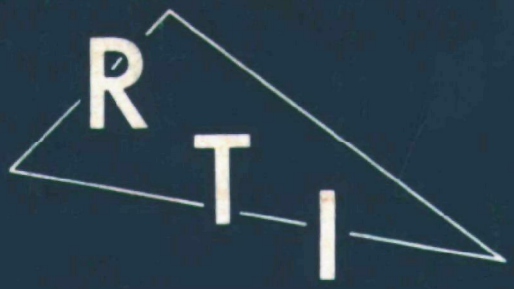


RESEARCH TRIANGLE INSTITUTE

Draft Report

Statistical Analysis of Mirex Special Study Data

June 3, 1980

by

Carol Leininger
Donna Lucas
Charles Sparacino
Stephen Williams

Prepared for

Task Manager: Cindy Stroup
Project Officer: Dr. Joseph Carra

Design and Development Branch
Survey and Analysis Division
Office of Pesticides and Toxic Substances
U. S. Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460

Under EPA Contract Number 68-01-5848

DRAFT COPY

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont.)

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Mirex is an insecticide which has a specific geographic region of application in the United States. The Mirex Special Study was undertaken in 1975 as part of the National Human Monitoring Program in an attempt to obtain information about the levels of mirex in human adipose tissues in areas of mirex application. A sample of human adipose tissue specimens was selected from the mirex treated areas in eight southern States. Detectable levels of mirex were found in 141 chemical analyses of 624 human adipose tissue specimens in the sample. The data were analyzed with respect to three demographic variables (race, age, and sex) and two geographic variables (Census Division and State). Geographic divisions appear to be the most salient factors in levels of concentration of mirex in the sample studied.

1. INTRODUCTION

1.1 Foreword

This report presents a synopsis of the research activities involved in Task 17 of EPA contract number 68-01-5848, Statistical Analysis of Mirex Special Study Data. Chapter 2 describes the survey design employed in the Mirex Special Study. A rationale of the statistical analysis approach is also included in Chapter 2. Chapter 3 is a discussion of the chemical analysis. Chapters 4 and 5 are presentations of unweighted and weighted statistical analyses, respectively.

1.2 Summary of Study

Mirex is an organochlorine insecticide widely used for the control of the imported fire ant in large areas of the southern United States. It is chemically resistant to degradation, which, in combination with the substance's pharmacodynamics, leads to environmental mobility and potential incorporation into various food chains.

Mirex residues have been found in specimens of human adipose tissue, a discovery which led to the Mirex Special Study within the organization of the National Human Monitoring Program (NHMP). From October 1975 through September 1976, 630 human adipose tissue specimens were collected from selected surgical patients and cadavers. The sample was selected within a study area defined as all counties having some application of the insecticide mirex between 1965 and 1974. Mirex residues were found in 141 of the 624 tissue specimens analyzed.

The definitions of the study area, sample design, sample selection, specification of the procedures for collecting specimens in the field, and specification of chemical analysis were undertaken by the NHMP within the Environmental Protection Agency (EPA).

The statistical analyses and assessment of chemical analysis were performed by Research Triangle Institute, Research Triangle Park, North Carolina, under EPA contract number 68-01-5848. EPA Task Manager was Cindy Stroup and EPA technical assistant was Sandra Strassman-Sundy.

RTI personnel with major responsibilities were: S. R. Williams, Project Director; Dr. D. Lucas, Task Leader; Dr. C. Sparacino, chemist; and C. Leininger, statistician.

1.3 RTI Objectives and Findings

1.3.1 Objectives

RTI had several primary objectives for Task 17, Statistical Analysis of MSS Data. These were: investigate and document the MSS survey design, calculate quasi selection weights, assess the assumptions and limitations of the design, verify the chemical analysis results, and analyze the weighted and unweighted data for all 624 specimens and for the 123 quantifiable positive values.

1.3.2 Findings

This report and the accompanying appendices draw together the information about the MSS provided to RTI by EPA staff and the results of the RTI staff work on design assessment. The method of selection weight calculation is outlined briefly.

The chemical analysis results are quite solid and are not a problem in this study.

RTI considers the weighted regression and weighted chi-square to be the most sensitive and most appropriate tests for describing the study population. Usually, results of an unweighted analysis should not even be presented, especially in the face of extreme disproportionate sampling, because it is easy to misinterpret such results. The unweighted results

are presented here because the selection weights could not be founded entirely on probability of selection.

The results of the unweighted analysis indicate that the location of the collection site is the most important factor in the differences in number of positive detections of mirex in human adipose tissue. In unweighted regression analysis of the 123 quantifiable observations, demographic factors and geographic factors did not show any effect in the degree of mirex concentration in the sample specimens. The results of the unweighted chi-square tests are significant ($p<0.0001$) for State and Census Division, and marginally significant for the factors age and race ($p<0.10$).

In the weighted analyses location of the selection site is again the major variable of interest. State is a significant ($p<0.0001$) factor in both weighted chi-square and weighted regression analyses of the proportion of positive mirex detection. The weighted regression analysis for the quantifiable residue amounts indicates an interaction between geographic location and race. This suggests that there is a race effect for some geographic divisions that is not particularily strong throughout the mirex treated area. This interpretation is corroborated by the results of the weighted chi-square tests.

2. THE NATIONAL HUMAN MONITORING PROGRAM'S SPECIAL STUDY OF MIREX

2.1 Detailed Description of the Study

2.1.1 Introduction

Human adipose tissue specimens for the Mirex Special Study (MSS) were collected from October 1, 1975 through September 30, 1976 (FY76). The target population was the human population of all counties where mirex was sprayed at any time during the 10-year period 1965 to 1974 [1]. A list of these counties was provided to the NHMP manager by the U.S. Department of Agriculture. Maps of the eight southern States containing these counties are displayed in Appendix A. A list of the mirex counties, by State, is given in Appendix B. The pattern of mirex use can be charted directly to the areas of infestation of imported fire ants in this country. This infestation pattern does not conform to large-scale geographic or political boundaries, such as Census divisions or States, as can be seen by examining the maps in Appendix A. No information was provided concerning the amount or frequency of mirex application over the 10-year period. Such information might be extremely important in determining levels of expected exposure to this insecticide; for further discussion of this point see Appendix C.

2.1.2 Sample Design

Mirex residues were found in human adipose tissue specimens submitted to the NHMP, which was designed to provide estimates at the national level. There were eight NHMP cities in mirex areas where established contacts (hospital pathologists or medical examiners) were already submitting adipose tissue specimens for the NHMP. A special study was designed to increase representation from mirex regions by increasing the number of sample

sites in mirex areas. 32 supplementary sites were selected, bringing the total number of sites contacted in the MSS to 40.

To select the 32 supplementary areas for the MSS, a listing was made of all 1974 Ranally Metropolitan Areas (RMA's) in the mirex area: RMA's were defined on a township and locality basis. All areas with a total population of 50,000 or more were considered to be RMA's. Other areas that presently had populations close to 50,000, had populations greater than 50,000 in the past, or had special significance in their States were also defined as RMA's [2]. The complete listing of the population from which a sample will be selected is called a frame. The eight NHMP sites were on the frame of 68 RMA's located in the mirex area. These 8 cities were not included as possible selections for the mirex supplementary sample because they were already included as part of the MSS sample by virtue of their initial selection into the NHMP.

Of the 60 RMA's in the mirex supplementary list, 32 were apparently selected by simple random sampling (SRS). The 60 RMA's were listed in alphabetical order (not pertinent to the selection process), and then a SRS was selected from the list. See Appendix D for a discussion of the site selection methodology employed. Nine additional sites were selected, in the same manner, to be used as alternates in case there were nonresponding sites. These alternates were to be substituted for nonresponding sites in the order in which they were selected. One site was replaced by an alternate selection. A list of the selected sites and alternates is given in Appendix E.

The selection of sites within mirex areas produced 40 sample RMA's. Of these, 5 of the 8 NHMP sites and 23 of the 32 mirex supplementary sample RMA's responded with valid tissue specimens. It is not known if any of the

nonresponding RMA's sent tissue samples that did not fit EPA specifications. The treatment of nonresponding sites is described in section 2.3. Figure 2.1 outlines the path of the sample selection procedures for obtaining tissue specimens for the MSS.

Data collection for the MSS after the selection of first-stage RMA's follows the methods used in the Adipose Tissue Survey (ATS) of the NHMP. For the convenience of the readers, a brief discussion of the NHMP is now included; a more detailed description is contained in a 1979 EPA document [3].

2.2 National Human Monitoring Program

2.2.1 Introduction

The statistical design used to collect data for the ATS of the NHMP has two stages. The contiguous 48 states were stratified into several regions. Sampling sites were selected from a list of eligible places with probability proportional to population. Within the sampling sites, the subsampling was performed by cooperating pathologists and medical examiners.

2.2.2 Strata

In FY70-FY72 the contiguous 48 states were stratified by Census Region. Beginning in FY73 the strata were changed to Census divisions. In the earlier period, the number of sites selected within each stratum was determined by its population as given in the 1960 Census. In FY73, the allocations were revised based on 1970 Census data.

2.2.3 Eligible Places

In FY70-72, the eligible places were cities with populations greater than 25,000 persons based on the 1960 Census. In FY73-76, the eligible places were cities greater than 25,000 based on the 1970 Census. Sample sites were selected in FY73 and followed through FY76. The sample sites

