using the HRGC/MS and GC/ECD Methods

Recovery measures the proportion of the PCBs in the sample which were detected by the measurement method. The PCB recovery can be estimated from measurements on samples which were spiked with PCBs. These samples include the porcine tissue samples (measured using the GC/ECD method) and the dichloromethane samples

and all samples spiked with surrogate compounds (measured with the HRGC/MS method). Because the multisplit samples were not spiked with PCBs, these samples cannot be used to estimate PCB recovery.

For the GC/ECD method, only the porcine samples in batches 4 through 10 were spiked with PCBs. These porcine samples had known PCB concentrations of 1 ug/g. The PCB recovery measurements in these seven porcine samples were, in order: 68%, 75%, 75%, 78%, 80%, 80%, and 95%. The average recovery was 78%, with a 95% confidence interval from 71% to 85%. The PCB recovery was lower than for most primary compounds measured in the same porcine samples (see Table 8).

For the HRGC/MS method, the PCB recovery can be determined from measurements on the dichloromethane spiked samples and measurements of the surrogate compounds in all samples. One spiked dichloromethane sample was analyzed with each of the 10 batches. Because the HRGC/MS method provides a measurement for each of the 10 PCB homologs, the total PCB concentration was determined by adding the measurements for the homologs. The total PCB concentration was used to calculate recovery[19]. The dichloromethane samples were spiked with equal amounts of each homolog. This even distribution of PCBs among homologs may not reflect the distribution of PCBs found in naturally occurring samples. In addition, recovery of PCBs from dichloromethane may be different than that from lipid material. The PCB recoveries for the 10 dichloromethane samples were, in order: 57%, 61%, 62%, 65%, 69%, 70%, 74%, 75%, 79%, and 80%. The average recovery was 69%, with a 95% confidence interval from 64% to 75%. This recovery is similar to or higher than that for most other compounds spiked into the dichloromethane samples (see Table 8).

[19]As can be seen in Table A-2 in the appendix, the PCB recovery in the Dichloromethane spiked samples tended to increase as the number of chlorine atoms increased, ranging from 51% to 83% recovery.

Another means of estimating the PCB recovery using the HRGC/MS method uses surrogate compounds which were added to each sample. Four stable isotope labeled surrogate compounds, representing PCB homologs with 1, 4, 8, and 10 chlorine atoms, were spiked into all HRGC/MS lipid samples at levels of 2, 5, 8, and 10 micrograms respectively. Because PCB recovery may be different in the presence of lipid, only measurements on adipose tissue samples were used to estimate PCB recovery. The recovery estimates are for the sum of the four surrogate homologs. Figure 36 shows a histogram of the PCB recovery measurements for lipid samples. The average recovery for the surrogate PCBs in the 57 adipose tissue samples was 62%, with a 95% confidence interval from 59% to 66%.

The PCB recovery estimates for the HRGC/MS and GC/ECD methods are summarized in Table 13. The average recoveries of spiked PCBs using the HRGC/MS and the GC/ECD methods ranged of 62% to 78%, with the GC/ECD recovery slightly higher than the HRGC/MS recovery.

9.3 Comparison of HRGC/MS and GC/ECD Paired Measurements

In order to compare the HRGC/MS and GC/ECD measurements, the total HRGC/MS PCB concentration across all homologs was calculated. Figure 37 shows a histogram of the HRGC/MS PCB concentrations for paired samples. The corresponding GC/ECD coded concentrations are shown using shading.

As can be seen from Figure 37, the HRGC/MS measurements are skewed, with many low measurements and a few high measurements. The sample with the largest measurement had a concentration of 1.29 ug/g, almost twice the next highest concentration in a paired sample. The GC/ECD concentration for this sample was between 1.0 and 3.0 ug/g, in agreement with the HRGC/MS measurement. The remaining GC/ECD measurements were between .33 and 1.0 ug/g and the corresponding HRGC/MS measurements ranged from nondetect to 0.67 ug/g, with an average of 0.28 ug/g and a standard deviation

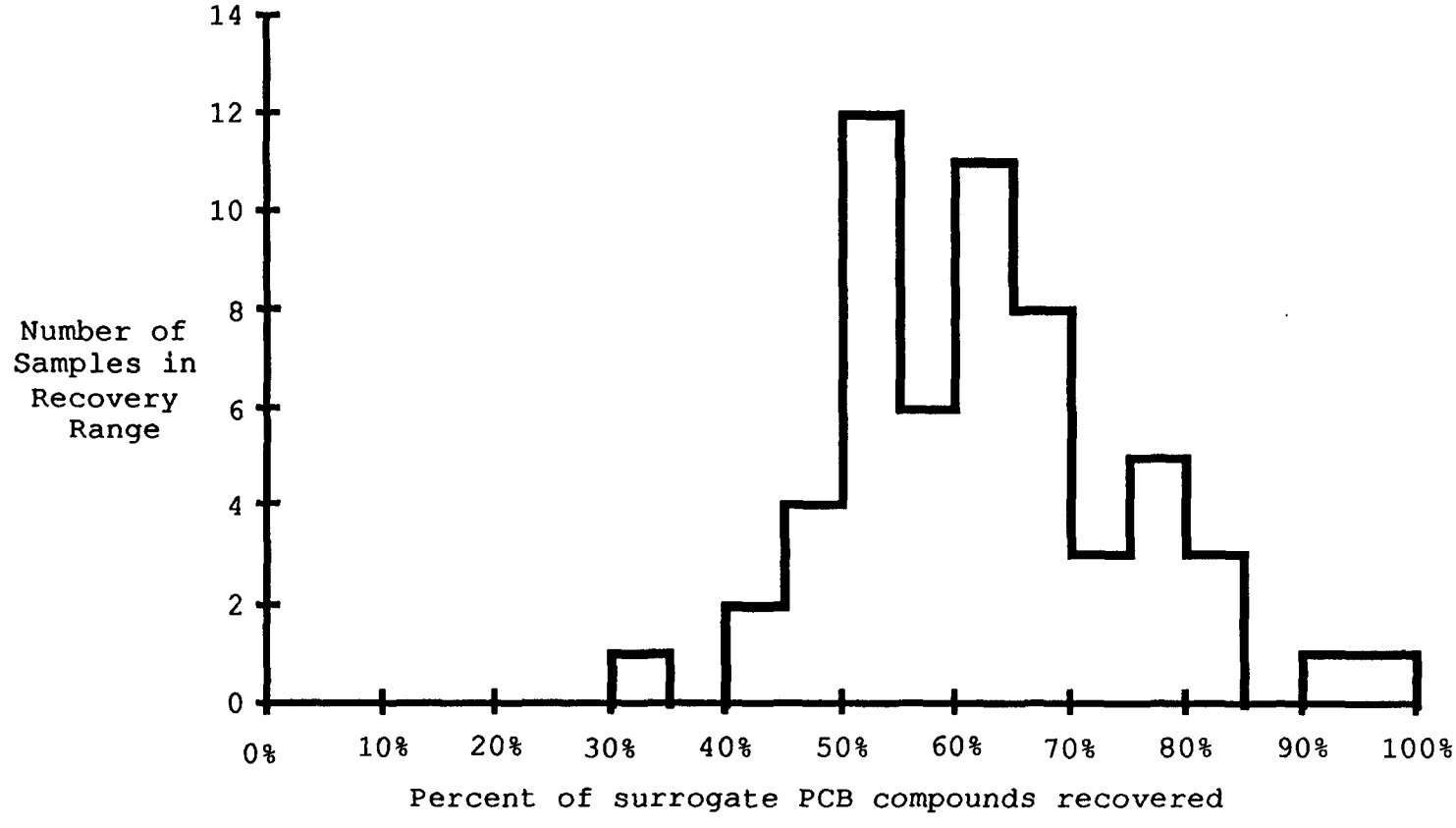

Figure 36. Histogram of HRGC/MS surrogate compound recoveries in lipid samples.

Table 13. Summary of PCB Recovery Measurements

Analytical method	Sample type	Mean recovery	95% Confidence interval
GC/ECD	Porcine samples	78%	71% to 85%
HRGC/MS	Dichloromethane samples	69%	64% to 75%
	Surrogate compounds	62%	59% to 66%

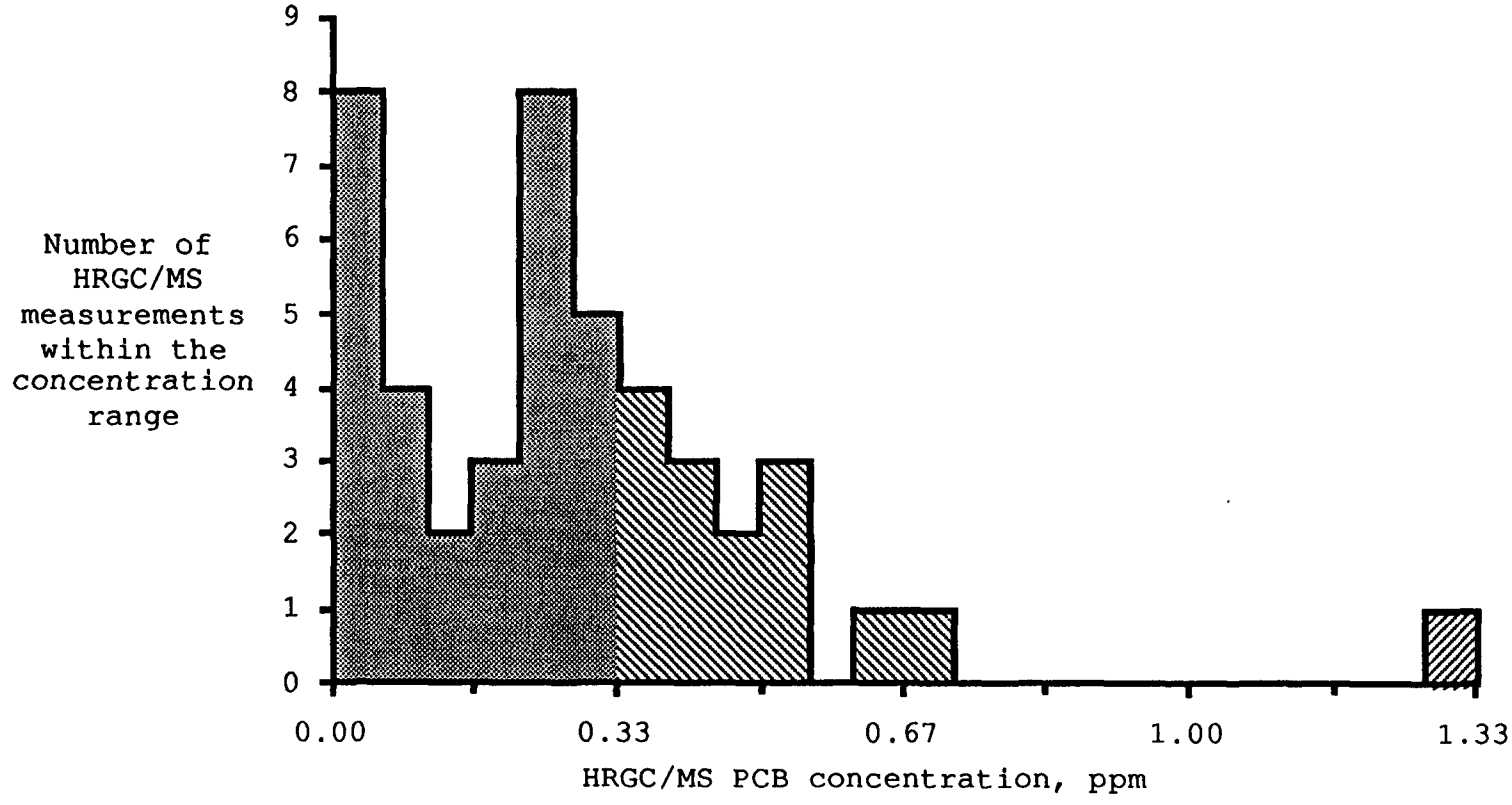

Figure 37. Histogram of HRGC/MS PCB measurements with shading to indicate coded GC/ECD concentration in paired samples.

of 0.24 ug/g. Two-thirds of the HRGC/MS measurements were below 0.33 ug/g, indicating that concentrations measured using the HRGC/MS method are generally below those from the GC/ECD method.

A cross tabulation of the GC/ECD and HRGC/MS data in paired samples, coded using the same interval categories as the GC/ECD data, is shown in Table 14. The GC/ECD and HRGC/MS measurements are in the same concentrations intervals for 15 samples. In the remaining 30 paired samples, the GC/ECD measurements are in the range from 0.33 to 1.0 ug/g and the HRGC/MS measurements are lower, less than 0.33 ug/g.

The PCB measurements on the multisplit samples can also be used to compare the HRGC/MS and GC/ECD methods. Because the spiking solution contained no PCBs, the PCB measurements on all five multisplit samples for each of three composites were comparable. For these samples, all GC/ECD measurements were within the 0.33 to 1.0 ug/g range[20]. Table 15 shows the number of multisplit samples with HRGC/MS measurements either below, in the same range as or above the GC/ECD measurement. As can be seen from the table, the HRGC/MS measurements are either below or similar to the GC/ECD measurements. No HRGC/MS measurements fall into a concentration category greater than that for the paired GC/ECD measurement.

The data do not allow a determination of the ratio of the HRGC/MS to the GC/ECD measurements due to the interval nature of the reported data and the small range of the reported concentrations. However, the results for the paired and multisplit samples supports the conclusion that the ratio of the GC/ECD to HRGC/MS measurements is greater than 1.0.

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[20]For one unspiked multisplit sample, one of two extract PCB measurements had a coded concentration between 1.0 and 3.0 ug/g, higher than the other two measurements on the same extract. This measurement was ignored for this analysis. Its inclusion would not change the result that the HRGC/MS measurements are less than or similar to the GC/ECD measurements.

Table 14. Coded HRGC/MS versus Coded GC/ECD PCB Measurements in Paired Samples

Coded HRGC/MS measurements	Coded GC/ECD measurements		
	Not Detected	.33 to 1 ug/g	1 to 3 ug/g
Not Detected or less than 0.33	0	30	0
.33 to 1 ug/g	0	14	0
1 to 3 ug/g	0	0	1

Table 15. Comparison of HRGC/MS and GC/ECD Measurements in Multisplit Samples

Composite[a]	Number of HRGC/MS measurements		
	HRGC/MS lower than GC/ECD	HRGC/MS Similar to GC/ECD	HRGC/MS greater than GC/ECD
A	5	0	0
B	1	4	0
C	1[b]	3	0

Note. All GC/ECD samples had coded PCB measurements between 0.33 and 1.0 ug/g.

[a]Other than distinguishing composites from which the multisplit samples were prepared, the composite identifier has no meaning.

[b]In one sample, five homologs could not be measured. Because the HRGC/MS concentration based on the remaining five homologs may be significantly lower than the actual PCB concentration, it has been left out of the total for this cell.