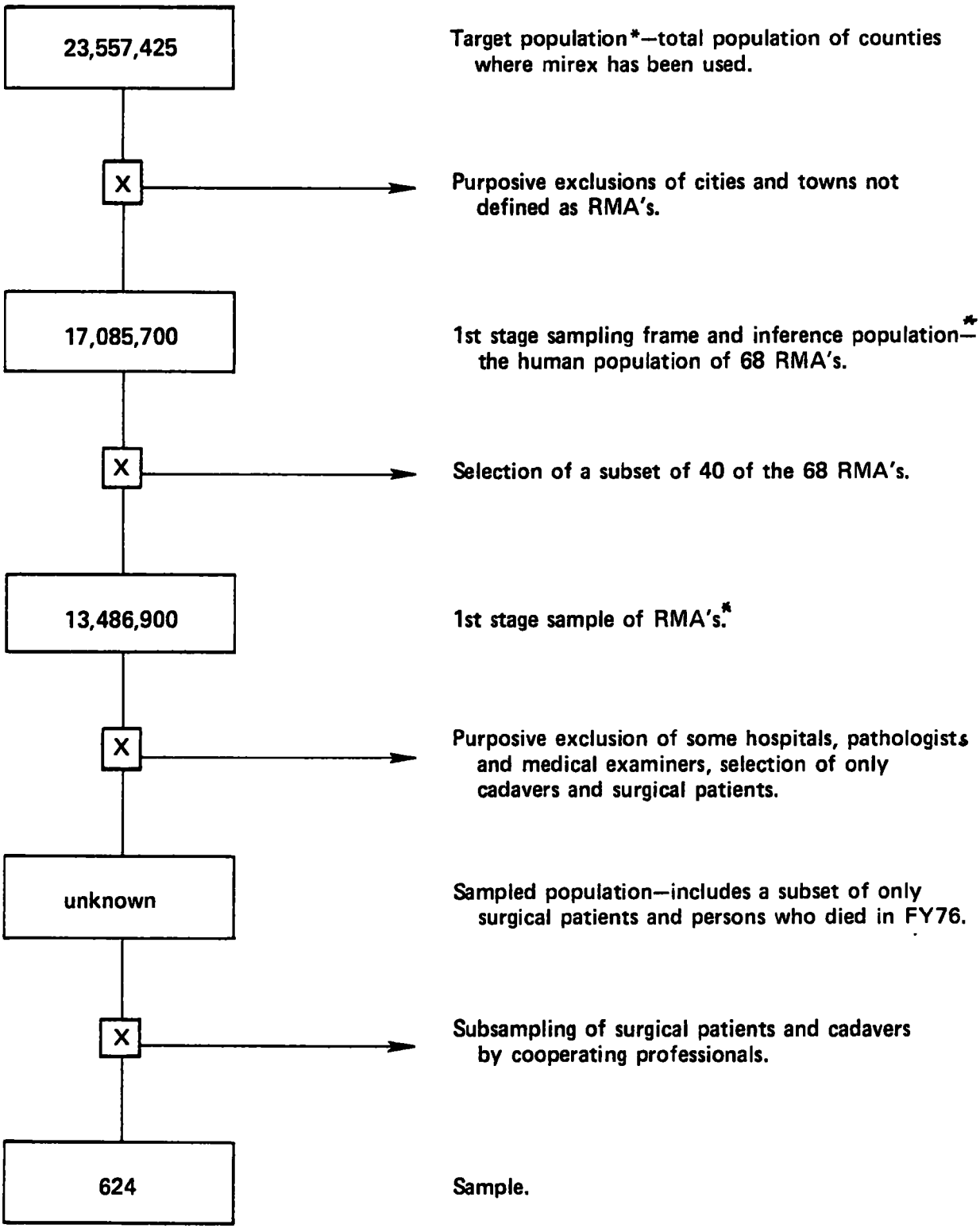


Figure 2.1. Sampling frame and selection procedures for Mirex Special Study.

Source Based on information contained in references 1, 4.

*Population values based on 1970 census data.

were independently selected from each stratum with probability proportional to size of the city. This was done by listing the sites in random order along with their cumulative population totals. An interval for each stratum was calculated by dividing the total population for all sites listed by the number of sites to be selected in the stratum. A random number was obtained between 0 and the length of the interval to give a starting point. The sites were selected by matching their cumulative totals with the starting point or integer multiples of the interval plus the starting point.

2.2.4 Pathologists

The subsampling within each site was done by a cooperating pathologist or medical examiner. When no cooperative pathologist or medical examiner could be found in a selected site, an alternate site was selected. Alternate sites were chosen by position in the listing of eligible sites with respect to the nonrespondent site. The first alternate was the site immediately below the originally selected site, the second alternate the one immediately above, the third alternate was the second site following and so on.

2.2.5 Quotas

Each site was assigned a quota based on the demographic characteristics of age, sex, and race. The ages were grouped into three ranges: 0-14, 15-44, and greater than 44. The races were classified as white and nonwhite. The quotas for FY70-FY72 were based on the national demographic characteristics according to the 1960 Census. The quotas for FY73-FY76 were determined by the appropriate demographic characteristics for each Census division.

Below the sample city level there was no probability structure for obtaining the participants in the survey. The hospitals, pathologists and medical examiners were selected subjectively. Also, the tissue samples

from cadavers and surgical patients were selected subjectively by the cooperating professionals. Guidelines for selecting patients were distributed by EPA. Appendix F contains a copy of the letter sent to each cooperating professional containing these guidelines, which outline the procedures for screening specimens and the types of individuals from whom tissue specimens should not be obtained (for example, suspected victims of pesticide poisoning and institutionalized patients). The MSS quotas are given in Appendix G.

2.3 Selection Weight Construction

In order to use the information obtained in the MSS to estimate the level of mirex concentration in the population of RMA's in the target area and to account for disproportionate sampling rates, it is necessary to weight the original values before analysis. This presents some problems, for the nature of the selection of sites, which includes at some points purposive selection (subjective, not based on any known probability of selection), is incompatible with the formation of statistically valid sampling weights. A method of "quasi" weighting has been devised and used for the weighted analyses. A simple method of weighting was chosen to facilitate the weighted analyses, but it is by no means necessarily the best or only way to weight the data. The method of weighting chosen and some of that particular method's limitations are described here.

The first stage selection frame, consisting of 68 1974 RMA's from the designated mirex counties in eight southern States, was actually split between two lists, which were considered separately in constructing weights. One list consisted of eight mirex area cities that were originally part of the NHMP, and the second list consisted of 60 sites (RMA's),

from which the supplementary MSS sample was drawn. The eight cities that had been part of the NHMP were included with certainty in the sample. These eight cities are in RMA's, but were not considered among the possible selections for the supplementary sample.

Even if one assumes the selection process within sample sites is approximately random, the sample design of the MSS does not assign equal "probabilities" of selection to all elements (cadavers and surgical patients) in the sample. This introduces the possibility of sampling bias in calculating population estimates. Bias refers to the difference between an estimated population value and the true population value. It can occur as a result of sampling error or measurement error, or a combination of both. To facilitate bias reduction in this situation, a sample weight for each observation was calculated, which attempts to reflect its approximate probability of selection. Including weights in the analysis should reduce bias in estimating means or proportions. For probability sampling this results in strictly unbiased estimators for such statistics as means, proportions, and totals. In the following paragraphs, procedures are given for computing weights for the MSS. Again, note that true sampling weights cannot be calculated because some stages of selection involve non-probability sampling.

The population from which tissue specimens might be obtained is not actually all people residing in a selected RMA; only surgical patients or individuals who die from certain causes at the selected hospital in any sample RMA have a chance of being selected. The surgical patients and cadavers that are available to the selected pathologist may not necessarily be residents of the RMA that they are purported to represent, because individuals may travel to another city for medical treatment. The possibility of medical care migration is particularly problematic for RMA's that

are located on or near the borders of mirex areas (such as Dallas).

To estimate the probability of a given individual being selected within a sample site, one might use the number of pathologists and hospitals, so that the probability of selecting any one pathologist at any hospital in an RMA could be estimated. Then the population of suitable surgical patients and cadavers to which the pathologist had access would need to be estimated, so that a probability of selection of a tissue specimen by that pathologist could be estimated. Even with this type of information, the estimates of selection probability would be very rough because the pathologists and tissue specimens in the MSS were not selected in any random or systematic method but by purposive selection. At this time, there is no reasonable adjustment that can be made to take the above facts into consideration. Hence, equal probability of selection is assumed for the within-site stage of sampling. This method involves dividing the number of specimens received by the population of the RMA.

The probability of an RMA being selected in the MSS is dependent upon the study for which the site was originally selected because the selection criteria for the sites that were already part of the NHMP were different from those for the sites that were sampled specifically for the MSS.

The probability of selection of a site at the first stage is the number of selections made divided by the number of possible selections; this is the number of selected RMA's divided by the number of RMA's in the frame, or

$$\Pr\left\{\begin{matrix}\text{1st stage}\\ \text{selection}\end{matrix}\right\} = \frac{\text{number (n) of sample RMA's}}{\text{number (N) of RMA's on the list}}$$

For each of the eight RMA's that are part of the NHMP, the probability of selection is equal to 8/8, or a probability of selection of one. These eight sites were included with certainty in the sample because data collection systems already existed at these locations. For RMA's that were selected as part of the supplementary or additional MSS sample, the probability of selection is 32/60, or 0.53. The supplementary sites were selected by simple random sampling, which does not consider the size of the population of the RMA's in the selection process. When equal selection probability is used at the first stage and approximately equal sample sizes are selected from each of the primary sampling units, this can result in oversampling small RMA's and cause underrepresentation of RMA's that have large populations.

Weighting the observations within the sample site is used to adjust for this. The weight of a given sampling unit is the inverse of the "quasi" sampling rate. Hence, the weight for a given observation is

$$\text{Selection weight} = \frac{\text{number of RMA's on list}}{\text{number of sample RMA's}} \times \frac{\text{population of RMA}}{\text{number of specimens selected in RMA}}$$

In this study, as noted previously, some of the sample sites selected did not submit valid tissue samples for analysis. To compensate for this nonresponse, the inverse of the proportion of selected sample sites which responded was used to adjust the weights. For example, data were submitted from five of the eight NHMP sites ($\frac{5}{8}$ or 62.5 percent responded), which results in a nonresponse adjustment factor of 1.6 for NHMP sites. The nonresponse adjustment for supplementary RMA's is 1.23, the inverse of 26/32. This factor, multiplied by the selection weight for an observation, produces a weight adjusted for nonresponse at the first stage level. There

was no information upon which to base any nonresponse adjustments for selection of tissue specimens within selected sites.

3. EVALUATION OF CHEMICAL ANALYSIS

3.1 Introduction

The objective of this portion of the study is to assess the reliability of the results obtained from the chemical analytical procedures employed in the Mirex Special Study. The analysis of adipose tissue specimens for mirex was carried out at the Michigan Department of Public Health under the direction of Mr. Robert L. Welch.

The analytical procedure used for the determination of mirex is described in the EPA Pesticides Methods manual [5]. Specific details regarding the collection, storage, and shipping of specimens were described in the NHMP study report for mirex [1]. In conformance with the Work Plan requests for needed data, Mr. Welch shipped a number of raw mirex chromatograms to RTI. These chromatograms were examined in sufficient detail to allow for a meaningful assessment of the quality of reported mirex levels. The identification numbers (not patient identifers) and dates of analysis for these chromatograms are provided in Appendix H.

3.2 Discussion

The method used for the determination of mirex levels was based on the use of an external standard. Reference standards were injected before, during, and at the end of each mirex specimen group. The standard consisted, usually, of a mixture of known amounts of mirex and Aroclor 1260. This mixture served not only for purposes of quantitation, but also provided a constant check on the degree of separation between mirex and a late-eluting Aroclor peak. This peak represents an interference found in most tissue specimens due to the ubiquitous nature of the Aroclors. In an effort to maximize the separation of the two peaks, the gas chromatographic

procedure was modified, and the analysis was conducted at a temperature slightly below that recommended in the Pesticides Manual. This procedure was approved by memo (Appendix I) prior to chemical analysis. Chromatograms of Aroclor 1260, mirex, and a mixture of 1260/mirex are depicted in Figures 3.1 through 3.3. Baseline separation was achieved for mirex and the Aroclor 1260 peak, and this separation was maintained throughout the analysis period covered by the chromatograms examined (May 1976-November 1977).

The same quantity of Aroclor 1260 and mirex was injected for each check. Thus, a measure of the reproducibility of injection could be determined by measuring detector response (peak height) for these standards. A check of 17 such injections showed acceptably close agreement (differences < 10 percent) between injections for a given specimen group. This agreement and the reproducible retention times observed for the compounds over a period of several months are indicative of a stable analytical system. Greater reliability can be attached to results obtained from such a system.

For a significant number of chromatograms, an interference peak other than Aroclor 1260 was noted. Of some 50 chromatograms from which a quantitative assessment of mirex was made, approximately half (26) showed mirex peak interference. The degree of interference varied (Figures 3.4 through 3.6) but was not so severe as to prevent estimation of levels of target compound.

Of the chromatograms examined, only one blank and two control specimens were included. These specimens were not described so no significance could be attached to them, except that the blank chromatogram was "clean" in the area of interest.