10 ANALYSIS OF PRECISION AND COMPONENTS OF VARIANCE

Precision refers to the variability of the measurements. The variability may be measured in terms of the variance, standard deviation, or coefficient of variation. An analysis of precision attempts to quantify the variability of the measurements and factors which affect that variability. The precision of the measurements may be a function of concentration, the compound being measured, and/or factors associated with the sample processing steps, such as the batch in which a sample is analyzed.

The model in Chapter 5 was used as a basis for the analysis. The variance components are estimated using only the positively quantified measurements, unless otherwise indicated. Thus samples with trace measurements and measurements below the detection limit are not included in the analysis of variance components. Although the decision to use only the positively quantified measurements and the rounding of the reported GC/ECD concentrations will have some affect on the variance estimates, the importance of these factors is expected to be small.

10.1 Standard Deviation Versus Mean

Experience has shown that, for concentration data which cannot be negative, the measurement error increases with the size of the measurement. If the data have a lognormal distribution, as assumed in Chapter 5, the standard deviation of the data will be linearly related to the concentration as:

$$\sigma = K\ C \qquad (10.1)$$

where:

σ = the standard deviation of the measurements;

K = the proportionality constant; and

C = concentration being measured.

For measurements on a group of samples with the same expected concentration, equation (10.1) can be approximated by:

$$s_j = K\,\bar{x}_j \qquad (10.2)$$

where:

s_j = the standard deviation of the measurements in group j;

$\bar{x}_j$ = the average of the measurements in group j.

Taking the log of both sides of equation (10.2) gives:

$$\ln(s_j) = \ln(K) + \ln(\bar{x}_j) \qquad (10.3)$$

The variance of $\ln(s_j)$ is roughly inversely proportional to the degrees of freedom. Therefore, the following equation can be fit using weighted regression, with the weights equal to the degrees of freedom:

$$\ln(s_j) = \alpha + \gamma \ln(\bar{x}_j) + \varepsilon. \qquad (10.4)$$

For each compound, the slope, γ, can be tested to determine if equation (10.3) fits the data. The assumption that the data can be described by a lognormal distribution is consistent with the data if the confidence interval for γ includes 1.0. An assumption that the measurements have a constant variance is consistent with the data if the confidence interval for γ includes zero.

Data from the spiked multisplit and paired samples were used to estimate the slope in equation (10.4). These analyses are described in the following sections.

10.1.1 Spiked multisplit samples

Four spiked multisplit samples were prepared at each of three spiking levels. The mean and standard deviation of measurements for the three spiking levels were used to estimate the parameters in equation (10.4). The mean and standard deviation were calculated using the following formulas:

$$\text{Mean} = \bar{x}_j = \frac{\sum_{i=1}^{n_j} x_{ij}}{n_j} \qquad (10.5)$$

$$\text{Standard Deviation} = s_j = \sqrt{\frac{\sum_{i=1}^{n_j} (x_{ij}-\bar{x}_j)^2}{n_j-1}} \qquad (10.6)$$

where:

j = the spike level, 1 = lowest spike level, 3 = highest spike level.

n_j = the number of split samples at the j^{th} spike level, in this case $n_j = 4$ for all spike levels unless some measurements are missing; and

x_{ij} = the measured concentration for the i^{th} sample, $i = 1$ to 4, for samples with the j^{th} spike level.

The estimates of standard deviation, s_j, each have three degrees of freedom and thus are not very accurate. With only three spiking levels, there is only one degree of freedom for calculating confidence intervals for γ. Under this condition, a precise estimate of γ is obtained only if the spacing between spiking levels is large. Equation (10.4) was fit to the spiked

multisplit data using all primary compounds except PCBs.[21] The results are presented in Section 10.1.3.

10.1.2 Paired samples

The procedure used for the multisplit samples was modified for the paired samples because there are no paired samples with the same expected concentration. Instead, the residuals and predicted values from regression were used to estimate the mean concentrations and standard deviations for application of equation (10.4).

The analysis assumed that the relationship between the GC/ECD and HRGC/MS measurements followed the simple regression model:

$$X_{Mcs} = A_c + B_c * X_{Ecs} + \text{error} \qquad (10.7)$$

where:

X_{Mcs} = measurement for compound c in sample s using the HRGC/MS method;

X_{Ecs} = measurement for compound c in sample s using the GC/ECD method;

A_c = intercept for the linear relationship between measurement methods for measurements on compound c; and

B_c = slope for the linear relationship between measurement methods for measurements on compound c.

The steps in the analysis were:

(1) Fit the model in equation (10.7) to all samples with positively quantified values for each method, determine the residuals and predicted values;

(2) Order the predicted values from smallest to largest;

(3) Divide the predicted values into four groups from smallest to largest;

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[21] p,p'-DDT was not spiked in the multisplit samples, however, the concentrations in the unspiked samples had a great enough range to fit equation (10.4).

(4) For each group, calculate the average predicted HRGC/MS concentration, $\bar{x}_j$, and the standard deviation of the residuals, s_j; and

(5) Use the four values of $\bar{x}_j$ and s_j to fit equation (10.4) and estimate γ.

Equation (10.7) was used to estimate the relationship between the standard deviation and the concentration for HRGC/MS measurements. The following similar equation was used to estimate precision for the GC/ECD measurements:

$$X_{Ecs} = A_c + B_c * X_{Mcs} + \text{error} \qquad (10.8)$$

Although the results of these procedures are only approximate, they provide more degrees of freedom for estimating the standard deviations and more data values for fitting equation (10.4).

10.1.3 Results

Plots of $\ln(s_j)$ versus $\ln(\bar{x}_j)$ were prepared for all compounds and groups of comparable measurements derived from the multisplit and paired samples. From the plots, the following conclusions are evident:

- The estimates of γ for each compound are not very precise; and
- There is a general increase in variability with increase in concentration.

To estimate a more stable confidence interval for γ, the calculations used a pooled variance across all compounds. To provide a possibly more precise estimate of γ, a pooled slope estimate was also calculated under the assumption that the slope γ was identical for all compounds.

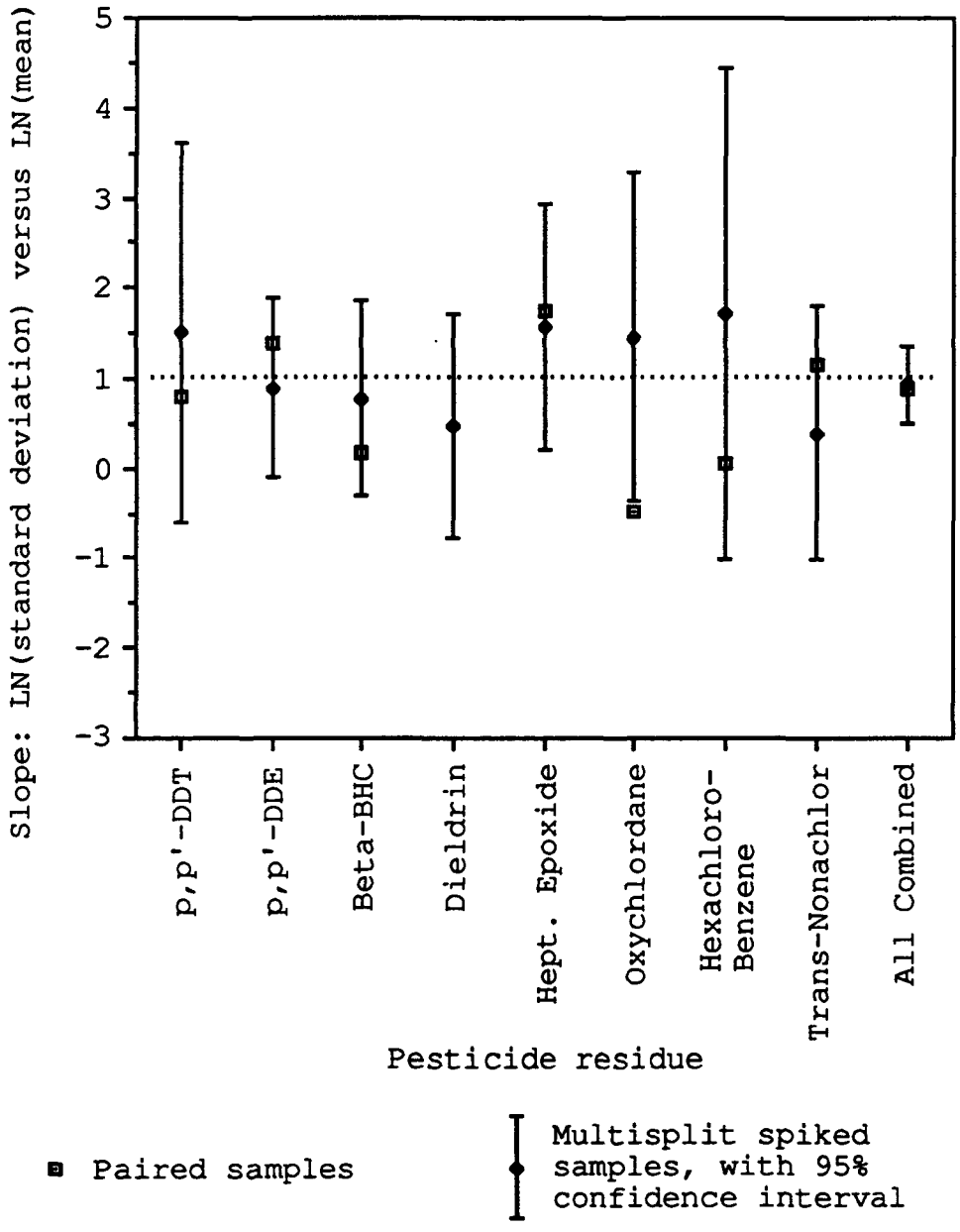

Figure 38. Slope of the linear relationship between the log of the standard deviation and the log of the mean for GC/ECD measurements on multisplit spiked and paired samples.

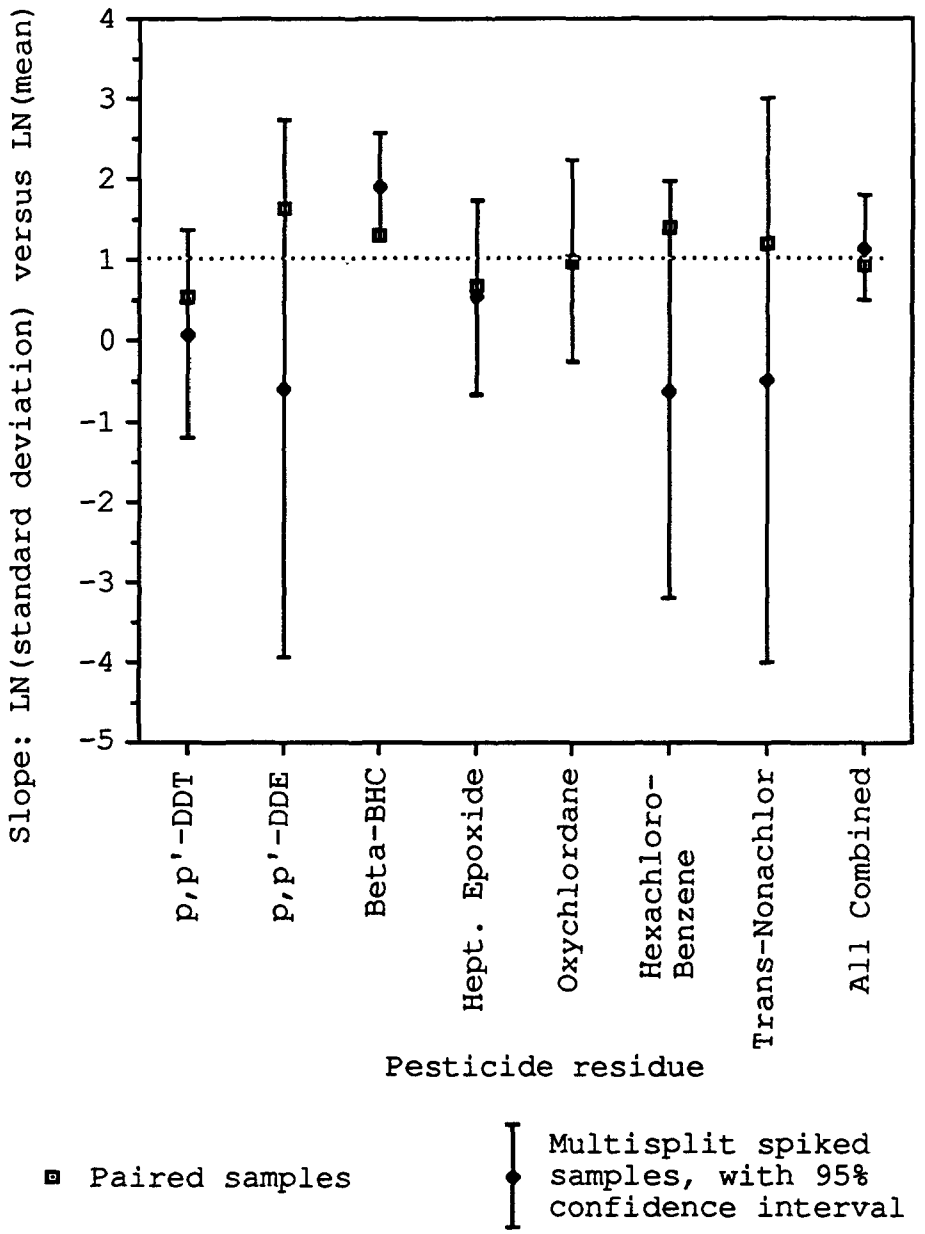

Figure 39. Slope of the linear relationship between the log of the standard deviation and the log of the mean for HRGC/MS measurements on multisplit spiked and paired samples.

Figures 38 and 39 show confidence intervals for γ based on the multisplit spiked samples and point estimates of γ based on the paired samples. Because the measurements within each group of paired samples are not true replicates, confidence intervals for the slopes would be approximate and are therefore not reported. Results for each primary compound and the pooled estimate for all compounds are shown. Figure 38 shows estimates for GC/ECD measurements. Figure 39 has estimates for HRGC/MS measurements. On both figures, a dotted line marks a slope of 1.0 corresponding to a lognormal distribution.

As can be seen from Figure 38 and 39, the results do not contradict the assumption that the data have a lognormal distribution. Most confidence intervals include a slope of 1.0, consistent with the lognormal distribution, and fewer confidence intervals include a slope of zero, consistent with a normal distribution. The confidence interval for the pooled estimate is consistent with the assumption that the errors have a lognormal distribution. For subsequent analyses, the error components are assumed to have a lognormal distribution, or equivalently, the log transformed data are assumed to have a normal distribution.

10.2 Components of Variance

A components of variance analysis divides the variance of the measurements into components which can be associated with specific sample processing steps. The components add together. Thus, the variance of the measurement error is the sum of the variance associated with differences between batches and the variance associated with differences between measurements on samples within batches. The calculation of variance components uses the log transformed measurements from the QC samples, the spiked multisplit samples, and the surrogate compounds.

The model for the data, equation (5.10), includes components of variance associated with:

- Differences between batches, σ^2_{mb};
- Differences between batches which depend on the internal standard used to quantitate the measurement, σ^2_{Mbi} (this term is confounded with σ^2_{mb} in the GC/ECD data);
- Differences between batches which depend on the internal standard and compound being quantitated, σ^2_{mbic} (this term only applies to the HRGC/MS data);
- Differences between samples within batches which are the same for both the HRGC/MS and GC/ECD measurements, σ^2_s;
- Differences between samples within batches which are different for the HRGC/MS and GC/ECD measurements, σ^2_{ms};
- Differences between measurements quantitated using the same internal standard within a sample, σ^2_{msi} (this term is confounded with σ^2_{ms} in the GC/ECD data); and
- Unexplained measurement error, σ^2_m.

Not all of these components can be estimated using every subset of data. For most subsets of the data, the following two variance components can be estimated, 1) a between-batch component ($\sigma^2_{mb} + \sigma^2_{mbi}$ (+ σ^2_{mbic} for the HRGC/MS data)) and 2) a within-batch component ($\sigma^2_s + \sigma^2_{ms} + \sigma^2_{msi} + \sigma^2_m$). The second of these components is the sum of the individual components associated with samples, internal standards within samples, and unexplained measurement error.

There is no one optimal way to estimate the components of variance. Calculation procedures based on different assumptions about which estimation criteria to optimize give different results. Estimation of the variance components was performed on the computer using SAS PROC VARCOMP (SAS 1985) which provides four calculation procedures. In order to show the range of estimates which might be achieved using different estimation procedures,

Tables 16 through 19 present results using three procedures labeled MIVQUE, Type I, and REML[22]. Discussions of the results are based on the restricted maximum likelihood (REML) procedure, because this method provides information on the precision of the variance component estimates which are useful for interpretation. The discussion notes if the interpretation might change based on results from the MIVQUE or Type I methods.

All of the variance components are estimated under the assumption that the magnitude of the components are the same for all compounds. To the extent that this is not true, the variance components represent the average component across compounds. The summary tables in Appendix A provide information on each compound separately. In general, compounds within either Fraction 1 or Fraction 2 appear to have similar variability. However Fraction 1 compounds appear to have less variability than Fraction 2 compounds. Therefore, separate results are presented for Fraction 1 and Fraction 2 compounds.

10.2.1 Components of variance for the HRGC/MS measurements

Eleven surrogate standards were injected into all but 3 of the 80 samples analyzed using the HRGC/MS method. Eight of the 11 surrogate compounds were measured in Fraction 1 and three in Fraction 2. Because Fraction 2 compounds were analyzed only in three batches, less data are available for estimation of variance components for Fraction 2 compounds. The spiked dichloromethane and multisplit samples also provide data for estimation of the overall measurement variance.

[22]The unrestricted maximum likelihood procedure was not selected due to the longer computation times required and similarity to REML.

Variance components for HRGC/MS Surrogate compounds in Fraction 1

The model for determination of the components of variance for Fraction 1 compounds is:

$$\ln(X_{Mcbsi}) = \ln\left(\frac{S_c}{R_{Mct}}\right) + \delta_{Mbi} + \delta_{Mbic} + \delta_{Mb} + \delta_{Ms'} + \delta_{Msi} + \varepsilon_{Mcbsi} \quad (10.9)$$

where $\delta_{Ms'} = \delta_{Ms} + \delta_s$.

This model has components associated with batch, batch and internal standard, batch, internal standard, and compound, samples within batches, internal standards within samples, and unexplained error. The fixed effects for different compounds, $\ln\left(\frac{S_c}{R_{Mct}}\right)$, are removed before estimating the variance components.

The model assumes that (1) the measured concentrations in the blank samples, dichloromethane spike samples, and composite adipose tissue samples differ due to different recoveries in each sample matrix and (2) after correcting for differences in recovery, the variance components are the same for all subsets of measurements. The differences among the sample matrices are statistically significant (based on the output from the type I components of variance analysis, $p < .05$). The measured concentrations in the blank samples are lower than in the other two matrices.

The estimated components of variance are shown in Table 16. All components except the batch component are significantly different from zero. However, the component for batch effects which differ by internal standard is significant. Thus there are significant differences between batches. The differences depend on the internal standard used to quantitate the measurements. Visual inspection of the residuals indicates that the unexplained variance is greater for some compounds than others. Therefore,

Table 16. Variance Components for Log Transformed Measurements for HRGC/MS Fraction 1 Surrogate Compounds

Component to be estimated		Variance estimation procedure MIVQUE	Type I	REML ±95% CI	Percent of REML variance explained by each term
Batch	σ^2_{Mb}	.009	.006	.011 (±.018)	12%
Batch and Internal standard	σ^2_{Mbi}	.005	.015	.014 (±.013)	15%
Batch, Internal standard and compound	σ^2_{Mbic}	.013	.005	.005 (±.004)	5%
Sample within batch	$\sigma^2_{Ms} + \sigma^2_s$	.011	.008	.012 (±.008)	13%
Internal standard within sample	σ^2_{Msi}	.014	.024	.022 (±.008)	24%
Unexplained error	σ^2_M	.034	.028	.028 (±.004)	30%
Overall measurement error	Total	.085	.085	.092	100%

these variance estimates represent an average across all compounds.

For Fraction 1 compounds, the estimate of within-batch variance is .062.[23] The estimate of between-batch variance is .030.[24] Thus variation within batches (due to measurement error and differences between samples) contributes more to overall measurement error than differences between batches. Adding the components together, the overall measurement error variance for the surrogate compounds in Fraction 1 is .092. The corresponding coefficient of variation of an untransformed measurement is 31%.

The residuals from the analysis of variance had several low observations which might be designated outliers. To determine the effect of the more extreme observations on the variance components, the cases with the lowest six residuals were removed and the components were recalculated. With the extreme outliers removed there were no changes in the conclusions and the overall measurement error variance estimate drops slightly to .083 with a corresponding coefficient of variation for the untransformed measurements of 29%. The results based on all measurements were used in subsequent summaries.

Variance components for HRGC/MS Surrogate compounds in Fraction 2

The model for determination of the components of variance is:

$$\ln(X_{Mcbsi}) = \ln\left(\frac{S_c}{R_{Mct}}\right) + \delta_{Mb'} + \delta_{Mbic} + \delta_{Msi'} + \varepsilon_{Mcbsi} \qquad (10.10)$$

[23]This is the sum of the REML variance estimates for the three within-batch variance components, .012, 022, and .028.
[24]This is the sum of the REML variance estimates for the two between-batch variance components, .011, .014, and 005.

where

$\delta_{Msi'} = \delta_s + \delta_{Ms} + \delta_{Msi}$, the variance associated with both sample differences and differences in the within-sample internal standard responses. This term is assumed to have a variance of $\sigma_s^2 + \sigma_{Ms}^2 + \sigma_{Msi}^2$; and

$\delta_{Mb'} = \delta_{Mb} + \delta_{Mbi}$, the variance associated with batch differences which are common to all measurements in a batch and which are associated with internal standards. This term is assumed to have a variance of $\sigma_{Mb}^2 + \sigma_{Mbi}^2$.

In this model, the sample and internal standard effects are combined because all surrogate compounds in Fraction 2 were quantitated on the same internal standard. The model assumes that the average concentration in the blank samples, dichloromethane spike samples, and adipose tissue samples differ due to different recoveries in the each sample matrix.

This model has components associated with batch, batch and compound, samples within batches, and unexplained error. The fixed effects for different compounds, $\ln\left(\frac{S_c}{R_{Mct}}\right)$, are removed before estimating the variance components. The estimated variance components are shown in Table 17.

The component associated with differences between samples and internal standards within samples is significantly greater than zero. The variance components for sample and unexplained error in Fraction 2 compounds are much larger than for Fraction 1 compounds. For Fraction 2 compounds, the overall estimate of within-batch variance is .749. The overall estimate of between-batch variance is .006. Adding the components together, the overall measurement error variance for the surrogate compounds in Fraction 2 is .754. The corresponding coefficient of variation of one untransformed measurement is 106%. Differences between measurements in different batches is due mostly to differences between the samples within batches and not differences between the batches.

Table 17. Variance Components for Log Transformed Measurements for HRGC/MS Fraction 2 Surrogate Compounds

Component to be estimated		Variance estimation procedure			Percent of REML variance explained by each term
		MIVQUE	Type I	REML ±95% CI	
Between Batch	$\sigma^2_{Mb} + \sigma^2_{Mbi}$	.016	.017	.006 (±.169)	1%
Batch and compound	σ^2_{Mbic}	-.032	-.033	0	0%
Sample and internal standard	$\sigma^2_s + \sigma^2_{Ms} + \sigma^2_{Msi}$	.423	.423	.430 (±.363)	57%
Unexplained error	σ^2_M	.341	.342	.319 (±.136)	42%
Overall measurement error	Total	.748	.748	.754	100%

Variance components for HRGC/MS spiked dichloromethane samples

One spiked dichloromethane sample was analyzed in each batch using the HRGC/MS method. Because there is only one sample per batch, the within-batch and between-batch components for the dichloromethane samples cannot be estimated independently using the dichloromethane data. The model for the data is:

$$\ln(X_{Mcbsi}) = \ln\left(\frac{S_c}{R_{Mct}}\right) + \delta_{Mb'+s} + \delta_{Mb+si'} + \varepsilon_{Mcbsi'} \qquad (10.11)$$

where:

$\delta_{Mb'+s} = \delta_{Mb} + \delta_{Ms} + \delta_s$ a random effect associated with batch b and sample s, assumed to have a variance of $\sigma^2_{Mb} + \sigma^2_{Ms} + \sigma^2_s$; and

$\delta_{Mb+si'} = \delta_{Mbi} + \delta_{Mbic} + \delta_{Msi}$ a random effect associated with the internal standard used to quantitate the measurements, assumed to have a variance of $\sigma^2_{Mbi} + \sigma^2_{Mbic} + \sigma^2_{Msi}$.

$\varepsilon_{Mcbsi'} = \delta_{Mbic} + \varepsilon_{Mcbsi}$ a random effect associated with the batch and compound and unexplained error, assumed to have a variance of $\sigma^2_{Mbic} + \sigma^2_M$.

The variances for Fraction 1 and Fraction 2 compounds differ for the QC compounds. On the assumption that they may differ for the dichloromethane samples also, Fraction 1 compounds are analyzed separately. This model has components associated with batch and sample, internal standards, and unexplained error. The fixed effects for different compounds, $\ln\left(\frac{S_c}{R_{Mct}}\right)$, were removed before estimating the variance components. The estimated components of variance are shown in Table 18.

Table 18. Variance Components for Log Transformed Measurements for Fraction 1 Compounds in HRGC/MS Spiked Dichloromethane Samples

Component to be estimated		Variance estimation procedure			Percent of REML variance explained by each term
		MIVQUE	Type I	REML ±95% CI	
Batch and sample	$\sigma^2_{Mb} + \sigma^2_{Ms} + \sigma^2_s$	.011	.002	.011 (±.033)	12%
Internal standard	$\sigma^2_{Mbi} + \sigma^2_{Msi}$	.021	.047	.061 (±.041)	64%
Unexplained error	$\sigma^2_{Mbic} + \sigma^2_M$	.040	.023	.023 (±.003)	24%
Overall measurement error	Total	.072	.072	.095	100%

The overall measurement error variance for Fraction 1 compounds in the dichloromethane spiked samples is .095. This corresponds to a coefficient of variation for an untransformed measurement of 32%. The component associated with internal standards is significant, indicating that compounds within a sample quantitated using the same internal standard will have correlated measurement errors.

The recovery measurement for p,p'-DDE in batch 2 is 155%. This measurement is unusually large compared to the recovery measurements for p,p'-DDE in dichloromethane samples in other batches. Because removing this measurement from the calculations reduces the overall error variance by less than 2%, the results for all data including this unusual value are reported.

Because Fraction 2 compounds were analyzed in only three batches, relatively little data was available to estimate variance components. Therefore, only the overall measurement error variance for the log transformed measurements is estimated for the Fraction 2 compounds. As with the surrogate compounds, the variance for Fraction 2 compounds is greater than for Fraction 1 compounds. The overall measurement error variance for Fraction 2 compounds in the dichloromethane spiked samples is .396. This corresponds to a coefficient of variation for an untransformed measurement of 70%.

Variance components for Fraction 1 compounds in the spiked multisplit samples.

Due to the sample design for the multisplit samples (three composites, each with four splits analyzed in three batches), estimated variance components would have few degrees of freedom and not be very precise. Therefore, only the overall measurement variance was calculated for the multisplit samples. The average measurement error variance for the log transformed measurements on primary Fraction 1 compounds in the spiked multisplit composite

samples is .176. The corresponding coefficient of variation for the untransformed concentration is 44%.

10.2.2 Components of variance for the GC/ECD measurements

Aldrin was injected into all but 3 of the 86 samples analyzed using the GC/ECD method. The recovery for the aldrin can be used to estimate overall batch and sample effects. Porcine samples and spiked multisplit samples analyzed in each batch can be used to estimate the overall measurement variance.

Variance components for GC/ECD aldrin measurements

Only one compound, aldrin, was spiked into the GC/ECD samples to estimate recovery. Because there is only one compound per sample, only the within- and between-batch components of variance can be estimated. Therefore, the model is:

$$\ln(X_{Ecbs}) = \ln\left(\frac{S_c}{R_{Ect}}\right) + \delta_{Eb}' + \varepsilon_{Ecbs} \qquad (10.12)$$

where δ_{Eb}' is the between-batch component and ε_{Ecbs} is the within-batch component.

The fixed effects for different compounds, $\ln\left(\frac{S_c}{R_{Ect}}\right)$, were removed before estimating the variance components. The estimated components of variance are shown in Table 19. The model fit to the aldrin measurements assumes that the average concentration in the blank samples, porcine fat samples, and adipose tissue samples differs even though the spiking levels were the same. Although the differences between these groups of samples are not statistically significant ($p < .058$), the differences were modeled because they were close to significant and differences were found in the HRGC/MS measurements.

Table 19. Variance Components for Log Transformed Aldrin Measurements in GC/ECD Samples

Component to be estimated		Variance estimation procedure			Percent of REML variance explained by each term
		MIVQUE	Type I	REML ±95% CI	
Between batch	$\sigma^2_{mb} + \sigma^2_{mbi}$	.003	.003	.003(±.006)	12%
Within batch	$\sigma^2_s + \sigma^2_{ms} + \sigma^2_{msi} + \sigma^2_m$	.022	.022	.022(±.008)	88%
	Total	.025	.025	.025	100%

For the aldrin data, the between-batch variance component, .003, is not significantly greater than zero. The estimate of within-batch variance is .022. Adding the components together, the overall measurement error variance for aldrin is .025. The corresponding coefficient of variation of an untransformed measurement is 16%.