To assess the reliability of the chromatographic and, to a limited extent, the quantitative procedures used, all mirex peaks were measured

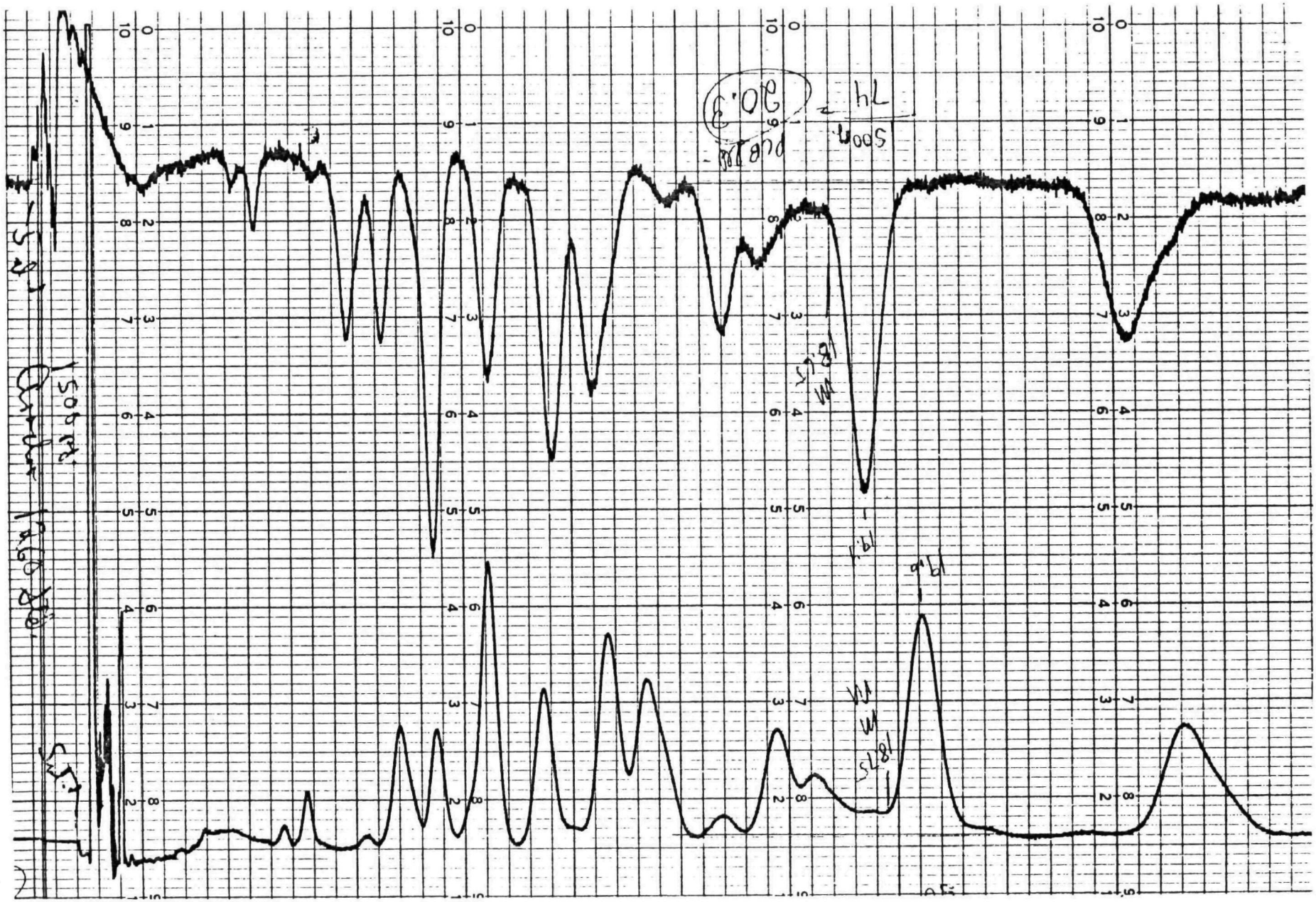


Figure 3.1. Gas chromatogram - Aroclor 1260 standard.

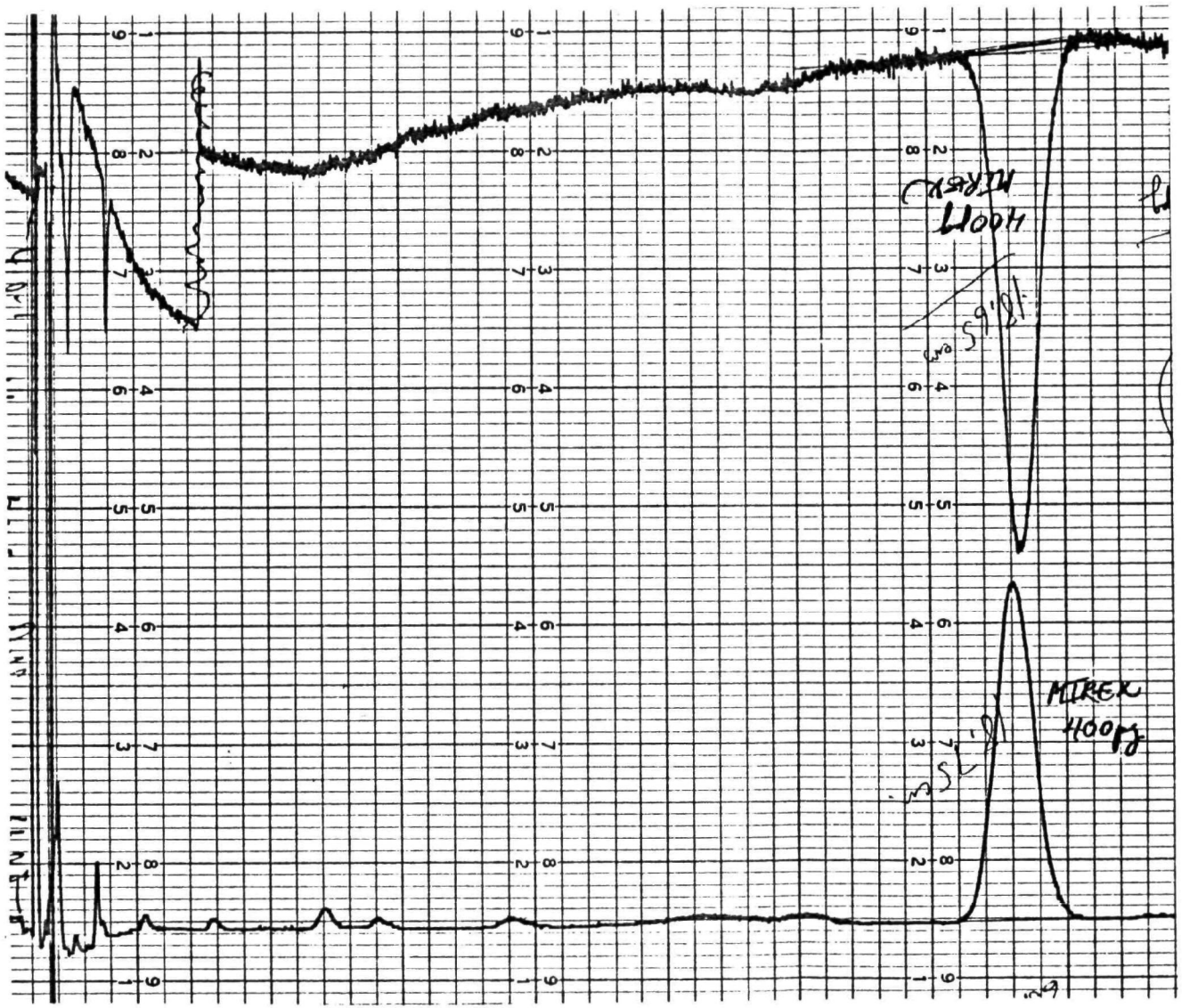


Figure 3.2. Gas chromatogram - mirex standard. Conditions as in Figure 1.

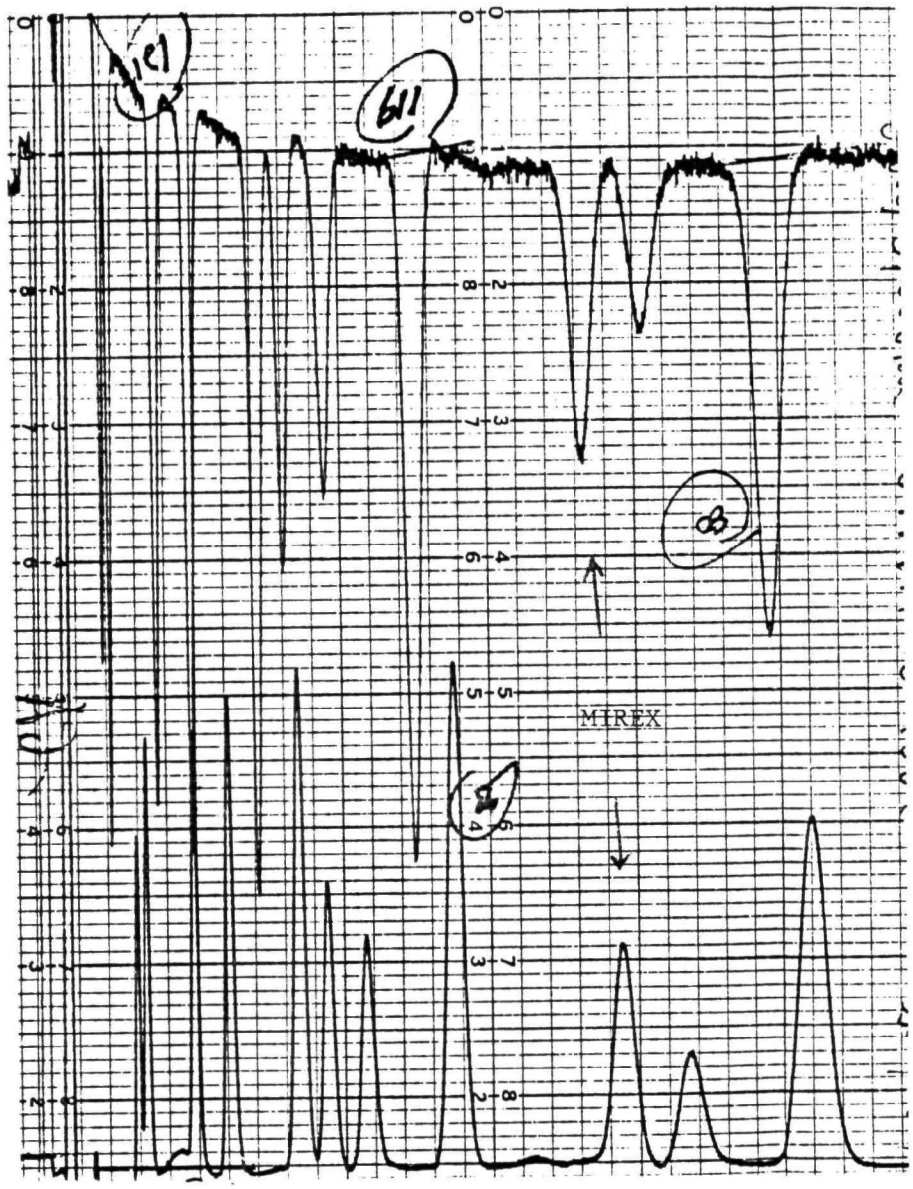


Figure 3.3. Gas chromatogram - mixture of Aroclor 1260 and mirex standards. Conditions as in Figure 1 except chart speed is 0.5X.

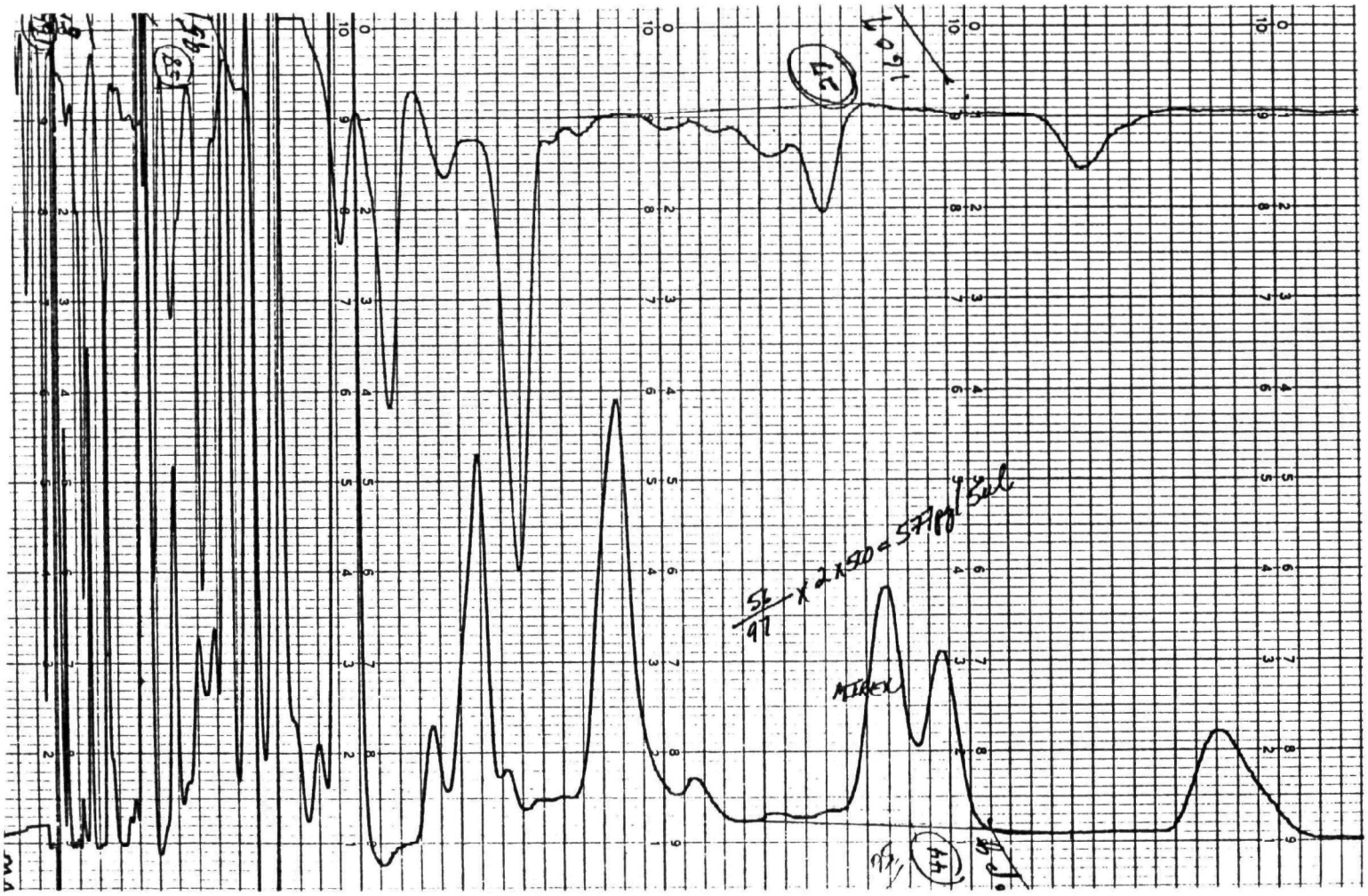


Figure 3.4. Gas chromatogram - mirex containing sample (moderate level) with interference peak (moderate level). Conditions as in Figure 3.1.

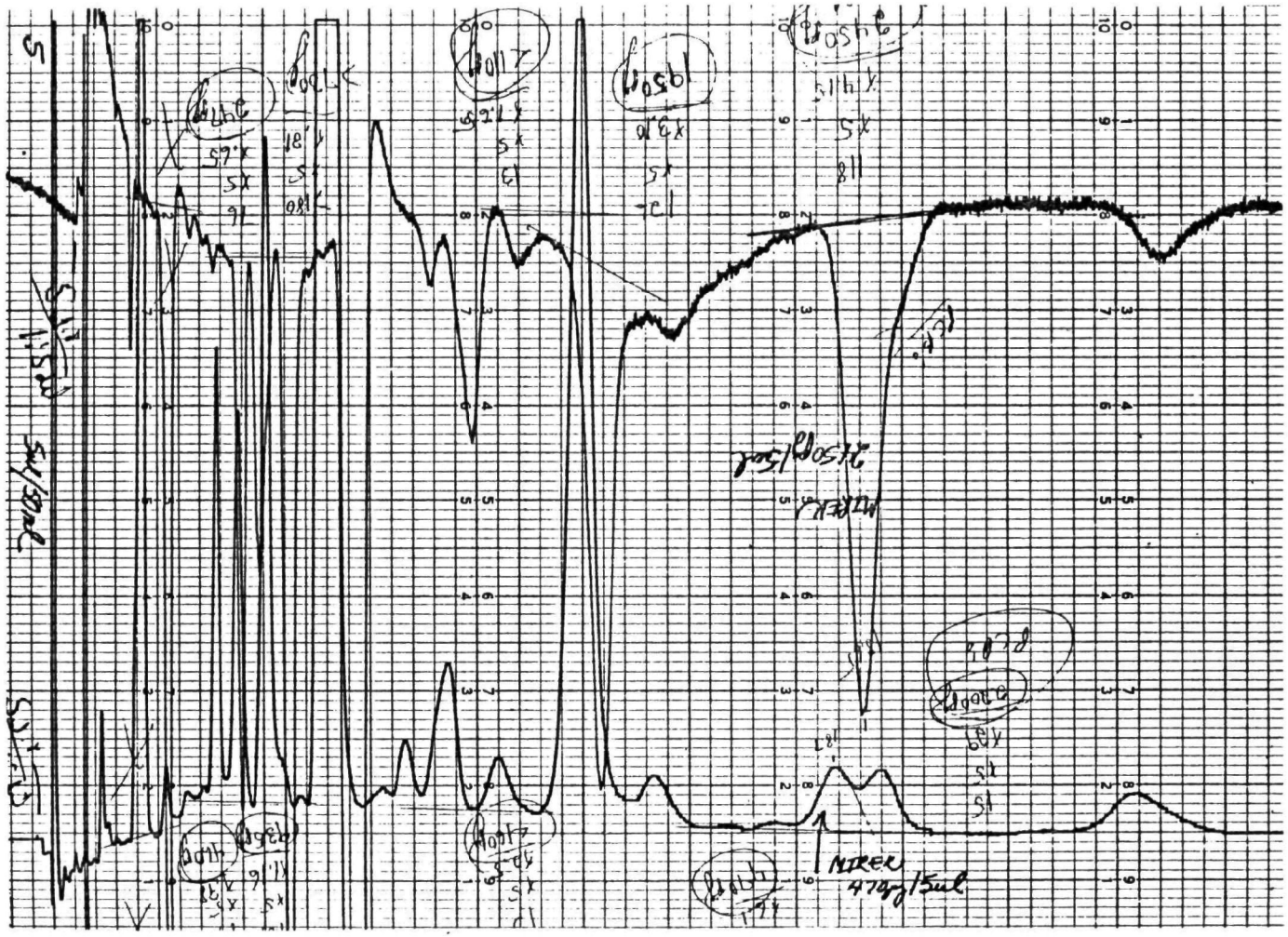


Figure 3.5. Gas chromatogram - mirex containing sample, (low level) with interference peak, (low level). Conditions as in Figure 3.1.

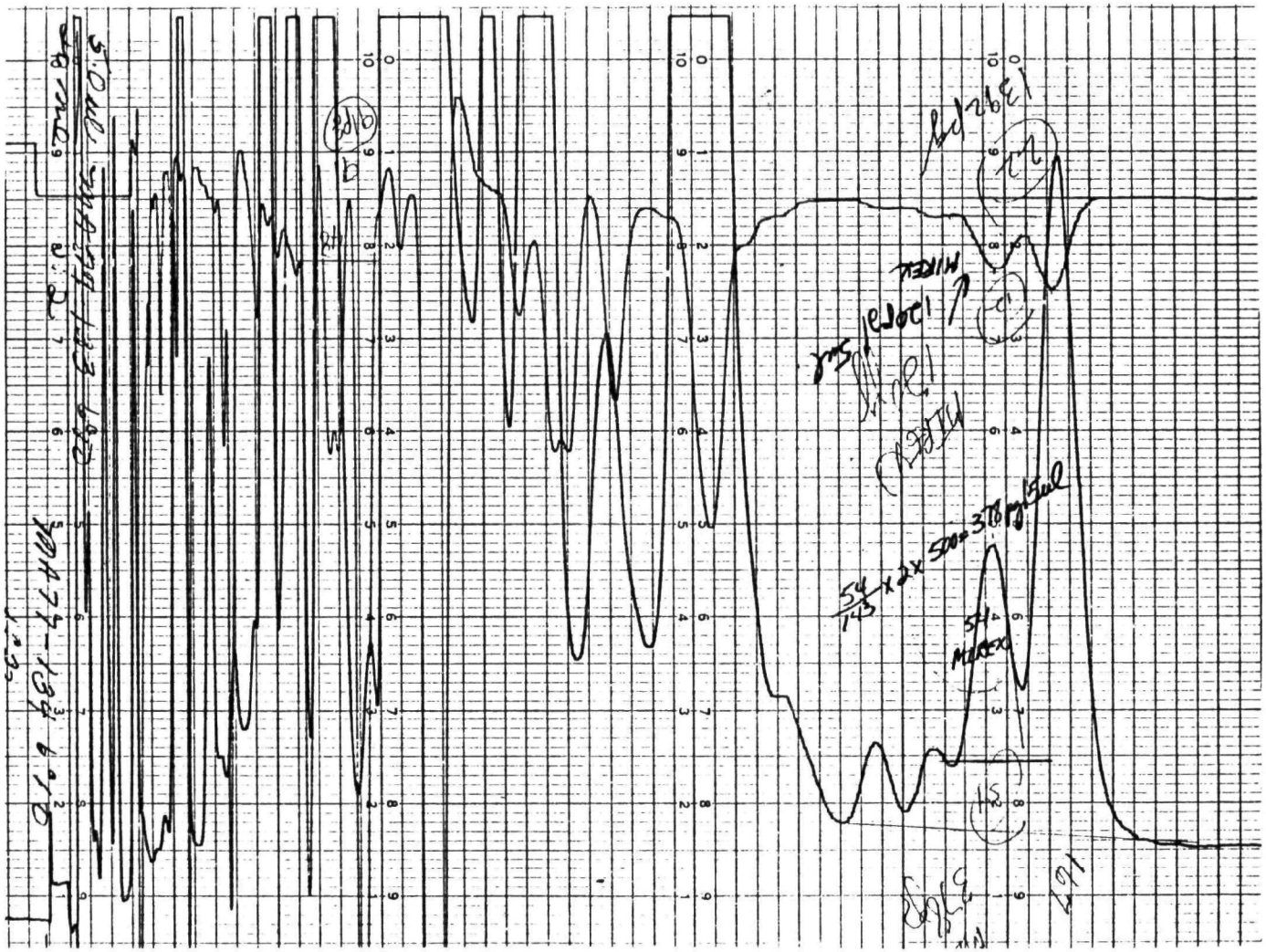


Figure 3.6. Gas chromatogram - mirex containing sample (moderate level) with interference (high level). Conditions as in Figure 3.1.