Measurement error variance for the GC/ECD porcine fat samples

The model for the QC samples is simpler than that for the HRGC/MS measurements because only one internal standard was used. As with the dichloromethane samples, with only one sample per batch, the batch and sample components of variance cannot be estimated independently. Therefore, only the overall measurement error variance, pooled across spiked compounds, was estimated from the porcine sample data. Because the porcine fat samples had different sources and spiking levels for the first 3 batches than the last 7 batches, the error estimates were made separately for batches 1 to 3 and 4 through 10.

For the porcine fat samples from batches 1, 2, and 3, the overall measurement error variance for the log transformed data is .009. The corresponding coefficient of variation for an untransformed measurement is 10%.

For the porcine fat samples from batches 4 through 10, the overall measurement error variance for the log transformed data is .008. The corresponding coefficient of variation for the untransformed concentration is 9%.

Variance components for GC/ECD measurements on Fraction 1 compounds in the spiked multisplit samples

Because only one of the Fraction 2 compounds (dieldrin) had positively quantified measurements, variance estimates from the

GC/ECD spiked multisplit samples were calculated for Fraction 1 compounds only. On the assumption that the variances are similar for all primary Fraction 1 compounds in the spiked multisplit composite samples, the overall measurement error variance for the log transformed data is .013. The corresponding coefficient of variation for the untransformed concentration is 11%.

10.3 Summary and Comparison of HRGC/MS and GC/ECD Precision

Based on the analysis of standard deviation versus concentration and plots of the data, the variability of the HRGC/MS and GC/ECD measurements increases as the concentration being measured increases. The decision to model the log concentration and assume that the log transformed concentration had constant variance was based on the relationship between the standard deviation and mean concentration from spiked multisplit and paired samples, theoretical considerations, and observations on the residuals from analyses using the log transformed data. Modeling of the log transformed data results in a statistical model which is relatively easy to fit to the data using standard statistical techniques.

Where possible, the overall measurement error variance was divided into components of variance associated with sample processing steps. Of particular interest is the within- and between-batch estimates of variance. The within-batch component of variance is the variance of measurements on split samples within the same batch. This includes errors associated with sample handling within a batch or day, injection of internal standards, and quantitation. The between-batch component of variance is the portion of the measurement variance which is attributed to differences between batches. This includes errors associated with calibration and possible changes in the preparation of different solutions or equipment setup between batches or days. The between-batch components of variance and overall measurement error variance for the transformed data are summarized in Table 20. The

Table 20. Summary of Variance Components for GC/ECD and HRGC/MS Measurements

Analysis method and subset of the data		N	Log transformed measurements		Untransformed measurements	
			Between batch variance	Overall error variance	Coef. of variation	95% Pred. Interval
HRGC/MS	Surrogate compounds Fraction 1	620	.030	.092	31%	±61%
HRGC/MS	Surrogate compounds Fraction 2	66	.006	.754	106%	±208%
HRGC/MS	Spiked dichloromethane samples Fraction 1	428		.095	32%	±62%
HRGC/MS	Spiked dichloromethane samples Fraction 2	27		.396	70%	±137%
HRGC/MS	Spiked multi-split samples Fraction 1	82	Assumed zero	.176	44%	±86%
GC/ECD	Aldrin	73	.003	.025	16%	±31%
GC/ECD	Porcine fat samples Batches 1 to 3 Fraction 1	27		.009	10%	±19%
GC/ECD	Porcine fat samples Batches 4 to 10 Fraction 1	62		.008	9%	±17%
GC/ECD	Spiked multi-split samples Fraction 1	84	Assumed zero	.013	11%	±22%

N is the number of measurements from which the variance components are estimated.

The within-batch variance component can be calculated from the difference between the between batch and overall variance.

The 95% prediction interval for an individual measurement is calculated as 1.96 times the coefficient of variation.

overall measurement variance is also expressed as a coefficient of variation for an untransformed measurement.

The components of variance were estimated from different subsets of the data according to how the samples were prepared. The components based on different subsets may differ due to many factors including 1) different compounds analyzed in the different subsets, 2) different matrices in which the compounds are found, and 3) different analytical procedures used to process the samples.

The variances were calculated assuming that all compounds in the subset of data being analyzed have the same variance. Review of the data suggests that this assumption is not unreasonable but that some differences may exist. Therefore, the components of variance and overall variance represent averages across compounds. Additional information about individual compounds can be obtained from the tables in Appendix A.

As can be seen in Table 20, measurements for Fraction 2 compounds using the HRGC/MS procedures have greater variances than those for Fraction 1 compounds. The three variance estimates for Fraction 1 compounds are .092, .095, and .176. The estimates for Fraction 2 compounds are .396 and .754.

Both the Fraction 1 and Fraction 2 compounds measured using the HRGC/MS procedures have overall variance estimates greater than those for the GC/ECD measurements. The overall variance estimates for the GC/ECD compounds are all less than .03. The HRGC/MS overall variance estimates range from .092 to .176 for Fraction 1 compounds. As a rough rule of thumb, the variance of the HRGC/MS measurements is nine times greater than that of the GC/ECD measurements, resulting in a coefficient of variation three times greater for the HRGC/MS measurements than the GC/ECD measurements. Based on a weighted average of the variance estimates

in Table 20[25] approximate 95% prediction intervals for the Fraction 1 GC/ECD and HRGC/MS measurements are 22% and 63% respectively.

Systematic differences between batches are small relative to the differences between measurements on samples in the same batch. However, batch differences for the HRGC/MS measurements on the surrogate compounds are statistically significant. Therefore, the calculation of recovery and determination of the relationship between the GC/ECD and HRGC/MS measurements (Chapters 6 and 7) accounted for the presence of any batch effects.

The proposed model for the data hypothesizes sample and internal standard effects, in addition to batch effects. The statistical analysis indicates that both the sample and internal standard effects are significant for the HRGC/MS measurements. Although these effects can be ignored for this Comparability Study and many uses of the data, comparison of measurements for different compounds in the same sample and different samples in the same batch must take these variance components into account.

[25]The average was calculated using the number of measurements, N, for the weights.

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APPENDIX A: SUMMARY DATA TABLES

Tables A-1 through A-18 summarize the measurements used in the Comparability Study. The following guide is provided to help locate the appropriate table:

Tables A-1 to A-7 summarize the HRGC/MS measurements:

Tables A-8 to A-18 summarize the GC/ECD measurements:

Table A-1. Summary of HRGC/MS Measurements on Method Blanks, Nominal Concentration in ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
p,p -DDT	0	0	12	0%	.016	ND					
o,p -DDT	0	0	12	0%	.012	ND					
p,p -DDE	1	0	11	8%	.012	ND	.013	.013		.013	.013
o,p -DDE	0	0	12	0%	.012	ND					
p,p -DDD	0	0	12	0%	.012	ND					
o,p -DDD	0	0	12	0%	.091	ND					
alpha-BHC	0	0	12	0%	.012	ND					
beta-BHC	0	0	12	0%	.016	ND					
delta-BHC	0	0	12	0%	.012	ND					
gamma-BHC(LINDANE)	0	0	12	0%	.012	ND					
ALDRIN	0	0	12	0%	.012	ND					
HEPTACHLOR	0	0	12	0%	.016	ND					
HEPTACHLOR EPOXIDE	0	0	12	0%	.012	ND					
OXYCHLORDANE	0	0	12	0%	.012	ND					
trans-NONACHLOR	0	0	12	0%	.012	ND					
gamma-CHLORDANE	0	0	12	0%	.012	ND					
MIREX	0	0	12	0%	.012	ND					
1,2,3-TRICHLOROBENZENE	0	0	12	0%	.012	ND					
1,2,4-TRICHLOROBENZENE	2	0	10	17%	.012	ND	.018	.018	.001	.017	.019
1,3,5-TRICHLOROBENZENE	0	0	12	0%	.012	ND					
1,2,3,4-TETRACHLOROBENZENE	0	0	12	0%	.012	ND					
1,2,3,5-TETRACHLOROBENZENE	0	0	12	0%	.012	ND					
1,2,4,5-TETRACHLOROBENZENE	0	0	12	0%	.012	ND					
PENTACHLOROBENZENE	0	0	12	0%	.012	ND					
HEXACHLOROBENZENE	0	0	12	0%	.012	ND					
NAPHTHALENE	0	0	12	0%	.012	ND					
ACENAPHTHYLENE	0	0	12	0%	.012	ND					
ACENAPHTHENE	0	0	12	0%	.012	ND					
FLUORENE	0	0	12	0%	.012	ND					
PHENANTHRENE	0	0	12	0%	.012	ND					
FLUORANTHENE	0	0	12	0%	.012	ND					

Table A-1. (Continued)

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
PYRENE	0	0	12	0%	.012	ND					
CHRYSENE	0	0	12	0%	.016	ND					
MONOCHLOROBIPHENYLS	0	0	12	0%	.012	ND					
DICHLOROBIPHENYLS	0	0	12	0%	.012	ND					
TRICHLOROBIPHENYLS	0	0	12	0%	.012	ND					
TETRACHLOROBIPHENYLS	0	0	12	0%	.012	ND					
PENTACHLOROBIPHENYLS	0	0	12	0%	.012	ND					
HEXACHLOROBIPHENYLS	0	0	12	0%	.012	ND					
HEPTACHLOROBIPHENYLS	0	0	12	0%	.012	ND					
OCTACHLOROBIPHENYLS	0	0	12	0%	.012	ND					
NONACHLOROBIPHENYLS	0	0	12	0%	.016	ND					
DECACHLOROBIPHENYL	0	0	12	0%	.016	ND					
DIELDRIN	0	0	3	0%	.012	ND					
ENDRIN	0	0	3	0%	.043	ND					
DIMETHYL PHTHALATE	0	0	3	0%	.012	ND					
DIETHYL PHTHALATE	0	0	3	0%	.012	ND					
DI-n-BUTYL PHTHALATE	3	0	0	100%		.092	.092	.122	.061	.081	.192
BUTYL BENZYL PHTHALATE	0	0	3	0%	.015	ND					
BIS(2-ETHYLHEXYL) PHTHALATE	0	0	3	0%	.047	ND					
DI-n-OCTYL PHTHALATE	1	0	2	33%	.012	ND	.019	.019		.019	.019
TRIBUTYL PHOSPHATE	0	0	3	0%	.012	ND					
TRIS(2-CHLOROETHYL) PHOSPHATE	0	0	3	0%	.043	ND					
TRIS(DICHLOROPROPYL) PHOSPHATE	0	0	3	0%	.012	ND					
TRIBUTOXYETHYL PHOSPHATE	0	0	3	0%	.078	ND					
TRIPHENYL PHOSPHATE	0	0	3	0%	.012	ND					
TRITOLYL PHOSPHATE	0	0	3	0%	.043	ND					

For Blanks, the nominal concentration is calculated assuming a wet weight of 20 grams and percent lipid of 85%.
Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.
The mean LOD is based on ND and TR measurements.

Table A-2. Summary of HRGC/MS Measurements on Dichloromethane Spike Samples, as Percent Recovery.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
p,p -DDT	10	0	0	100%		64%	64%	67%	18%	46%	94%
o,p -DDT	10	0	0	100%		77%	77%	81%	12%	70%	107%
p,p -DDE	10	0	0	100%		70%	70%	80%	30%	55%	155%
o,p -DDE	10	0	0	100%		72%	72%	72%	10%	59%	89%
p,p -DDD	10	0	0	100%		69%	69%	73%	14%	58%	91%
o,p -DDD	4	0	4*	50%	100%	35%	75%	77%	9%	70%	89%
alpha-BHC	10	0	0	100%		60%	60%	62%	10%	51%	86%
beta-BHC	10	0	0	100%		70%	70%	74%	21%	53%	118%
delta-BHC	10	0	0	100%		65%	65%	69%	11%	59%	89%
gamma-BHC(LINDANE)	10	0	0	100%		65%	65%	65%	10%	53%	87%
ALDRIN	10	0	0	100%		67%	67%	65%	8%	54%	79%
HEPTACHLOR	10	0	0	100%		52%	52%	51%	14%	31%	72%
HEPTACHLOR EPOXIDE	10	0	0	100%		81%	81%	81%	12%	68%	107%
OXYCHLORDANE	10	0	0	100%		72%	72%	71%	8%	60%	82%
trans-NONACHLOR	10	0	0	100%		72%	72%	73%	12%	57%	90%
gamma-CHLORDANE	10	0	0	100%		83%	83%	83%	15%	62%	108%
MIREX	10	0	0	100%		71%	71%	71%	10%	59%	88%
1,2,3-TRICHLOROBENZENE	10	0	0	100%		27%	27%	29%	13%	12%	50%
1,2,4-TRICHLOROBENZENE	10	0	0	100%		25%	25%	28%	13%	12%	49%
1,3,5-TRICHLOROBENZENE	10	0	0	100%		25%	25%	25%	11%	10%	47%
1,2,3,4-TETRACHLOROBENZENE	10	0	0	100%		36%	36%	37%	15%	18%	60%
1,2,3,5-TETRACHLOROBENZENE	10	0	0	100%		31%	31%	34%	14%	15%	57%
1,2,4,5-TETRACHLOROBENZENE	10	0	0	100%		31%	31%	34%	14%	15%	57%
PENTACHLOROBENZENE	10	0	0	100%		45%	45%	45%	12%	31%	71%
HEXACHLOROBENZENE	10	0	0	100%		52%	52%	51%	7%	41%	66%
NAPHTHALENE	10	0	0	100%		29%	29%	30%	13%	12%	51%
ACENAPHTHYLENE	10	0	0	100%		45%	45%	45%	15%	27%	69%
ACENAPHTHENE	10	0	0	100%		41%	41%	42%	14%	23%	72%
FLUORENE	10	0	0	100%		56%	56%	60%	14%	43%	86%
PHENANTHRENE	10	0	0	100%		66%	66%	68%	8%	52%	80%
FLUORANTHENE	10	0	0	100%		82%	82%	80%	12%	58%	93%

Table A-2. (Continued)

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
PYRENE	10	0	0	100%		26%	26%	33%	16%	16%	59%
CHRYSENE	10	0	0	100%		66%	66%	67%	6%	59%	79%
MONOCHLOROBIPHENYLS	10	0	0	100%		51%	51%	52%	14%	35%	76%
DICHLOROBIPHENYLS	10	0	0	100%		60%	60%	63%	10%	47%	80%
TRICHLOROBIPHENYLS	10	0	0	100%		59%	59%	59%	8%	48%	75%
TETRACHLOROBIPHENYLS	10	0	0	100%		66%	66%	66%	7%	55%	78%
PENTACHLOROBIPHENYLS	10	0	0	100%		73%	73%	72%	8%	63%	84%
HEXACHLOROBIPHENYLS	10	0	0	100%		73%	73%	72%	9%	60%	85%
HEPTACHLOROBIPHENYLS	10	0	0	100%		74%	74%	72%	8%	61%	81%
OCTACHLOROBIPHENYLS	10	0	0	100%		73%	73%	73%	10%	55%	84%
NONACHLOROBIPHENYLS	10	0	0	100%		83%	83%	83%	16%	50%	106%
DECACHLOROBIPHENYL	10	0	0	100%		82%	82%	79%	20%	40%	109%
DIELDRIN	3	0	0	100%		85%	85%	77%	30%	43%	102%
ENDRIN	3	0	0	100%		115%	115%	93%	50%	36%	128%
DIMETHYL PHTHALATE	3	0	0	100%		51%	51%	56%	11%	49%	68%
DIETHYL PHTHALATE	3	0	0	100%		56%	56%	57%	11%	46%	67%
DI-n-BUTYL PHTHALATE	3	0	0	100%		126%	126%	133%	23%	114%	158%
BUTYL BENZYL PHTHALATE	3	0	0	100%		32%	32%	26%	19%	4%	41%
BIS(2-ETHYLHEXYL) PHTHALATE	0	0	3	0%	23%	ND					
DI-n-OCTYL PHTHALATE	2	0	1	67%	10%	4%	13%	13%	12%	4%	22%
TRIBUTYL PHOSPHATE	0	0	3	0%	10%	ND					
TRIS(2-CHLOROETHYL) PHOSPHATE	0	0	3	0%	37%	ND					
TRIS(DICHLOROPROPYL) PHOSPHATE	1	0	2	33%	10%	ND	1%	1%		1%	1%
TRIBUTOXYETHYL PHOSPHATE	1	0	2	33%	75%	ND	3%	3%		3%	3%
TRIPHENYL PHOSPHATE	3	0	0	100%		95%	95%	99%	11%	92%	112%
TRITOLYL PHOSPHATE	2	0	1	67%	10%	19%	24%	24%	7%	19%	29%

* One measurement with an ND qualifier had a comment indicating the chemical is present but not quantifiable.