(peak heights) and the results compared with those obtained from the chromatograms. Comparisons were also made of the amount of mirex as calculated from the chromatograms and as determined by the RTI staff. These data are shown in Table 3.1. As can be seen, the results are consistent with average absolute errors and standard errors (Δ%, see Table 3.1) of 9.2 $\pm$ 2.1% for peak height measurements, and 8.1 $\pm$ 1.2% for calculated amounts. A check on the interpersonal variability was determined by measuring and calculating the same parameters, i.e., peak height and amount of mirex, using a different RTI staff member. Some 10 percent of the RTI-measured chromatograms were replicated in this manner. These results are shown in Table 3.2. Again the data are very consistent, with the average absolute error and standard error calculated at 3.0 $\pm$ 1.0 %. Thus, the measurement of mirex peaks and the calculation of raw mirex amounts were straightforward, and, at least for the chromatograms examined, no gross errors (inaccurate measurement, faulty calculation, peak assignment error) were evident.

As no completely adequate method exists for the quantitative statistical treatment of "trace" levels of compound, an attempt was made to ascertain the minimum detectable quantity (MDQ) of mirex as analyzed by Welch. Since the MDQ was not calculated or made available for RTI use, an approximation was made adopting the usual assumption of MDQ = 3 (signal/noise). Since two detection systems were used in this study, MDQs were determined for each at the beginning of the analytical runs (May 1976) and again at the end (November 1977). For system 1, the MDQs were 16 pg at the beginning and 20 pg at the end of the study; for system 2, the MDQs were 9 pg and 12 pg at the beginning and end, respectively. The similarity of these values bespeaks an extremely stable analytical system, with more than

TABLE 3.1. COMPARISON OF MIREX PEAK HEIGHTS (mm) AND CALCULATED AMOUNTS (pg) AS DETERMINED BY WELCH AND RTI

Specimen	Ht (Welch)	Ht (RTI)	Δ%*	Amt (Welch)	Amt (RTI)	Δ%*
MA76-98 (1:2)	17	16.5	-2.9	187	159	-15.0
MA76-100 (1:5)	108	113.0	4.6	2967	2729	-8.0
MA76-101 (1:2)	19	20.5	7.9	284	271	-4.6
MA76-123 (1:2)	54	57.0	5.6	?	1326	-
MA76-124 (1:2)	58	77.0	32.8	X	488	-
MA76-125 (1:2)	43	43.5	1.2	X	423	-
MA76-126 (1:2)	13	13.0	0	236	236	0
MA76-127 (1:5)	75	76.5	2.0	X	1855	-
MA76-128 (1:2)	75	82.0	9.3	986	982	-0.4
MA76-129 (1:2)	20	20.0	0	213	185	-13.1
MA76-130 (1:2)	20	20.0	0	274	240	-12.4
MA76-131 (1:2)	78	81.5	4.5	829	755	-8.9
MA76-160 (1:2)	15	15.0	0	221	190	-14.0
MA76-161 (1:2)	98	99.5	1.5	2020	2030	0.5
MA76-162 (1:2)	28	33.0	17.9	412	418	1.5
MA76-164 (1:2)	0	0	-	0	0	-
MA76-165 (1:2)	0	0	-	0	0	-
MA76-166 (1:2)	0	0	-	0	0	-
MA76-167 (1:2)	0	0	-	0	0	-
MA76-80 (1:5)	15.5	15.0	-3.2	470	458	-2.6
MA76-81 (?)	32.5	32.5	0	198	198	0
MA76-82 (1:5)	118	119.0	0.8	2450	2466	0.7
MA76-83 (1:5)	6.5	6.5	0	198	198	0
MA76-157 (1:2)	22	23.5	6.8	224	209	-6.7
MA76-158 (1:2)	0	0	-	0	0	-
MA76-159 (1:2)	32	32.5	1.6	327	289	-11.6
MA77-9 (1:2)	12	16.5	37.5	102	115	12.7
MA77-11 (1:1)	60	67.0	11.7	254	205	-19.5
MA77-12 (1:2)	21	23.0	9.5	244	217	-11.1

TABLE 3.1. (cont'd)

Specimen	Ht (Welch)	Ht (RTI)	Δ%*	Amt (Welch)	Amt (RTI)	Δ%*
MA77-13 (1:2)	71	80.0	12.7	602	557	-7.5
MA77-14 (1:2)	124	126.5	2.0	1442	1193	-17.3
MA77-60 (1:2)	56	60.0	7.1	577	517	-10.4
MA77-61 (1:2)	107	120.0	12.4	973	906	-6.9
MA77-129 (1:2)	15	11.0	-26.7	?	282	-
MA77-130 (1:2)	18	17.5	-2.8	?	280	-
MA77-133 (1:2)	12	17.0	41.7	120	142	18.3
MA77-134 (1:2)	54	70.5	30.6	378	400	5.8

* $\Delta\ \% = \frac{X\ (RTI) - X\ (Welch)}{X\ (Welch)}\ (100)$

TABLE 3.2. INTERPERSON COMPARISON OF MIREX PEAK HEIGHT MEASUREMENTS

Specimen	Peak height (mm) KB	SF	Δ %
MA 76-128 (1:2)	85.0	82.0	3.7
MA 76-129 (1:2)	21.0	20.0	5.0
MA 76-130 (1:2)	20.0	20.0	0
MA 76-131 (1:2)	79.5	81.5	-2.5
MA 76-12 (1:2)	22.5	23.0	-2.2
MA 76-11 (1:5)	25.5	23.5	8.5
MA 76-60 (1:2)	60.0	60.0	0
MA 76-60 (1:5)	29.0	29.5	-1.7

adequate sensitivity for these types of analyses. Unfortunately, these values could not be translated into the concentration values (ppm) reported on the data file. This was due to the inability to relate the specimen identifiers (specimen numbers) on the same chromatograms with the identifiers (patient numbers) reported on the raw data file. In addition, information was not available regarding the exact analytical procedures followed, and, thus, specimen dilution factors could not be ascertained. The absolute amounts of mirex, as shown on most of the chromatograms, could not, therefore, be converted to ppm fat.

Although an assurance check could not be made on the final, reported mirex concentration levels, the raw data indicate that sound analytical quantitation procedures were followed, and, in general, the results are of acceptable quality. Further assurance of the quality of the data is provided by independent confirmation of the mirex levels using, primarily, gas chromatography/mass spectrometry (GC/MS). Results of analysis of some 23 specimens (Appendix J) showed reasonably good agreement between the values reported by Welch and those reported by the confirming laboratories.

The quality of the raw data, the results of the confirmational analyses, and the stability of the analytical system are taken as good indicators of reliable and accurate results. Although every aspect of the analysis could not be checked (for example, calculations leading to concentration values), no errors were noted from the available chemical analytical data. There is no indication that the information as reported on the raw data file is suspect.

4. UNWEIGHTED ANALYSIS

4.1 Data Description

The MSS data received by RTI consisted of 664 observations from 31 responding sites. 40 observations were excluded from analysis: 34 records were of specimens collected outside the reference time period, and the lipid extractable amount for 6 observations was less than 10 percent. There were no data exclusions on the basis of inadequate chemical analysis confidence codes. After exclusions, there were 624 observations available for statistical analysis.

In Chapter 2, there is brief mention of desired specimen quotas by age, race, and sex (Appendix G). These quotas guided the field contact in submitting specimens from a range of individuals. It should be kept in mind that representativeness for factors of occupation and length of residence in an area is not accomplished through balancing on age, race, and sex information. The quotas were established in an attempt to obtain information that would reflect the age, race, and sex distribution of the living population.

Tissue specimens that fit the quota specifications were referred to as design specimens, and those tissue specimens received in excess of the quota guidelines were called surplus specimens. None of the sites filled their quota of 27 design specimens even though 6 of the 31 responding sites submitted 27 or more tissue specimens. There were 479 design specimens and 145 surplus specimens. There is no difference in methodology of selection or collection between the design and surplus specimens; the designation was made in order of date of receipt of a specimen as the study year progressed.

There seems to be no obvious reason to exclude the surplus tissue specimens (23.2 percent of the data) from weighted analysis and probably not from unweighted analysis because design quotas were never satisfied.

Table 4.1 contains classifications for all tissue specimens within Census Divisions by age, race, and sex. The desired quotas for racial representation were met reasonably well within the South Atlantic and West South Central Census Division and almost exactly within the East South Central Division. The Census Division level desired distribution by age group was not met; the 0-14 age group was consistently underrepresented in all three divisions. The actual distribution of specimens classified by sex was roughly in keeping with the desired quotas on the Census Division level.

For the various sample sites the number of valid tissue specimens submitted ranged from 1 to 37. Dallas, Texas, one of the NHMP sites and the largest RMA in the MSS (2,455,000 people), was the location that contributed only one tissue specimen. Dallas presents further analysis problems because of its location, for it falls on the edge of a mirex area and part of the defined RMA including Dallas falls outside of the mirex boundary. Because Dallas is the largest city in that part of Texas (see maps, Appendix A), the population that relies on the medical facilities in Dallas extends far beyond the boundaries of the mirex counties. Houston and San Antonio are similarly situated in both geographical and medical terms. The locations of other RMA's are subject to the same cautions in interpreting the residence of the individuals from whom adipose tissue specimens were obtained. Some sites, such as those in Louisiana, are clearly located in a heavily fire-ant-infested region, and for these, little ambiguity exists about the potential mirex exposure of the population.

Table 4.1 Specimens Collected for Each Census Division By Age, Race, and Sex*

East South Central: Alabama and Mississippi

Number of Specimens

	Caucasian		Non-Caucasian		
Age Group	Male	Female	Male	Female	Total
0-14	5	7	1	2	15
15-44	22	29	6	5	62
≥45	36	43	7	12	98
	63	79	14	19	
	142		33		175

desired Non-Caucasian quota = 5/27 = 18.5%
actual Non-Caucasian representation = 33/175 = 18.9%

South Atlantic: Florida, Georgia, North Carolina, and South Carolina

Number of Specimens

	Caucasian		Non-Caucasian		
Age Group	Male	Female	Male	Female	Total
0-14	13	8	6	10	37
15-44	35	29	25	11	100
≥45	55	52	18	20	145
	103	89	49	41	
	192		90		282

desired Non-Caucasian quota = 6/27 = 22.2%
actual Non-Caucasian representation = 90/282 = 31.9%

West South Central: Louisiana and Texas

Number of Specimens

	Caucasian		Non-Caucasian		
Age Group	Male	Female	Male	Female	Total
0-14	7	9	2	0	18
15-44	18	34	7	8	67
≥45	28	41	7	6	82
	53	84	16	14	
	137		30		167

desired Non-Caucasian quota = 4/27 = 14.8%
actual Non-Caucasian representation = 30/167 = 18.0%

*Based on the results of the MSS and information in Appendix G.