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).

All concentrations are lipid adjusted.

The mean LOD is based on ND and TR measurements.

Table A-3. Summary of HRGC/MS Measurements on Paired Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
p,p -DDT	39	0	5*	89%	.049	.063	.069	.099	.086	.017	.460
o,p -DDT	0	0	45	0%	.013	ND					
p,p -DDE	41	0	2*	95%	.352	.671	.728	.846	.542	.180	2.487
o,p -DDE	2	0	43	4%	.013	ND	.019	.019	.009	.013	.026
p,p -DDD	1	0	44	2%	.013	ND	.015	.015		.015	.015
o,p -DDD	0	0	44*	0%	.127	ND					
alpha-BHC	0	0	45	0%	.020	ND					
beta-BHC	40	0	5	89%	.212	.086	.097	.135	.125	.014	.541
delta-BHC	0	0	45	0%	.018	ND					
gamma-BHC(LINDANE)	1	0	44	2%	.019	ND	.018	.018		.018	.018
ALDRIN	0	0	45	0%	.013	ND					
HEPTACHLOR	0	0	45	0%	.018	ND					
HEPTACHLOR EPOXIDE	37	0	8	82%	.016	.043	.052	.059	.039	.015	.202
OXYCHLORDANE	37	0	8	82%	.016	.040	.049	.057	.031	.023	.131
trans-NONACHLOR	42	0	2*	95%	.014	.069	.072	.079	.046	.020	.242
gamma-CHLORDANE	0	0	45	0%	.013	ND					
MIREX	2	0	43	4%	.013	ND	.027	.027	.013	.018	.035
1,2,3-TRICHLOROBENZENE	0	0	45	0%	.013	ND					
1,2,4-TRICHLOROBENZENE	2	0	43	4%	.013	ND	.024	.024	.007	.019	.028
1,3,5-TRICHLOROBENZENE	0	0	45	0%	.013	ND					
1,2,3,4-TETRACHLOROBENZENE	0	0	45	0%	.013	ND					
1,2,3,5-TETRACHLOROBENZENE	0	0	45	0%	.013	ND					
1,2,4,5-TETRACHLOROBENZENE	0	0	45	0%	.013	ND					
PENTACHLOROBENZENE	0	0	45	0%	.013	ND					
HEXACHLOROBENZENE	37	0	8	82%	.015	.021	.023	.028	.024	.011	.163
NAPHTHALENE	11	0	34	24%	.013	ND	.019	.019	.006	.013	.029
ACENAPHTHYLENE	0	0	45	0%	.013	ND					
ACENAPHTHENE	0	0	45	0%	.013	ND					
FLUORENE	1	0	44	2%	.014	ND	.066	.066		.066	.066
PHENANTHRENE	10	0	35	22%	.013	ND	.023	.071	.100	.016	.262
FLUORANTHENE	1	0	44	2%	.014	ND	.013	.013		.013	.013

Table A-3. (Continued)

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
PYRENE	2	0	43	4%	.013	ND	.317	.317	.306	.101	.533
CHRYSENE	2	0	43	4%	.015	ND	.056	.056	.002	.054	.057
MONOCHLOROBIPHENYLS	0	0	45	0%	.013	ND					
DICHLOROBIPHENYLS	0	0	45	0%	.013	ND					
TRICHLOROBIPHENYLS	17	0	28	38%	.014	ND	.015	.017	.005	.011	.026
TETRACHLOROBIPHENYLS	19	0	26	42%	.032	ND	.056	.060	.034	.014	.111
PENTACHLOROBIPHENYLS	38	0	7	84%	.016	.065	.073	.090	.068	.018	.330
HEXACHLOROBIPHENYLS	43	0	1	98%	.013	.120	.121	.138	.091	.015	.446
HEPTACHLOROBIPHENYLS	37	0	7	84%	.015	.085	.094	.111	.094	.014	.571
OCTACHLOROBIPHENYLS	8	0	36	18%	.013	ND	.031	.041	.036	.010	.122
NONACHLOROBIPHENYLS	10	0	34	23%	.019	ND	.023	.031	.020	.012	.067
DECACHLOROBIPHENYL	16	0	28	36%	.021	ND	.016	.028	.019	.011	.081
Total PCBs	44	0	1	98%	.011	.310	.309	.363	.300	.014	1.560
DIELDRIN	5	0	7	42%	.020	ND	.028	.051	.040	.020	.114
ENDRIN	0	0	12	0%	.041	ND					
DIMETHYL PHTHALATE	0	0	12	0%	.014	ND					
DIETHYL PHTHALATE	0	0	12	0%	.014	ND					
DI-n-BUTYL PHTHALATE	12	0	0	100%		.153	.153	.209	.165	.023	.491
BUTYL BENZYL PHTHALATE	8	0	4	67%	.021	.030	.068	.086	.066	.016	.185
BIS(2-ETHYLHEXYL) PHTHALATE	0	0	12	0%	.041	ND					
DI-n-OCTYL PHTHALATE	3	0	9	25%	.022	ND	.040	.042	.029	.014	.072
TRIBUTYL PHOSPHATE	0	0	12	0%	.014	ND					
TRIS(2-CHLOROETHYL) PHOSPHATE	0	0	12	0%	.041	ND					
TRIS(DICHLOROPROPYL) PHOSPHATE	0	0	12	0%	.015	ND					
TRIBUTOXYETHYL PHOSPHATE	0	0	12	0%	.089	ND					
TRIPHENYL PHOSPHATE	7	0	5	58%	.013	.018	.031	.115	.234	.015	.645
TRITOLYL PHOSPHATE	0	0	12	0%	.052	ND					

* One measurement for p,p'-DDT, o,p'-DDE, and trans-nonachlor and 2 measurements for p,p'-DDE with an ND qualifier had a comment indicating the chemical is present but not quantifiable.

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).

All concentrations are lipid adjusted.

The mean LOD is based on ND and TR measurements.

Table A-4. Summary of HRGC/MS Measurements on the High Level Spiked Multisplit Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
p,p -DDT	4	0	0	100%		.143	.143	.141	.019	.118	.160
o,p -DDT	0	0	4	0%	.013	ND					
p,p -DDE	4	0	0	100%		1.139	1.139	1.371	.540	1.033	2.172
o,p -DDE	0	0	4	0%	.013	ND					
p,p -DDD	0	0	4	0%	.013	ND					
o,p -DDD	0	0	4	0%	.071	ND					
alpha-BHC	0	0	4	0%	.013	ND					
beta-BHC	4	0	0	100%		.849	.849	.869	.784	.168	1.610
delta-BHC	0	0	4	0%	.013	ND					
gamma-BHC(LINDANE)	0	0	4	0%	.013	ND					
ALDRIN	0	0	4	0%	.013	ND					
HEPTACHLOR	0	0	4	0%	.013	ND					
HEPTACHLOR EPOXIDE	4	0	0	100%		.217	.217	.231	.047	.192	.300
OXYCHLORDANE	4	0	0	100%		.146	.146	.138	.041	.081	.180
trans-NONACHLOR	4	0	0	100%		.261	.261	.233	.074	.124	.287
gamma-CHLORDANE	0	0	4	0%	.013	ND					
MIREX	0	0	4	0%	.013	ND					
1,2,3-TRICHLOROBENZENE	0	0	4	0%	.013	ND					
1,2,4-TRICHLOROBENZENE	0	0	4	0%	.013	ND					
1,3,5-TRICHLOROBENZENE	0	0	4	0%	.013	ND					
1,2,3,4-TETRACHLOROBENZENE	0	0	4	0%	.013	ND					
1,2,3,5-TETRACHLOROBENZENE	0	0	4	0%	.013	ND					
1,2,4,5-TETRACHLOROBENZENE	0	0	4	0%	.013	ND					
PENTACHLOROBENZENE	0	0	4	0%	.013	ND					
HEXACHLOROBENZENE	4	0	0	100%		.076	.076	.078	.007	.073	.089
NAPHTHALENE	0	0	4	0%	.013	ND					
ACENAPHTHYLENE	0	0	4	0%	.013	ND					
ACENAPHTHENE	0	0	4	0%	.013	ND					
FLUORENE	0	0	4	0%	.013	ND					
PHENANTHRENE	0	0	4	0%	.013	ND					
FLUORANTHENE	0	0	4	0%	.013	ND					

Table A-4. (Continued)

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
PYRENE	1	0	3	25%	.013	ND	.013	.013		.013	.013
CHRYSENE	0	0	4	0%	.013	ND					
MONOCHLOROBIPHENYLS	0	0	4	0%	.013	ND					
DICHLOROBIPHENYLS	0	0	4	0%	.013	ND					
TRICHLOROBIPHENYLS	1	0	3	25%	.013	ND	.023	.023		.023	.023
TETRACHLOROBIPHENYLS	0	0	4	0%	.078	ND					
PENTACHLOROBIPHENYLS	4	0	0	100%		.079	.079	.069	.031	.025	.093
HEXACHLOROBIPHENYLS	4	0	0	100%		.101	.101	.097	.013	.079	.110
HEPTACHLOROBIPHENYLS	4	0	0	100%		.089	.089	.090	.008	.082	.101
OCTACHLOROBIPHENYLS	0	0	4	0%	.013	ND					
NONACHLOROBIPHENYLS	2	0	2	50%	.065	.016	.031	.031	.000	.031	.032
DECACHLOROBIPHENYL	2	0	2	50%	.065	.014	.028	.028	.000	.028	.028
DIELDRIN	2	0	0	100%		.542	.542	.542	.158	.431	.654
ENDRIN	0	0	2	0%	.065	ND					
DIMETHYL PHTHALATE	0	0	2	0%	.013	ND					
DIETHYL PHTHALATE	0	0	2	0%	.013	ND					
DI-n-BUTYL PHTHALATE	2	0	0	100%		.335	.335	.335	.054	.297	.374
BUTYL BENZYL PHTHALATE	0	0	2	0%	.013	ND					
BIS(2-ETHYLHEXYL) PHTHALATE	0	0	2	0%	.039	ND					
DI-n-OCTYL PHTHALATE	2	0	0	100%		.051	.051	.051	.012	.043	.060
TRIBUTYL PHOSPHATE	0	0	2	0%	.013	ND					
TRIS(2-CHLOROETHYL) PHOSPHATE	0	0	2	0%	.065	ND					
TRIS(DICHLOROPROPYL) PHOSPHATE	0	0	2	0%	.013	ND					
TRIBUTOXYETHYL PHOSPHATE	0	0	2	0%	.098	ND					
TRIPHENYL PHOSPHATE	1	0	1	50%	.013	.016	.032	.032		.032	.032
TRITOLYL PHOSPHATE	0	0	2	0%	.076	ND					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.
The mean LOD is based on ND and TR measurements.

Table A-5. Summary of HRGC/MS Measurements on the Low Level Spiked Multisplit Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
p,p -DDT	2	0	2	50%	.060	.008	.031	.031	.021	.016	.045
o,p -DDT	0	0	4	0%	.012	ND					
p,p -DDE	4	0	0	100%		.821	.821	.952	.339	.720	1.447
o,p -DDE	0	0	4	0%	.012	ND					
p,p -DDD	0	0	4	0%	.012	ND					
o,p -DDD	0	0	4	0%	.039	ND					
alpha-BHC	0	0	4	0%	.012	ND					
beta-BHC	4	0	0	100%		.434	.434	.405	.210	.126	.627
delta-BHC	0	0	4	0%	.012	ND					
gamma-BHC(LINDANE)	0	0	4	0%	.012	ND					
ALDRIN	0	0	4	0%	.012	ND					
HEPTACHLOR	0	0	4	0%	.036	ND					
HEPTACHLOR EPOXIDE	4	0	0	100%		.074	.074	.072	.027	.037	.102
OXYCHLORDANE	4	0	0	100%		.059	.059	.054	.012	.037	.061
trans-NONACHLOR	4	0	0	100%		.128	.128	.154	.084	.087	.273
gamma-CHLORDANE	0	0	4	0%	.012	ND					
MIREX	0	0	4	0%	.012	ND					
1,2,3-TRICHLOROBENZENE	0	0	4	0%	.012	ND					
1,2,4-TRICHLOROBENZENE	0	0	4	0%	.012	ND					
1,3,5-TRICHLOROBENZENE	0	0	4	0%	.012	ND					
1,2,3,4-TETRACHLOROBENZENE	0	0	4	0%	.012	ND					
1,2,3,5-TETRACHLOROBENZENE	0	0	4	0%	.012	ND					
1,2,4,5-TETRACHLOROBENZENE	0	0	4	0%	.012	ND					
PENTACHLOROBENZENE	0	0	4	0%	.012	ND					
HEXACHLOROBENZENE	4	0	0	100%		.047	.047	.045	.010	.031	.054
NAPHTHALENE	0	0	4	0%	.012	ND					
ACENAPHTHYLENE	0	0	4	0%	.012	ND					
ACENAPHTHENE	0	0	4	0%	.012	ND					
FLUORENE	0	0	4	0%	.012	ND					
PHENANTHRENE	0	0	4	0%	.012	ND					
FLUORANTHENE	0	0	4	0%	.012	ND					

Table A-5. (Continued)

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
PYRENE	0	0	4	0%	.012	ND					
CHRYSENE	0	0	4	0%	.036	ND					
MONOCHLOROBIPHENYLS	0	0	4	0%	.012	ND					
DICHLOROBIPHENYLS	0	0	4	0%	.012	ND					
TRICHLOROBIPHENYLS	0	0	4	0%	.012	ND					
TETRACHLOROBIPHENYLS	3	0	1	75%	.029	.043	.052	.058	.028	.035	.089
PENTACHLOROBIPHENYLS	4	0	0	100%		.064	.064	.069	.038	.029	.119
HEXACHLOROBIPHENYLS	4	0	0	100%		.131	.131	.140	.080	.053	.244
HEPTACHLOROBIPHENYLS	4	0	0	100%		.130	.130	.107	.064	.014	.156
OCTACHLOROBIPHENYLS	3	0	1	75%	.012	.029	.029	.032	.006	.028	.040
NONACHLOROBIPHENYLS	3	0	1	75%	.012	.015	.015	.016	.002	.015	.018
DECACHLOROBIPHENYL	2	0	2	50%	.012	.007	.018	.018	.005	.014	.022
DIELDRIN	3	0	0	100%		.116	.116	.113	.007	.106	.118
ENDRIN	0	0	3	0%	.028	ND					
DIMETHYL PHTHALATE	0	0	3	0%	.012	ND					
DIETHYL PHTHALATE	0	0	3	0%	.012	ND					
DI-n-BUTYL PHTHALATE	3	0	0	100%		.094	.094	.099	.022	.080	.122
BUTYL BENZYL PHTHALATE	2	0	1	67%	.048	.030	.077	.077	.067	.030	.124
BIS(2-ETHYLHEXYL) PHTHALATE	0	0	3	0%	.028	ND					
DI-n-OCTYL PHTHALATE	0	0	3	0%	.012	ND					
TRIBUTYL PHOSPHATE	0	0	3	0%	.012	ND					
TRIS(2-CHLOROETHYL) PHOSPHATE	0	0	3	0%	.028	ND					
TRIS(DICHLOROPROPYL) PHOSPHATE	0	0	3	0%	.012	ND					
TRIBUTOXYETHYL PHOSPHATE	0	0	3	0%	.101	ND					
TRIPHENYL PHOSPHATE	0	0	3	0%	.020	ND					
TRITOLYL PHOSPHATE	0	0	3	0%	.123	ND					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.
The mean LOD is based on ND and TR measurements.

Table A-6. Summary of HRGC/MS Measurements on the Mid Level Spiked Multisplit Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
p,p -DDT	4	0	0	100%		.068	.068	.067	.012	.053	.080
o,p -DDT	0	0	4	0%	.011	ND					
p,p -DDE	4	0	0	100%		1.374	1.374	1.419	.159	1.283	1.644
o,p -DDE	0	0	4	0%	.011	ND					
p,p -DDD	0	0	4	0%	.037	ND					
o,p -DDD	0	0	4	0%	.088	ND					
alpha-BHC	0	0	4	0%	.011	ND					
beta-BHC	4	0	0	100%		.094	.094	.097	.012	.086	.113
delta-BHC	0	0	4	0%	.011	ND					
gamma-BHC(LINDANE)	0	0	4	0%	.011	ND					
ALDRIN	0	0	4	0%	.011	ND					
HEPTACHLOR	0	0	4	0%	.011	ND					
HEPTACHLOR EPOXIDE	4	0	0	100%		.156	.156	.174	.049	.138	.247
OXYCHLORDANE	4	0	0	100%		.164	.164	.160	.028	.124	.188
trans-NONACHLOR	4	0	0	100%		.194	.194	.199	.045	.155	.254
gamma-CHLORDANE	0	0	4	0%	.011	ND					
MIREX	0	0	4	0%	.011	ND					
1,2,3-TRICHLOROBENZENE	0	0	4	0%	.011	ND					
1,2,4-TRICHLOROBENZENE	1	0	3	25%	.011	ND	.015	.015		.015	.015
1,3,5-TRICHLOROBENZENE	0	0	4	0%	.011	ND					
1,2,3,4-TETRACHLOROBENZENE	0	0	4	0%	.011	ND					
1,2,3,5-TETRACHLOROBENZENE	0	0	4	0%	.011	ND					
1,2,4,5-TETRACHLOROBENZENE	0	0	4	0%	.011	ND					
PENTACHLOROBENZENE	0	0	4	0%	.011	ND					
HEXACHLOROBENZENE	4	0	0	100%		.062	.062	.056	.014	.035	.066
NAPHTHALENE	0	0	4	0%	.011	ND					
ACENAPHTHYLENE	0	0	4	0%	.011	ND					
ACENAPHTHENE	0	0	4	0%	.011	ND					
FLUORENE	0	0	4	0%	.011	ND					
PHENANTHRENE	0	0	4	0%	.011	ND					
FLUORANTHENE	0	0	4	0%	.011	ND					

Table A-6. (Continued)

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Mean LOD	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
PYRENE	0	0	4	0%	.011	ND					
CHRYSENE	0	0	4	0%	.011	ND					
MONOCHLOROBIPHENYLS	0	0	4	0%	.011	ND					
DICHLOROBIPHENYLS	0	0	4	0%	.011	ND					
TRICHLOROBIPHENYLS	3	0	1	75%	.011	.013	.013	.014	.003	.012	.017
TETRACHLOROBIPHENYLS	2	0	2	50%	.049	.046	.104	.104	.017	.093	.116
PENTACHLOROBIPHENYLS	4	0	0	100%		.154	.154	.125	.063	.030	.160
HEXACHLOROBIPHENYLS	4	0	0	100%		.218	.218	.193	.066	.095	.239
HEPTACHLOROBIPHENYLS	4	0	0	100%		.162	.162	.162	.009	.152	.173
OCTACHLOROBIPHENYLS	1	0	3	25%	.011	ND	.025	.025		.025	.025
NONACHLOROBIPHENYLS	2	0	2	50%	.011	.006	.017	.017	.008	.011	.023
DECACHLOROBIPHENYL	3	0	1	75%	.011	.016	.020	.018	.006	.011	.022

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.
The mean LOD is based on ND and TR measurements.
These multisplit samples were in the batches in which the fraction 2 extract was not analyzed.
Therefore, the fraction 2 compounds are not shown in the table.

Table A-7. Summary of HRGC/MS Measurements on Surrogate QA Compounds in all Samples, Percent Recovery.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
CHRYSENE-D12	77	0	0	100%	66%	66%	65%	10%	30%	96%
1,2,4-TRICHLOROBENZENE-d3	78	0	0	100%	29%	29%	29%	13%	9%	74%
13C6-1,2,4,5-TETRACHLOROBENZENE	78	0	0	100%	42%	42%	42%	14%	14%	80%
13C6-HEXACHLOROBENZENE	78	0	0	100%	57%	57%	56%	10%	30%	84%
13C6-4-CHLOROBIPHENYL	78	0	0	100%	63%	63%	63%	14%	34%	115%
13C12-3,3 ,4,4-TETRACHLOROBIPHENYL	77	0	0	100%	63%	63%	65%	15%	20%	108%
13C12-2,2 ,3,3 ,5,5 ,6,6-OCTACHLOROBIPHENYL	77	0	1*	99%	62%	62%	66%	14%	31%	111%
13C12-DECACHLOROBIPHENYL	77	0	1*	99%	68%	68%	68%	22%	24%	130%
DIETHYL PHTHALATE -3,4,5,6-d4	22	0	0	100%	69%	69%	64%	21%	23%	104%
DI-N-BUTYL PHTHALATE-3,4,5,6-d4	22	0	0	100%	38%	38%	41%	26%	5%	94%
BUTYL BENZYL PHTHALATE-3,4,5,6-d4	22	0	0	100%	26%	26%	41%	36%	1%	141%

* The measurement for 13C12-2,2 ,3,3 ,5,5 ,6,6-OCTACHLOROBIPHENYL and 13C12-DECACHLOROBIPHENYL with an ND qualifier had a comment indicating the chemical is present but not quantifiable.

Surrogate QA standards with positive measurements are presented as PQ even through no data qualifier was reported

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).

All concentrations are lipid adjusted.

Table A-8. Summary of GC/ECD Measurements on Method Blanks, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	0	0	12	0%	ND					
trans-NONACHLOR	0	0	12	0%	ND					
p,p -DDT	0	0	12	0%	ND					
o,p -DDT	0	0	12	0%	ND					
p,p -DDE	0	0	12	0%	ND					
o,p -DDE	0	0	12	0%	ND					
p,p -DDD	0	0	12	0%	ND					
o,p -DDD	0	0	12	0%	ND					
alpha-BHC	0	0	12	0%	ND					
beta-BHC	0	0	12	0%	ND					
gamma-BHC(LINDANE)	0	0	12	0%	ND					
delta-BHC	0	0	12	0%	ND					
DIELDRIN	0	0	12	0%	ND					
ENDRIN	0	0	12	0%	ND					
HEPTACHLOR	0	0	12	0%	ND					
HEPTACHLOR EPOXIDE	0	0	12	0%	ND					
OXYCHLORDANE	0	0	12	0%	ND					
MIREX	0	0	12	0%	ND					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-9. Summary of GC/ECD Measurements on the Porcine Fat Samples in Batches 1, 2, and 3, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	3	0	0	100%	.020	.020	.023	.006	.020	.030
HEXACHLOROBENZENE (Corrected)	3	0	0	100%	.040	.040	.037	.006	.030	.040
trans-NONACHLOR	3	0	0	100%	.060	.060	.060	.000	.060	.060
p,p -DDT	3	0	0	100%	.150	.150	.153	.015	.140	.170
o,p -DDT	0	0	3	0%	ND					
p,p -DDE	3	0	0	100%	1.200	1.200	1.177	.059	1.110	1.220
o,p -DDE	0	0	3	0%	ND					
p,p -DDD	0	0	3	0%	ND					
o,p -DDD	0	0	3	0%	ND					
alpha-BHC	0	0	3	0%	ND					
beta-BHC	3	0	0	100%	.090	.090	.093	.006	.090	.100
gamma-BHC(LINDANE)	0	0	3	0%	ND					
delta-BHC	0	0	3	0%	ND					
DIELDRIN	3	0	0	100%	.090	.090	.090	.010	.080	.100
ENDRIN	0	0	3	0%	ND					
HEPTACHLOR	0	0	3	0%	ND					
HEPTACHLOR EPOXIDE	3	0	0	100%	.030	.030	.030	.000	.030	.030
OXYCHLORDANE	3	0	0	100%	.040	.040	.040	.000	.040	.040
MIREX	0	3	0	100%	.100					
MIREX (Corrected)	0	3	0	100%	.100					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-10. Summary of GC/ECD Measurements on the Porcine Fat Samples in Batches 4 through 10, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	7	0	0	100%	.030	.030	.030	.000	.030	.030
HEXACHLOROBENZENE (Corrected)	7	0	0	100%	.050	.050	.049	.004	.040	.050
trans-NONACHLOR	7	0	0	100%	.120	.120	.117	.011	.100	.130
p,p -DDT	7	0	0	100%	.230	.230	.237	.020	.210	.260
p,p -DDT (Corrected)	7	0	0	100%	.170	.170	.171	.007	.160	.180
o,p -DDT	0	0	7	0%	ND					
p,p -DDE	7	0	0	100%	2.040	2.040	2.029	.110	1.820	2.150
o,p -DDE	0	0	7	0%	ND					
p,p -DDD	0	0	7	0%	ND					
o,p -DDD	0	0	7	0%	ND					
alpha-BHC	0	0	7	0%	ND					
beta-BHC	7	0	0	100%	.290	.290	.293	.018	.270	.320
gamma-BHC(LINDANE)	0	0	7	0%	ND					
delta-BHC	0	0	7	0%	ND					
DIELDRIN	6	0	0	100%	.040	.040	.042	.004	.040	.050
ENDRIN	0	0	7	0%	ND					
HEPTACHLOR	0	0	7	0%	ND					
HEPTACHLOR EPOXIDE	7	0	0	100%	.070	.070	.073	.011	.060	.090
PCBs	7	0	0	100%	.780	.780	.787	.083	.680	.950
OXYCHLORDANE	7	0	0	100%	.100	.100	.107	.013	.090	.120
MIREX	7	0	0	100%	.090	.090	.087	.005	.080	.090
MIREX (Corrected)	7	0	0	100%	.140	.140	.137	.010	.120	.150

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-11. Summary of GC/ECD Measurements on the paired Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	45	0	0	100%	.030	.030	.038	.036	.012	.216
HEXACHLOROBENZENE (Corrected)	45	0	0	100%	.044	.044	.056	.053	.013	.324
trans-NONACHLOR	44	0	0	100%	.181	.187	.172	.090	.026	.389
p,p -DDT	45	0	0	100%	.292	.292	.331	.191	.052	.943
o,p -DDT	0	0	45	0%	ND					
p,p -DDE	45	0	0	100%	1.821	1.821	2.322	1.784	.302	8.295
o,p -DDE	0	0	45	0%	ND					
p,p -DDD	0	0	45	0%	ND					
o,p -DDD	0	0	45	0%	ND					
alpha-BHC	0	0	45	0%	ND					
beta-BHC	42	3	0	100%	.139	.141	.143	.089	.023	.340
gamma-BHC(LINDANE)	0	0	45	0%	ND					
delta-BHC	0	0	45	0%	ND					
DIELDRIN	45	0	0	100%	.074	.074	.087	.070	.017	.492
ENDRIN	0	0	45	0%	ND					
HEPTACHLOR	0	0	45	0%	ND					
HEPTACHLOR EPOXIDE	45	0	0	100%	.120	.120	.122	.062	.026	.304
OXYCHLORDANE	41	4	0	100%	.091	.101	.097	.040	.030	.182
MIREX	0	0	45	0%	ND					
Total PCB	1	44	0	100%	TR	Coded Concentrations				

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-12. Summary of GC/ECD Measurements of Aldrin Recovery in All Samples Except Extracts, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
ALDRIN	73	0	0	100%	95%	95%	97%	16%	67%	146%