In future investigations it would be useful to obtain the zip code of the home address of the patients from whom the tissue specimens are obtained so that residence in a mirex area can be verified. However, this still would not give any indication of the length of time an individual resided in an area exposed to mirex. Level of exposure and intensity of application of mirex to an area are also not known but are of interest.

4.2 Data Analysis

4.2.1 Data Distribution

In the interpretation of estimates and statistical tests in this section, which are based on unweighted analyses of MSS data, it must be recognized that at least two categories of individuals are not as prevalent (underrepresented) in the sample as in the population. These are people under age 15 and residents of the larger RMA's. In light of the correlations between geographic location and mirex positives, the latter imbalance may cause unweighted analysis to be seriously biased for purposes of extending the results to all persons in the study population. The weighted analyses in the following chapter should reduce this risk of bias.

In examining the data, it is important to consider the distribution of mirex levels before choosing summary statistics or conducting descriptive analyses. Of the 624 specimens, 141 (22.6 percent) contained detectable amounts of mirex. Eighteen (12.8 percent) of the 141 detectable values were trace quantities of mirex, present but not in sufficient quantity to be accurately measured (see discussion in section 3.2). These trace quantities were included in analyses where presence or absence of mirex was measured but not included in those statistical analyses where a specific numerical value was required.

The two histograms in Figure 4.1 illustrate the distribution of the frequency of mirex residue values (in parts per million) for all 624 specimens and for the 123 specimens for which there were definite measurable quantities. It is obvious that the levels of mirex in the sample do not follow a normal distribution (example at the bottom of Figure 4.1), even when the 501 zero (nondetectable) and trace values are excluded. The skewing of the distribution to the right indicates that the residue amounts follow a lognormal or exponential distribution, and that a transformation of the data might yield a distribution of values that would be more appropriate for meeting the assumption of normality underlying many statistical tests. Figure 4.2 contains histograms of the results of two logarithmic transformations of the measurable positive residue amounts: natural log ($\log_e$) and log base 10 ($\log_{10}$). The $\log_{10}$ transformation is slightly closer to normal than the $\log_e$ transformation and was chosen as the transformation to be used in computing summary statistics and in analyses involving the quantifiable residue amounts.

It should be noted that the actual distribution of mirex levels contained in human adipose tissues probably consists of fewer true zeros than current analytical techniques indicate. The skewing to the right would still exist, but the distribution of values would more closely resemble a true continuous distribution if the presence of mirex in any quantity, however small, could be measured.

No minimum detectable quantity could be determined for mirex in human adipose tissues; consequently, no specific numerical value could reasonably be substituted or inserted for the 18 trace observations. The zero values and trace values could be transformed to a logarithmic scale if a constant term was added to all 624 values before transformation. This was not done

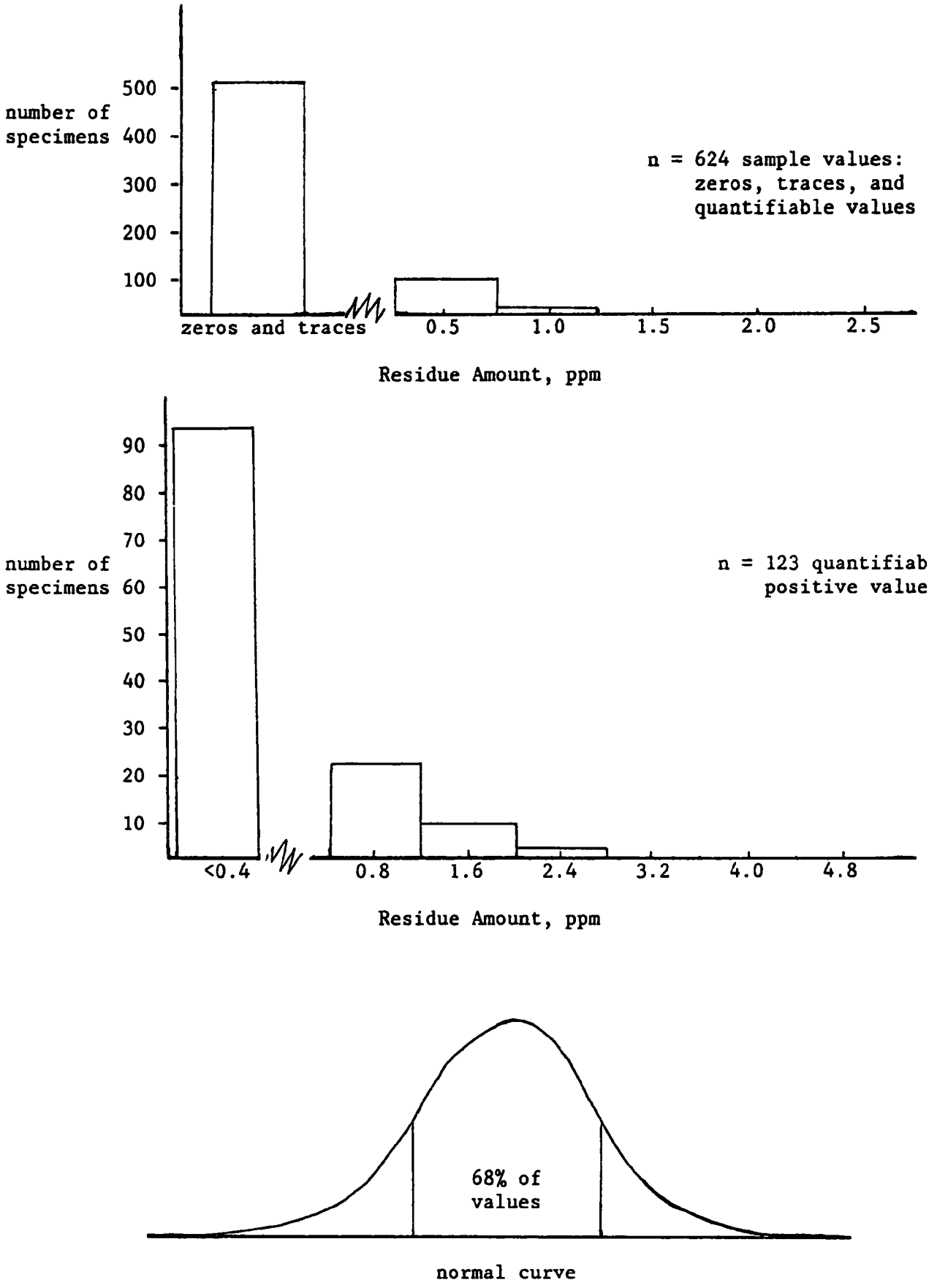


Figure 4.1 Frequency distribution for raw values of residue amount*
*Based on results of Mirex Special Study, FY76.

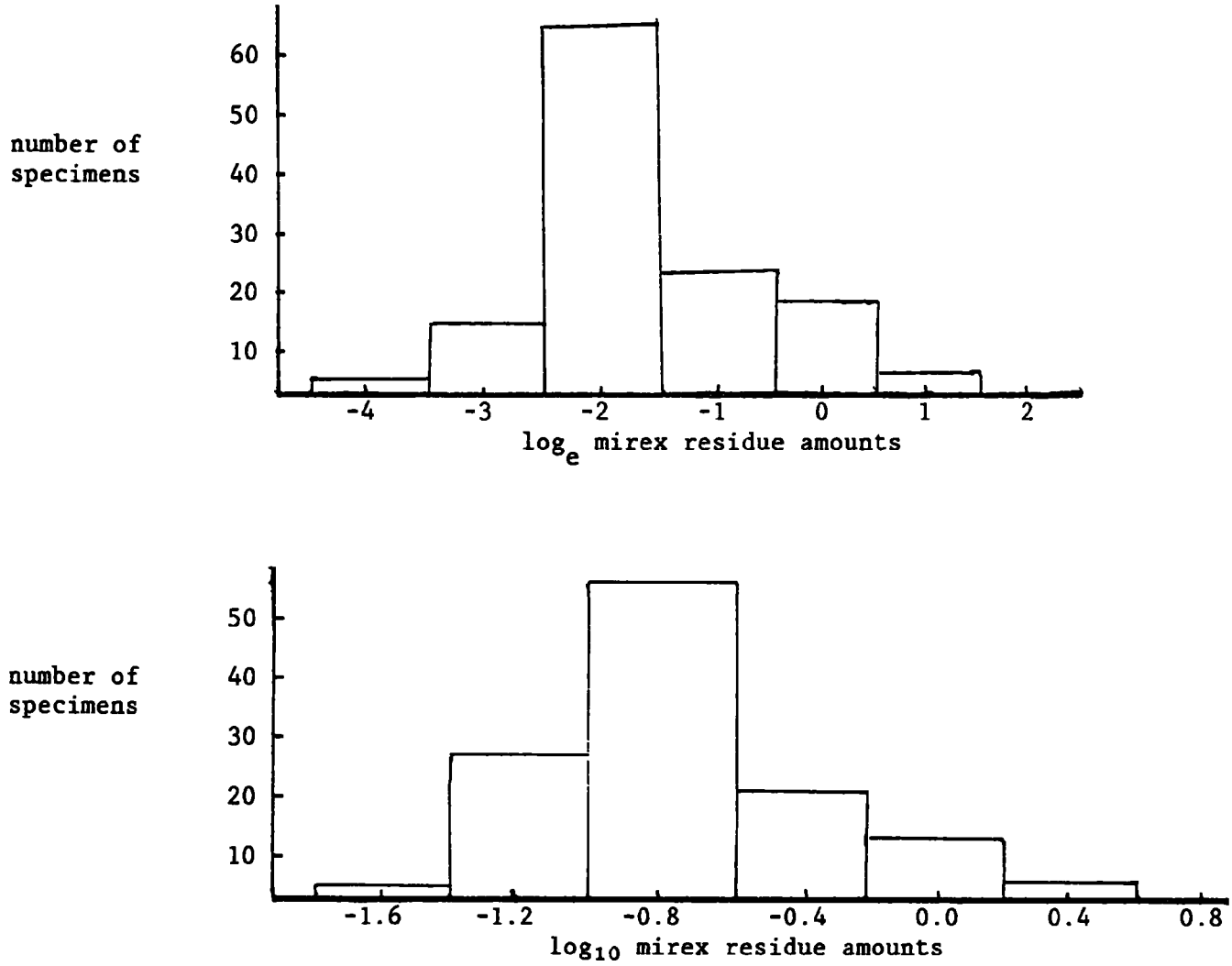


Figure 4.2 Frequency distributions for transformed values of mirex residue*

*Based on results of Mirex Special Study, FY76.

because there are so many nondetectable values that summary statistics for the entire sample would provide little information. Instead, it was decided to look at detectable versus nondetectable values and at the quantifiable residue amounts.

Both the proportion of positive values and the geometric mean of the transformed data were used as summary statistics. The geometric mean is an appropriate summary statistics for logarithmic data [6].

It has been specified by EPA that part of the analysis in this task is to be carried out on the residue amount divided by the percent lipid extractible material (LEM) in the specimen. This quantity is indicated by the term Y in the following pages. The natural log, $\log_e$, was obtained for all 123 quantifiable positive values of Y. The two histograms in Figure 4.3 are the distributions of Y and $\log_e$ Y.

4.2.2 Proportions of Positive Values

In Tables 4.2 and 4.3 are some simple classifications of the number of detectable and nondetectable values by demographic and geographic categories. In Table 4.2, which is a summary by State, the two States with the largest percent of detectable mirex levels are Mississippi and Louisiana, 46.3 and 35.7 percent positive, respectively. Georgia has the third largest percentage of detectable mirex levels, at 20.9 percent. The percentage of detectable mirex values drops to less than 8 percent for the 5 remaining States. There were no positive values for the specimens submitted from North Carolina and Texas.

This information by State suggests a possible connection between residence in a widespread mirex-treated area with detectable mirex levels in human adipose tissue. This is borne out by the highly significant results of a chi-square test on the zero/positive values by State in Table 4.4. The empty cells for positive values for Texas and North Carolina make

the chi-square test results less reliable than if there were some positive values for these states. However, it can still be used to infer that there are significant differences in the percent of positive mirex residues detected by State.

Census Division was the only other variable investigated which shows significant results in the chi-square test. Race, age, and sex did not show significant differences in the percent positive mirex residue values for the chi-square test results in Table 4.4.

The information in Table 4.3, which is a three-way classification by race, age, and detectable mirex residues, is less easily summarized than the data in the two-way classification in Table 4.2. When considering additional categories, it becomes necessary to consider possible interactions between variables as well as simple main effects. This can be done by computing an analysis of variance (ANOVA) or a regression equation. A model such as the following can be used.

$$P_{ijk\ell m} = \mu + A_i + S_j + R_k + L_\ell + \varepsilon_{ijk\ell m}$$

where

$P_{ijk\ell m}$ = mirex level in tissue for the m^{th} individual, the ℓ^{th} location, in the (ijk) age-race-sex group;

μ = mean mirex level;

A_i = age effect for the i^{th} age group;

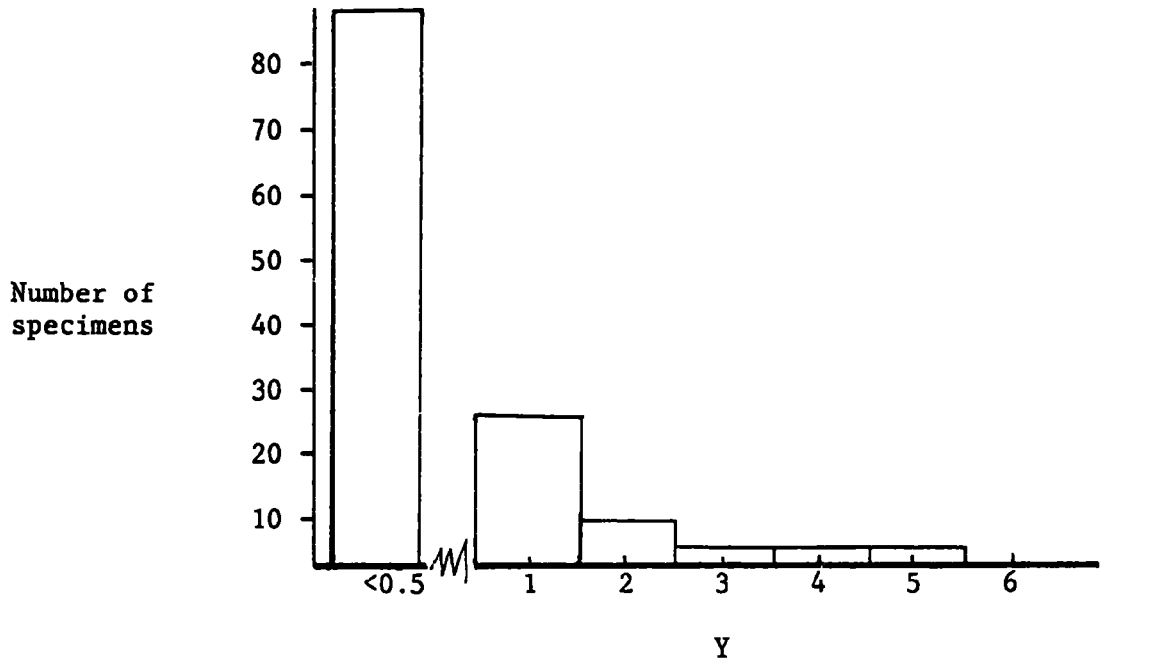
S_j = sex effect for the j^{th} sex group;

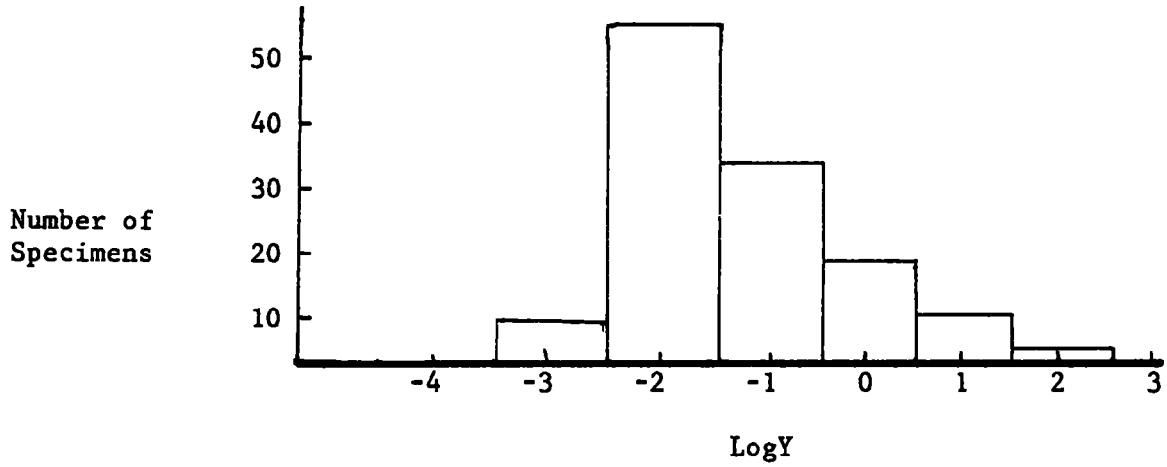
R_k = race effect for the k^{th} race group;

L_ℓ = location effect of the ℓ^{th} location;

$\varepsilon_{ijk\ell m}$ = random error.

In the above model, testing for age, sex, race, and locations effects corresponds to testing the equality of the A_i, S_j, R_k, and L_ℓ. Standard





Y = Mirex Residue Amount (ppm)/% Lipid Extractible Material

Figure 4.3 Frequency distributions of lipid adjusted mirex residue amounts and transformed values (n=123 quantifiable positives)*

*Based on results of Mirex Special Study, FY 76.

Table 4.2 Detection of Mirex Residue by State: Number of Specimens with Positive and Zero Detectable Levels*

State/number of responding RMA's	Zero	Positive	Total	Percent Positive
Alabama (3)	36	3	39	7.7
Florida (6)	129	8	137	5.8
Georgia (5)	72	19	91	20.9
Louisiana (5)	83	46	129	35.7
Mississippi (6)	73	63	136	46.3
North Carolina (1)	18	0	18	0.0
Texas (3)	38	0	38	0.0
South Carolina (2)	34	2	36	5.6
	483	141	624	

*Based on results of the Mirex Special Study, FY 76.

Table 4.3 Detection of Mirex Residue by Race and Age: Number of Specimens with Positive and Zero Detectable Levels*

Race	Age Group	Number of Specimens		
		Zero	Positive	Total
Caucasian				
	0-14	42	7	49
	15-44	128	39	167
	≥45	202	53	255
	Total	372	99	471
Non-Caucasian				
	0-14	19	2	21
	15-44	44	18	62
	≥45	48	22	70
	Total	111	42	153
Total over race and age		483	141	624

*Based on the results of the Mirex Special Study, FY76.

Table 4.4 Chi-Square Analysis Results for Zero/Positive Values: Geographic and Demographic Variables*

Variable	Number of specimens	% Positive within each level	X^2	df	Significance level (p)**
AGE					
0-14	70	12.7	4.53	2	0.103
15-44	229	24.5			
≥45	325	23.1			
CENSUS DIVISION					
East South Central	175	37.7	49.65	2	0.0001
South Atlantic	282	10.3			
West South Central	167	27.5			
RACE					
Caucasian	471	21.0	2.73	1	0.098
Non-Caucasian	153	27.5			
SEX					
Male	298	24.2	0.80	1	0.371
Female	326	21.2			
STATE					
Alabama	39	7.7	105.8	7	0.0001***
Florida	137	5.8			
Georgia	91	20.9			
Louisiana	129	35.7			
Mississippi	136	46.3			
North Carolina	18	0.0			
South Carolina	36	5.6			
Texas	38	0.0			

*Calculated at RTI, total number of specimens (N) = 624.

**Probability that a X^2 this large or larger might occur by chance, under the assumption of no difference among categories.

***Over 5% of the cells have expected counts less than 5. Sparse table indicates chi-square may not be a valid test.

statistical computer software was used to carry out these tests for unweighted data, and specialized software is available at RTI for carrying out the analyses for weighted data (see Chapter 5). A model similar to the above is used for testing the proportion of positive values and the geometric means of the positive values for mirex residues found in human tissue.

A regression model was tested for the main effects of race, age, sex, and location, with location being alternately defined as State or Census Division. Then the model was expanded to include all possible interaction terms. The dependent variable for this model was presence or absence of mirex, indicated as 0 or 1 in the model. The results of this analysis are summarized in Tables 4.5 and 4.6. When State was the location factor, the model gave a strong main effect ($p<0.0001$) for location, even considering interaction effects. When interaction terms were not considered, race and sex were both significant. After interaction terms were included in the model, these significant main effects for race and sex disappeared. When Census Division was used as the location factor, Census Division was highly significant ($p<0.0001$) for the main effects of the all possible interaction terms model. Race was the only other significant effect in either model involving Census Division, and there were no significant interaction terms.

In addition to examining the significance levels of the individual F ratios, the fit of the model to the data overall should be considered. The value of R^2 is one indication of the appropriateness of the model to describe the data. In general, the larger the value for R^2, the better the model is as a description of the relationship of the dependent variable (in this case, presence or absence of mirex residue in human tissue) to the independent factors (age, race, sex, and location). R^2 can range from zero

Table 4.5 Regression Model for Race, Age, Sex, and State:* Zero/Positive Residue Values

Source	df	F Value	Pr>F** Significance	R^2
Model	11	12.92	0.0001	0.188
Error	612			
Corrected Total	623			
State	7	18.89	0.0001	
Race	1	7.48	0.006	
Age	2	1.45	NS	
Sex	1	3.64	0.057	
Model	73	2.46	0.0001	0.246
Error	550			
Corrected Total	623			
State	7	6.85	0.0001	
Race	1	1.12	NS	
Age	2	1.18	NS	
Sex	1	0.21	NS	
St x R	7	0.80	NS	
St x A	13	0.37	NS	
St x Sx	7	0.21	NS	
R x A	2	0.60	NS	
R x Sx	1	0.00	NS	
A x Sx	2	0.58	NS	
St x R x A	10	1.02	NS	
St x R x Sx	7	0.89	NS	
St x A x Sx	11	0.37	NS	
R x A x Sx	2	0.53	NS	

*Calculated at RTI - unweighted and ignoring design effect.