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-13. Summary of GC/ECD Measurements on the High Level Spiked Multisplit Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	4	0	0	100%	.077	.077	.083	.022	.064	.116
HEXACHLOROBENZENE (Corrected)	4	0	0	100%	.116	.116	.125	.030	.103	.167
trans-NONACHLOR	4	0	0	100%	.520	.520	.523	.042	.475	.578
p,p -DDT	4	0	0	100%	.359	.359	.359	.023	.334	.385
o,p -DDT	0	0	4	0%	ND					
p,p -DDE	4	0	0	100%	8.947	8.947	9.002	.736	8.164	9.949
o,p -DDE	0	0	4	0%	ND					
p,p -DDD	0	0	4	0%	ND					
o,p -DDD	0	0	4	0%	ND					
alpha-BHC	0	0	4	0%	ND					
beta-BHC	4	0	0	100%	.661	.661	.677	.048	.642	.745
gamma-BHC(LINDANE)	0	0	4	0%	ND					
delta-BHC	0	0	4	0%	ND					
DIELDRIN	4	0	0	100%	.802	.802	.780	.082	.668	.847
ENDRIN	0	0	4	0%	ND					
HEPTACHLOR	0	0	4	0%	ND					
HEPTACHLOR EPOXIDE	4	0	0	100%	.481	.481	.485	.053	.424	.552
OXYCHLORDANE	4	0	0	100%	.327	.327	.331	.055	.270	.398
MIREX	0	0	4	0%	ND					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-14. Summary of GC/ECD Measurements on the Low Level Spiked Multisplit Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	4	0	0	100%	.060	.060	.057	.006	.048	.060
HEXACHLOROBENZENE (Corrected)	4	0	0	100%	.090	.090	.084	.017	.060	.096
trans-NONACHLOR	4	0	0	100%	.210	.210	.210	.029	.180	.240
p,p -DDT	4	0	0	100%	.234	.234	.228	.017	.204	.240
o,p -DDT	0	0	4	0%	ND					
p,p -DDE	4	0	0	100%	2.311	2.311	2.320	.223	2.101	2.557
o,p -DDE	0	0	4	0%	ND					
p,p -DDD	0	0	4	0%	ND					
o,p -DDD	0	0	4	0%	ND					
alpha-BHC	0	0	4	0%	ND					
beta-BHC	4	0	0	100%	.192	.192	.198	.021	.180	.228
gamma-BHC(LINDANE)	0	0	4	0%	ND					
delta-BHC	0	0	4	0%	ND					
DIELDRIN	4	0	0	100%	.282	.282	.270	.046	.204	.312
ENDRIN	0	0	4	0%	ND					
HEPTACHLOR	0	0	4	0%	ND					
HEPTACHLOR EPOXIDE	4	0	0	100%	.192	.192	.186	.012	.168	.192
OXYCHLORDANE	4	0	0	100%	.168	.168	.159	.018	.132	.168
MIREX	0	0	4	0%	ND					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-15. Summary of GC/ECD Measurements on the Mid Level Spiked Multisplit Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	4	0	0	100%	.090	.090	.090	.009	.079	.102
HEXACHLOROBENZENE (Corrected)	4	0	0	100%	.136	.136	.136	.009	.124	.147
trans-NONACHLOR	4	0	0	100%	.418	.418	.427	.034	.395	.475
p,p -DDT	4	0	0	100%	.424	.424	.421	.049	.373	.463
o,p -DDT	0	0	4	0%	ND					
p,p -DDE	4	0	0	100%	4.887	4.887	4.870	.583	4.305	5.401
o,p -DDE	0	0	4	0%	ND					
p,p -DDD	0	0	4	0%	ND					
o,p -DDD	0	0	4	0%	ND					
alpha-BHC	0	0	4	0%	ND					
beta-BHC	4	0	0	100%	.316	.316	.319	.014	.305	.339
gamma-BHC(LINDANE)	0	0	4	0%	ND					
delta-BHC	0	0	4	0%	ND					
DIELDRIN	4	0	0	100%	.520	.520	.520	.041	.475	.565
ENDRIN	0	0	4	0%	ND					
HEPTACHLOR	0	0	4	0%	ND					
HEPTACHLOR EPOXIDE	4	0	0	100%	.373	.373	.370	.037	.328	.407
OXYCHLORDANE	4	0	0	100%	.237	.237	.243	.022	.226	.271
MIREX	0	0	4	0%	ND					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-16. Summary of GC/ECD Measurements on Extracts of the Unspiked Samples Associated with the High Level Spiked Multisplit Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	3	0	0	100%	.039	.039	.034	.007	.026	.039
HEXACHLOROBENZENE (Corrected)	3	0	0	100%	.051	.051	.056	.007	.051	.064
trans-NONACHLOR	3	0	0	100%	.231	.231	.218	.046	.167	.257
p,p -DDT	3	0	0	100%	.462	.462	.454	.065	.385	.513
o,p -DDT	0	0	3	0%	ND					
p,p -DDE	3	0	0	100%	3.582	3.582	3.530	.235	3.273	3.736
o,p -DDE	0	0	3	0%	ND					
p,p -DDD	0	0	3	0%	ND					
o,p -DDD	0	0	3	0%	ND					
alpha-BHC	0	0	3	0%	ND					
beta-BHC	3	0	0	100%	.205	.205	.210	.032	.180	.244
gamma-BHC(LINDANE)	0	0	3	0%	ND					
delta-BHC	0	0	3	0%	ND					
DIELDRIN	3	0	0	100%	.103	.103	.094	.015	.077	.103
ENDRIN	0	0	3	0%	ND					
HEPTACHLOR	0	0	3	0%	ND					
HEPTACHLOR EPOXIDE	3	0	0	100%	.193	.193	.193	.039	.154	.231
OXYCHLORDANE	3	0	0	100%	.167	.167	.163	.020	.141	.180
MIREX	0	0	3	0%	ND					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-17. Summary of GC/ECD Measurements on Extracts of the Unspiked Samples Associated with the Low Level Spiked Multisplit Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	3	0	0	100%	.024	.024	.024	.000	.024	.024
HEXACHLOROBENZENE (Corrected)	3	0	0	100%	.036	.036	.036	.000	.036	.036
trans-NONACHLOR	3	0	0	100%	.120	.120	.124	.030	.096	.156
p,p -DDT	3	0	0	100%	.228	.228	.244	.028	.228	.276
o,p -DDT	0	0	3	0%	ND					
p,p -DDE	3	0	0	100%	1.417	1.417	1.381	.189	1.176	1.549
o,p -DDE	0	0	3	0%	ND					
p,p -DDD	0	0	3	0%	ND					
o,p -DDD	0	0	3	0%	ND					
alpha-BHC	0	0	3	0%	ND					
beta-BHC	3	0	0	100%	.096	.096	.096	.000	.096	.096
gamma-BHC(LINDANE)	0	0	3	0%	ND					
delta-BHC	0	0	3	0%	ND					
DIELDRIN	3	0	0	100%	.060	.060	.060	.000	.060	.060
ENDRIN	0	0	3	0%	ND					
HEPTACHLOR	0	0	3	0%	ND					
HEPTACHLOR EPOXIDE	3	0	0	100%	.084	.084	.088	.007	.084	.096
OXYCHLORDANE	3	0	0	100%	.072	.072	.068	.007	.060	.072
MIREX	0	0	3	0%	ND					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

Table A-18. Summary of GC/ECD Measurements on Extracts of the Unspiked Samples Associated with the Mid Level Spiked Multisplit Composite Samples, ug/g.

Chemical Name	Number of Measurements by Data qualifier PQ	TR	ND	Percent Detected	Overall Median	For Positively Quantified Measurements Median	Mean	Std.	Min.	Max.
HEXACHLOROBENZENE	3	0	0	100%	.034	.034	.034	.000	.034	.034
HEXACHLOROBENZENE (Corrected)	3	0	0	100%	.045	.045	.049	.007	.045	.057
trans-NONACHLOR	3	0	0	100%	.192	.192	.196	.017	.181	.215
p,p -DDT	3	0	0	100%	.407	.407	.411	.017	.395	.429
o,p -DDT	0	0	3	0%	ND					
p,p -DDE	3	0	0	100%	1.751	1.751	1.714	.162	1.537	1.853
o,p -DDE	0	0	3	0%	ND					
p,p -DDD	0	0	3	0%	ND					
o,p -DDD	0	0	3	0%	ND					
alpha-BHC	0	0	3	0%	ND					
beta-BHC	3	0	0	100%	.113	.113	.113	.000	.113	.113
gamma-BHC(LINDANE)	0	0	3	0%	ND					
delta-BHC	0	0	3	0%	ND					
DIELDRIN	3	0	0	100%	.090	.090	.094	.007	.090	.102
ENDRIN	0	0	3	0%	ND					
HEPTACHLOR	0	0	3	0%	ND					
HEPTACHLOR EPOXIDE	3	0	0	100%	.158	.158	.154	.007	.147	.158
OXYCHLORDANE	3	0	0	100%	.113	.113	.109	.007	.102	.113
MIREX	0	0	3	0%	ND					

Percent detected is defined as the number (PQ+TR)/(PQ+TR+ND).
All concentrations are lipid adjusted.

APPENDIX B: CONVERSION FROM STANDARD DEVIATION OF LOG TRANSFORMED DATA TO COEFFICIENT OF VARIATION

When fitting models to the log transformed data, the estimated error variance is for the transformed data. It may be desirable to convert the variance in the log scale to a coefficient of variation in the original scale. The following formula relates the variance of the log data, s^2, to the coefficient of variation of the original measurements, cv:

$$cv = \sqrt{\exp(s^2)-1} \qquad (B.1)$$

For reference, Table B-1 tabulates the coefficient of variation for selected values of s.

Table B-1. Coefficient of Variation for the Untransformed Data for Selected Values of s, the Standard Deviation of the Log Transformed Data

s	0.00	0.02	0.04	0.06	0.08
0.0	.000	.020	.040	.060	.080
0.1	.100	.120	.141	.161	.181
0.2	.202	.223	.243	.264	.286
0.3	.307	.328	.350	.372	.394
0.4	.417	.439	.462	.485	.509
0.5	.533	.557	.582	.607	.632
0.6	.658	.685	.711	.739	.767
0.7	.795	.824	.854	.884	.915
0.8	.947	.979	1.012	1.046	1.081
0.9	1.117	1.154	1.191	1.230	1.270
1.0	1.311	1.353	1.396	1.441	1.487

The coefficient of variation is read at the intersection of the "row" and "column" defined such that the value in the left column of the "row" and the top row in the "column" add to s.

APPENDIX C: RECOVERY FROM MULTISPLIT SAMPLES

Table C-1. Recovery for Spiked Compounds in Multisplit Samples Analyzed Using the HRGC/MS Method.

Compound	Average Recovery ±95% Confidence Interval	Average Recovery by Spike Level	Spike Level (ug/g)	Recovery Based on Samples in Same Batch	Recovery Based on Samples in Different Batches
p,p'-DDE (a)	26±50%	23%	1.00	42%	5%
		42%	3.00	45%	39%
		14%	5.00	9%	19%
Beta-BHC	99±50%	201%	0.10	322%	81%
		32%	0.20	30%	34%
		64%	0.40	196%	-68%
Dieldrin (b)	37±61%	43%	0.20	42%	44%
		-	0.40	-	-
		31%	0.60	62%	-
Heptachlor Epoxide	50±50%	46%	0.10	47%	44%
		59%	0.20	45%	72%
		46%	0.30	42%	49%
Oxychlordane (c)	42±50%	37%	0.10	42%	33%
		76%	0.15	78%	73%
		14%	0.20	23%	4%
trans-Nonachlor (a)	56±50%	48%	0.10	96%	-1%
		88%	0.20	104%	72%
		31%	0.30	38%	24%
Hexachlorobenzene (uncorrected)	41±50%	53%	0.05	56%	49%
		32%	0.08	41%	23%
		38%	0.11	35%	41%

(a) Unspiked sample for mid spike level is missing (footnote says "compound is present but cannot be quantitated" and no LOD was provided), replaced by 0. As a result of substituting zero for the missing unspiked concentration, the calculated recovery will tend to overestimate of the actual recovery.

(b) Unspiked sample for low spike level is below the LOD, replaced by LOD/2. Between batch recovery for low spike level based on a single value.

(c) Unspiked sample for low spike level is below the LOD, replaced by LOD/2.

Table C-2. Recovery for Spiked Compounds in Multisplit Samples Analyzed Using the GC/ECD Method.

Compound	Average Recovery ±95% Confidence Interval	Average Recovery by Spike Level	Spike Level (ug/g)	Recovery Based on Samples in Same Batch	Recovery Based on Samples in Different Batches
p,p'-DDE	82±19%	64%	1.00	49%	80%
		98%	3.00	97%	100%
		82%	5.00	74%	90%
Beta-BHC	89±19%	85%	0.10	75%	95%
		91%	0.20	93%	90%
		92%	0.40	99%	85%
Dieldrin	90±19%	88%	0.20	98%	78%
		95%	0.40	95%	95%
		88%	0.60	91%	85%
Heptachlor Epoxide	83±19%	75%	0.10	80%	70%
		99%	0.20	103%	95%
		76%	0.30	75%	77%
Oxychlordane	73±19%	73%	0.10	80%	65%
		83%	0.15	80%	87%
		64%	0.20	63%	65%
trans-Nonachlor	73±19%	45%	0.10	65%	25%
		104%	0.20	100%	107%
		69%	0.30	75%	63%
Hexachlorobenzene (corrected)	77±19%	80%	0.05	60%	100%
		100%	0.08	106%	94%
		52%	0.11	36%	68%
Hexachlorobenzene (uncorrected)	53±19%	55%	0.05	56%	60%
		63%	0.08	69%	56%
		41%	0.11	32%	50%

Table C-3. Recovery for Spiked Compounds in Multisplit Samples Analyzed Using the GC/ECD Method, with Unspiked Concentrations Measured in Extracts

Compound	Average Recovery ±95% Confidence Interval	Average Recovery by Spike Level	Spike Level (ug/g)	Recovery Based on Samples in Same Batch	Recovery Based on Samples in Different Batches
p,p'-DDE	85±14%	75%	1.00	49%	101%
		94%	3.00	97%	92%
		84%	5.00	74%	95%
Beta-BHC	89±14%	85%	0.10	75%	95%
		91%	0.20	93%	90%
		91%	0.40	99%	84%
Dieldrin	90±14%	88%	0.20	98%	78%
		94%	0.40	95%	94%
		89%	0.60	91%	87%
Heptachlor Epoxide	84±14%	80%	0.10	80%	80%
		96%	0.20	103%	90%
		76%	0.30	75%	77%
Oxychlordane	73±14%	75%	0.10	80%	70%
		80%	0.15	80%	80%
		65%	0.20	63%	68%
trans-Nonachlor	81±14%	65%	0.10	65%	65%
		103%	0.20	100%	105%
		77%	0.30	75%	78%
Hexachlorobenzene (corrected)	82±14%	83%	0.05	65%	100%
		94%	0.08	100%	88%
		69%	0.11	75%	64%
Hexachlorobenzene (uncorrected)	51±14%	55%	0.05	50%	60%
		63%	0.08	69%	56%
		36%	0.11	32%	41%

APPENDIX D: DESCRIPTION OF THE ANALYTICAL PROCEDURES

D.1 Summary of the MOG-GC/ECD Procedure

The modified Mills Olney Gaither (MOG) packed column gas chromatography/electron capture detector (PGC/ECD) procedure was used for a number of years as the standard NHATS method for detecting and quantifying chlorinated pesticides and PCBs in samples of adipose tissue. The MOG-PGC/ECD procedure has five major components:

(1) Extraction;

(2) Partitioning;

(3) Florisil clean-up;

(4) Dilution; and

(5) Identification and quantitation.

For the 1984 Comparability Study, the normal extraction step was modified. Extraction of composite samples was carried out by MRI. The extracts were split, with one portion reserved for the MOG-PGC/ECD analysis and another portion reserved for the HRGC/MS analysis. The MOG-PGC/ECD procedure in the 1984 Comparability Study is described below. This description is for single-split composite samples, the multisplit composite samples, the extracts of the multisplit composite samples, and the regenerated composite samples and their method blanks.

In the extraction step, a 25 gram composite was placed in a culture tube. Next, 10 milliliters (mL) of methylene chloride were added to the culture tube, and the entire contents of the tube were blended in a Tekmar tissuemizer. The blended mixture was allowed to separate and the methylene chloride layer was transferred to a filter funnel containing glass wool and sodium sulfate. The mixture resulting after eluting through the filter funnel was collected in a 100 mL flask.

Another 10 mL of methylene chloride were added to the sediment, and the process of blending, separating, transferring to funnel, and storing in flask was repeated. The addition of 10 mL of methylene chloride were repeated one or two more times.

The culture tube was rinsed with methylene chloride, the rinse mixture added to the funnel, the funnel rinsed, and the final output from the funnel was added to the 100 mL flask. A sufficient amount of methylene chloride was added to the flask to bring the volume of the mixture in the flask to 100 mL.

For some samples, particles remained in the mixture in the flask. These samples were refiltrated, and the final volume for these samples was 200 mL.

The percentage of the composite sample that was lipid tissue was determined as follows. A glass vial was weighed to the nearest 0.0001 gram. One mL of the final mixture was transferred to the vial. The volume of the mixture in the vial was reduced until only an oily residue was left. The vial was again weighed to the nearest 0.0001 gram, and the percentage of the composite sample that was lipid material was calculated.

Of the remaining volume for the sample, approximately 20% was designated for the MOG-PGC/ECD procedure, with the remainder designated for the HRGC/MS procedure. The appropriate volumes were concentrated to remove most of the methylene chloride. These concentrated volumes were transferred to vials for shipment to CSU.

After receipt at CSU, 1 microgram of the internal standard, aldrin, was added or "spiked" to the sample designated for the MOG-PGC/ECD method. The sample was then ready for the next step in the procedure.

In the partitioning step, the sample was transferred to a 125 mL separatory funnel by rinsing with hexane. Next, hexane and acetonitrile were mixed together in a separate container. About 30 mL of the hexane/acetonitrile mixture were added to the separatory funnel. The funnel was shaken for 2 minutes, and layers were allowed to separate. The lower layer was then drained into a 1-liter separator to which 550 mL of a 2% sodium sulfate solution and 100 mL of hexane had previously been added. The process of adding 30 mL of hexane/acetonitrile mixture to the funnel, shaking the funnel for 2 minutes, allowing layers to separate, and draining the lower layer into the 1-liter separator was repeated three more times.

Next, the 1-liter separator was inverted, and layers were allowed to separate. The lower layer was discarded. The contents of the 1-liter separator were washed twice with 100 mL of 2% sodium sulfate solution. After separation of layers in the 1-liter separator, the lowest layer was discarded. Excess water was drained. The remaining contents of the 1-liter separator were transferred to a 500 mL boiling flask. The contents of this flask were reduced to 3 to 5 mL through rapid evaporation.

The third step was Florisil cleanup. A reservoir column was prepared with one half to 1 inch of sodium sulfate on the bottom, 4 inches of Florisil in the middle, and an inch and a half of sodium sulfate on the top. A solution of 6% diethyl ether in hexane and a solution of 15% diethyl ether in hexane were prepared.

Approximately 100 mL of hexane was added to the column and allowed to flow through the column. This "rinse" hexane was discarded. A 500 mL boiling flask labeled "6%" was placed under the column to collect the output of the column. Then the 3 to 5 mL from the partitioning step was added to the column. Next, 200 mL of the 6% solution mix were added to the column.

At this point, a 500 mL boiling flask labeled "15%" was prepared with 0.2 micrograms of aldrin. When the 6% solution mix reached the sodium sulfate at the top of the column, the 6% receiver boiling flask was replaced with the 15% boiling flask. Two hundred mL of the 15 percent solution mix were added to the column, and the column was allowed to drain into the receiver flask labeled 15%.

The contents of both the 6% receiver flask and the 15% receiver flask were reduced to 3 to 5 mL through rapid evaporation.

In the dilution step, the contents of the 6% and the 15% flasks were transferred to separate centrifuge tubes. A volume of hexane was added to each centrifuge tube in accordance with an established algorithm.

In the identification and quantitation step, a 5-microliter sample from each centrifuge tube was injected separately into a gas chromatograph. Compounds were identified by their retention time relative to the chromatographic "peak" representing aldrin. Concentrations of compounds were quantitated based on the area of the peak representing the compound.

The limits of quantification and the limits of detection for the MOG-PGC/ECD method have been established from years of experience with the method. For samples for which quantitated concentrations were less than the limit of detection, a final concentration of zero was reported. For samples for which the quantitated concentration was between the limit of detection and the limit of quantification, the limit of quantification was reported as the final concentration of the sample. The label "Trace" was assigned in this case. For all other samples, the quantitated concentration was reported as the final concentration.

The recovery of aldrin was calculated as a quality assurance step on the entire process. However, this recovery was not used to correct the compound concentrations. Concentrations for hexachlorobenzene and, in some cases, mirex and p,p'-DDT were computed on both a corrected and an uncorrected basis. The correction factors were based on historical information on the recovery of these three compounds at the partitioning step. The concentrations of the other chemicals were not corrected for recovery.

D.2 Summary of the HRGC/MS Procedure

In order to expand the list of chemicals that could be monitored by the NHATS, the standard NHATS method for detection and quantification of chemical compounds was changed from MOG-PGC/ECD to High Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS). The HRGC/MS procedure has five major components:

(1) Extraction;

(2) Gel permeation chromatography;

(3) Florisil clean-up;

(4) Addition of internal standards; and

(5) Identification and quantification.

The HRGC/MS procedure used in the 1984 Comparability Study is described below. The description is for single-split composite samples. The same basic procedure, with appropriate modifications, was followed for the method blanks, the quality control samples, the multisplit composite samples, and the regenerated composite samples and their method blanks.

The HRGC/MS procedure began with the extraction step described for the MOG-PGC/ECD procedure. A volume designated for the HRGC/MS procedure was placed in a vial and shipped to CSU.

After receipt at CSU, the sample volume was spiked with a known amount of the 11 surrogate compounds. The extraction step was continued as follows.

Twenty mL of methylene chloride were added to the sample, and the sample and the methylene chloride were transferred to a culture tube. The rest of the extraction step followed the same process as described in the extraction step for the MOG-PGC/ECD procedure. Ten mL of methylene chloride were added to the culture tube, and the entire contents of the tube were blended in a Tekmar tissuemizer. The blended mixture was allowed to separate into a sediment and a methylene chloride layer. The methylene chloride layer was transferred to a filter funnel containing glass wool and sodium sulfate. The mixture resulting from the filter funnel was stored in a 100-mL flask.

Another 10 mL of methylene chloride were added to the sediment, and the process of blending, separating, transferring to funnel, and storing in flask was repeated. The addition of 10 mL of methylene chloride and the subsequent steps were repeated 1 or 2 more times.

The culture tube was rinsed with methylene chloride, the rinse mixture added to the funnel, the funnel rinsed, and the final output from the funnel was added to the 100-mL flask. A sufficient amount of methylene chloride was added to the flask to bring the volume of the mixture in the flask to 100 mL.

The mixture in the flask was transferred to a 250-mL evaporator. The volume in the evaporator was reduced to a volume of 10 to 20 mL. The contents of the evaporator were transferred to a vial. A sufficient amount of methylene chloride was added to the vial so that the vial contained about 0.25 grams of lipid material for each mL of mixture. The mixture in the vial was the final extract, and this extract was passed to the next step.

The next step in the procedure was gel permeation chromatography. The purpose of this step was to separate the target analytes from the lipid material. A column of Bio-Beads SX-3 in dichloromethane was prepared.

The sample extracts were filtered to remove particles that might interfere with the flow of the column. Then the extracts were transferred to the column in successive aliquots. The output of the column was collected in amber bottles sized 1 to 4 liters.

The contents of the amber bottles were transferred to an evaporator, and reduced to a volume of about 10 mL. Next, 50 mL of hexane were added to the evaporator. Then the volume of the evaporator was reduced, first to 10 mL and then to 1 mL.

If the 1 mL volume was unusually colored or viscous, 2 to 5 mL of methylene chloride were added, and the resulting volume of 3 to 5 mL was injected into the column. The output of the column was collected in amber bottles, placed in the evaporator, and again reduced to 1 mL.

A Florisil column was prepared by placing glass wool at the bottom of the column, adding 100 mL of hexane to the column, then adding 12.5 grams of Florisil, and finally placing a one-half inch layer of sodium sulfate on top of the Florisil. The hexane was drained so that the top of the hexane was level with the top of the sodium sulfate.

The 1 mL mixture from the previous step was transferred to the top of the column. A 500 mL flask was placed underneath the column. The column was drained until the sodium sulfate layer was almost exposed. At that point, 200 mL of a 6% solution of ethyl ether in hexane were added to the column at a rate of approximately 5 mL per minute. When the sodium sulfate layer was almost exposed, the flask underneath the column was replaced with a second 500 mL flask, and 200 mL of a 15% solution of ethyl ether

in hexane were added to the column. When the sodium sulfate layer was almost exposed, 200 mL of a 50% solution of ethyl ether in hexane were added to the column, without any change in the flask under the column.

After the column drained into the second collector flask, the output of the column was contained in two separate 500 mL flasks. The contents of the first collector flask are referred to as the "first fraction", and the contents of the second flask are referred to as the "second fraction". The first and second fractions were concentrated separately, first to 5 to 10 mL, then to less than 1 mL, and then to less than one-half mL. The final volume of each fraction was stored in two separate vials with Teflon-lined screw tops. The vials were stored at 4 degrees centigrade.

The volumes for the two fractions were each reduced to 0.2 mL. Known amounts of the three internal standards, anthracene-d10, naphthalene-d8, and benzo (a) anthracene-d12, were added to each of the fractions. A 1- to 2- microliter aliquot of each fraction was injected into the gas chromatograph/mass spectrometer.

The final step was identification and quantification. There were 57 target analytes for the HRGC/MS analysis. Associated with each of the 57 were a primary mass fragment and two secondary mass fragments. In addition, one of the three internal standards was designated as the appropriate internal standard for each target analyte for purposes of identification and quantification.

In order for an analyte to be identified, the following four criteria had to be satisfied:

(1) The primary and secondary masses had to achieve their maximum values within a specified time span;

(2) The retention time of the primary and secondary mass fragments relative to the designated internal standard

had to be within 10 seconds of the known relative retention time of the analyte;

(3) The relative abundances of the primary and secondary masses all had to be within 20% of the relative abundances in the reference spectrum of the analyte;

(4) The abundances of the primary and secondary masses all had to exceed three times the background signal to noise ratio.

Quantitation was carried out as follows. The chromatograph/mass spectrometer was calibrated. In calibration, known amounts of the target analyte and a known amount of the internal standard were injected into the instrument. The calibration was done with five different amounts of the target analyte. A relative response factor was calculated for each analyte from the calibration data for use in quantitation. The lower calibration limit was also used in quantitation, as described below.

For a composite sample, the amount of the target analyte present was calculated from the abundance of the primary mass of the analyte, the abundance of the primary mass of the internal standard, the amount of the internal standard, and the relative response factor. This calculated amount was given a label of "Positive Quantifiable" if the abundances of the primary and secondary masses all exceeded 10 times the background signal to noise ratio and the lower calibration limit was exceeded. A label of "Trace" was to be assigned to an amount for which 1) the primary and secondary masses were all above three times the signal to noise ratio, 2) one of the primary or secondary masses was between 3 and 10 times the background signal to noise ratio, and 3) the lower calibration limit was exceeded.

For samples for which 1) at least one of the mass abundances was less than three times the signal to noise ratio and 2) the lower calibration limit was exceeded, a label of "Not Detected" was assigned. The detection limit was the maximum of the lower

calibration limit and three times the signal to noise ratio. For samples for which there was no response or the response was less than the lower calibration limit, a label of "Not Detected" was assigned. The detection limit was the lower calibration limit. For samples for which a response above the lower calibration limit was observed, but the identification criteria were not met, a remark was made indicating an interference was present. The detection limit was quantitated based on the response observed.

Cases not covered above were handled by inclusion of written remarks indicating what was observed.

Concentrations of analytes were computed from the calculated amounts, the weight of the composite sample, and the percent lipid in the composite sample. No concentrations were corrected for recovery. The recoveries of the surrogate compounds were calculated as a check on method performance.

APPENDIX E: DISCUSSION OF THE VARIANCE COMPONENTS

In order to provide a more intuitive understanding of the variance components, this appendix discusses the various terms in the model in the context of the lab procedures which might have contributed to each term. This presentation is not meant to imply that the possible causes of error are limited to those discussed here or that the those discussed here are more important than other sources of error.

The discussion below assumes the following simplified procedure for the HRGC/MS measurement method:

> The analysis procedures, laboratory equipment, and solutions are prepared for each batch, including the internal standards spiking solution with three internal standards. The HRGC/MS equipment is calibrated using a set of six dilutions of a standard calibration solution. From the calibration data, a relative response factor (RRF) is calculated for each compound. The RRF relates the target compound response to the internal standard response.
>
> The sample preparation involves measuring the wet weight and percent lipid followed by several processing steps which move the target compounds from the tissue sample to the final extract and adjust the volume of the final extract to 200 uL. A measured amount of the internal standard solution is spiked into the final extract before a portion of the final extract is injected into the HRGC/MS.
>
> The primary output from the HRGC/MS is a response trace with multiple peaks. The area below the internal standard peak, the area below the peak for the target compound, the wet weight and percent lipid, and the relative response factor are used to quantitate the amount of each target compound (see equation (3.3)).

Measurement errors can result from these processing steps due to small variations in: (1) the measurement of weights and volumes or the area under a mass spectrometer peak (due partly to uncertainty in assessing the background response); (2) the rounding in calculation results; and (3) the equipment setup and sample processing steps (possibly due to age of the reagents, the equipment condition, or the time spent on the processing of each

sample). Large variations due to measuring, processing, or calculation errors will also contribute to the variance components.

The model assumes that the measurements for each compound (on a log scale) vary around a constant equal to the product of the true concentration and the method recovery, R_{mct}, which are constant for each compound.

$$\ln(X_{mcbsi}) = \ln(C_{cs}R_{mct}) + \text{error} \qquad \text{(E.1)}$$

Variation of the method recoveries from year to year or lab to lab are not considered. Variation or measurement error around this expected value can be separated into the components shown in the model. Table E-1 provides a description of each component and an example of processing steps which might contribute to each component.