**Probability that this might occur by chance under the assumption of no difference among categories.

Table 4.6 Regression Model for Race, Age, Sex, and Census Division: Zero/Positive Residue Values*

Source	df	F Value	Pr>F** Significance	R^2
Model	6	11.45	0.0001	0.100
Error	617			
Corrected Total	623			
Census Division	2	29.86	0.0001	
Race	1	8.05	0.005	
Age	2	1.74	NS	
Sex	1	2.54	NS	
Model	34	2.73	0.0001	0.136
Error	589			
Corrected Total	623			
Census Divsion	2	20.07	0.0001	
Race	1	6.82	0.0009	
Age	2	0.46	NS	
Sex	1	0.78	NS	
CD x R	2	1.10	NS	
CD x A	4	0.89	NS	
CD x S	2	0.59	NS	
R x A	2	0.19	NS	
R x S	1	0.50	NS	
A x S	2	1.11	NS	
CD x R x A	4	0.98	NS	
CD x R x S	2	0.23	NS	
CD x A x S	4	0.35	NS	
R x A x S	2	0.41	NS	
CD x R x A x S	3	1.64	NS	

*Calculated at RTI - unweighted and ignoring design effect.

**Probability that this might occur by chance under the assumption of no difference among categories.

to one; a good fit is indicated by values for R^2 that are greater than 0.4. The value for R^2 within a series of models of the same variable will always increase as more interaction or quadratic terms are added. This change in the magnitude of R^2 can be used to determine the relative contribution of an addition or deletion of a term to the model. In this case, the R^2 values for the 4 models fitted are small; the two models including State have higher R^2 values than do the models including Census Division as the geographic boundary of interest. This is logical, as the Census Division boundaries group States on a proximity basis that does not necessarily reflect concentrated mirex treatment area divisions. If percentage of a State's area treated by mirex were considered, it would be more reasonable to group Louisiana and Mississippi together for analysis than the Census Division grouping of Louisiana and Texas.

4.2.3 Quantifiable Positive Values

The summary statistics for the 123 quantifiable positive values are displayed in Tables 4.7 and 4.8. The data in these two tables are the summary statistics for both the $\log_{10}$ of the raw residue amount and the $\log_e$ of the lipid extractable material adjusted residue amount (Log Y). Both of these values were analyzed in an unweighted regression analysis. The model for this analysis is similar to the model used to detect differences in the presence or absence of mirex in the entire sample (624 observations).

The unweighted regression analysis did not show any significant differences either in a simple main effects model or in examination of interaction terms in the model. This indicates that if there are any statistically significant differences for the level of concentration of mirex residues among the categories of age, race, sex, and location of selection sites, these difference do not fit a linear model.

Table 4.7 Summary for Quantifiable Positive Residue Values: Geometric Means and Standard Errors*

Factor	Factor Levels	N	LOGY Geometric Mean (ppm)	Standard Error
All		123	0.319	0.029
AGE	0-14	8	0.226	0.084
	15-44	47	0.290	0.041
	≥45	68	0.356	0.044
CENSUS	East South Central	61	0.278	0.035
	South Atlantic	23	0.368	0.098
	West South Central	34	0.365	0.050
RACE	Caucasian	86	0.299	0.032
	Non-Caucasian	37	0.372	0.065
SEX	Male	65	0.362	0.049
	Female	58	0.278	0.033
STATE	Alabama	3	0.340	0.280
	Florida	30	0.262	0.116
	Georgia	14	0.419	0.148
	Louisiana	39	0.365	0.050
	Mississippi	58	0.275	0.035
	North Carolina	0	-	-
	South Carolina	1	0.897	-
	Texas	0	-	-

*Calculated at RTI-unweighted and ignoring design effect.

Table 4.8 Summary for Quantifiable Positive Residue Values: Geometric Means and Standard Errors*

Factor	Factor Levels	N	LOG_{10} Residue Amount Geometric Mean (ppm)	Standard Error
All		123	0.220	0.011
AGE	0-14	8	0.117	0.019
	15-44	47	0.205	0.012
	≥45	68	0.250	0.013
CENSUS	East South Central	61	0.190	0.010
	South Atlantic	23	0.237	0.026
	West South Central	34	0.267	0.016
RACE	Caucasian	86	0.211	0.010
	Non-Caucasian	37	0.245	0.017
SEX	Male	65	0.239	0.013
	Female	58	0.201	0.010
STATE	Alabama	3	0.290	0.106
	Florida	30	0.177	0.033
	Georgia	14	0.269	0.039
	Louisiana	39	0.267	0.016
	Mississippi	58	0.186	0.010
	North Carolina	0	-	-
	South Carolina	1	0.420	-
	Texas	0	-	-

*Calculated at RTI-unweighted and ignoring design effect.

4.2.4 Unweighted Analysis Conclusions

For this section, the results do not generalize to the target population but reflect only the actual sample selected. Within this group, it is observed that the most important factor in an individual showing mirex residues in adipose tissue is the location of the selection site. In this sample, there were no significant differences discovered differentiating any of the groups analyzed among the 123 observations with measurable levels of mirex.

5. WEIGHTED ANALYSIS

5.1 Introduction

The analyses presented in this chapter are weighted to produce population estimates for the target population. Quasi selection weights were calculated for each observation and used in estimating population totals. RTI has developed several software programs specifically for weighted analysis of sample survey data. These programs are used to produce the summary statistics and regression analyses [7,8].

5.2 Weight Formation

Weights were calculated separately for each site, as described in section 2.4. The observations within each site have the same selection weight, with no adjustments for age, race, and sex distribution within the site. The quotas established for each site were intended to create a self-weighting sample with respect to age, race, and sex distribution. These quotas were based on the demographic distribution of the living population of the Census Divisions in which the sample sites were located, rather than on the distribution of the population within the individual sites. These quotas were not achieved within site, although on a division level the desired proportions of race and sex were approximately met (see Table 4.1). The age distribution was not met, and this undoubtably reflects the source of the selection population. The population that is young is not proportionately represented in the segment of the population that undergo surgery or die in a large hospital.

After weights were calculated and added to the data file for each observation, the weighted analyses for the percent positive values and the quantifiable positives were carried out.

5.3 Estimates of Positive Mirex Detection

The parameters and statistical tests of interest are the same for the weighted and unweighted analyses. The use of weights to produce population based estimates affects the calculation of statistics and thus allows generalization of the statistics to the target population.

A weighted chi-square test was used to examine the estimated proportions of the target population with detectable mirex residues. The weighted chi-squares were produced by a weighted chi-square SAS procedure [9]. This produced an inflated statistic. To adjust for this inflation before evaluating the resulting statistic, the computed chi-squares were multiplied by the sampling fraction and then divided by the design effect. The sampling fraction is the number of specimens in the sample divided by the target population estimate. In this case, the sampling fraction = 624/23,371,372 = 2.67×10^{-5}. The design effect (DEFF) is the inverse of the ratio of the variance that would be obtained for a simple random sample to the variance for the design employed.

The results of this analysis are shown in Table 5.1. State was the only significant factor for differences in the proportion of the target population that has some detectable mirex residue in adipose tissue. Mississippi was the state that had a statistically significantly larger proportion of positive mirex detection.

A Taylorized weighted least squares analysis was used, which incorporates the sampling structure into the regression model calculations [8]. This analysis will not compute valid F statistics if the number of PSU's is smaller than the combined levels of the factors in the model. In the case of a model which included State as the location factor, there are 8 levels for the factor State. When interaction terms are included in this model,

Table 5.1 Weighted Chi-Square Analysis Results for Zero/Positive Values: Geographic and Demographic Variables*

Variable	Estimates of n	% Estimated Positive within each level	X^2	df	Significance level (p)**
AGE					
0-14	6,606,385	1.6	2.72	2	0.257
15-44	7,529,303	11.3			
≥45	9,235,645	15.4			
CENSUS DIVISION					
East South Central	1,727,307	37.6	2.6	2	0.273
South Atlantic	9,726,438	13.3			
West South Central	11,917,587	3.6			
RACE					
Caucasian	18,517,425	9.8	0.04	1	0.844
Non-Caucasian	4,853,897	11.5			
SEX					
Male	8,653,061	16.1	1.8	1	0.180
Female	14,718,264	6.7			
STATE					
Alabama	503,079	4.6	32.8	7	0.0001***
Florida	2,477,302	5.4			
Georgia	5,642,299	19.6			
Louisiana	3,229,595	13.4			
Mississippi	1,224,229	51.1			
North Carolina	210,692	0.0			
South Carolina	1,396,152	3.9			
Texas	8,687,994	0.0			

*Calculated at RTI, estimate of N = 23,371,332.

**Probability that a X^2 this large or larger might occur by chance, under the assumption of no difference among categories.

***Over 5% of the original cells have expected counts less than 5. Sparse table indicates chi-square may not be a valid test.

the sum of levels in the analysis rapidly increases beyond the limits imposed by the sampling structure. Consequently, only main effects models involving State as the location variable were computed at this time.

The interpretation of the weighted regression model requires some caution. The presence of significant main effects can be misleading in some cases. It is important to consider possible interaction effects in the model as well. Significant main effects in the presence of significant interaction terms involving the same factors that are in the main effects are not interpreted independent of the interaction terms.

The results for a weighted regression analysis for the estimated presence or absence of mirex when Census Division is the location of interest are in Table 5.2. A model containing all possible two-way interactions produced a significant sex and Census Division interaction ($p<0.017$).

The model containing State was limited to a simple main effects model, due to the restrictions noted above. Results for this model are shown in Table 5.3. The significant main effect for State ($p<0.0001$) in this analysis confirms the results of the weighted chi-square, which also shows State to be a significant factor in differences in proportion of the population with positive mirex residues. The main effect for sex in this model is marginally significant ($p<0.109$). There may be some interaction between sex and State; however, this was not tested.

5.4 Estimates of Quantifiable Positive Values

The estimates of the geometric means and standard errors for the $\log_e$ of the lipid extractable material (LEM) adjusted residue amounts and the the $\log_{10}$ residue amount are in Tables 5.4 and 5.5. These variables were also analyzed by the weighted regression technique described in section 5.2.

Table 5.2 Regression Model for Race, Age, Sex, and Census Division: Zero/Positive Residue Values*

Source	Degrees of freedom	F Value	Pr>F** Significance
Overall Model	6	9.30	0.0001
Census Division	2	3.53	0.042
Race	1	0.14	NS
Age	2	1.93	0.162
Sex	1	3.03	0.092
Overall Model	19	46.41	0.0001
Census Divsion	2	***	
Race	1	***	
Age	2	***	
Sex	1	***	
CD x R	2	0.26	NS
CD x A	4	1.13	NS
CD x S	2	3.45	0.044
R x A	2	0.28	NS
R x S	1	0.11	NS
A x S	2	1.22	NS

*Calculated at RTI.

**Probability that this might occur by chance under the assumption of no difference among categories.

***Not testable in the presence of interactions.

Table 5.3 Weighted Regression Model for Race, Age, Sex, and State: Zero/Positive Residue Values*

Source	Degrees of freedom	F Value	Pr>F** Significance
Overall Model	11	11.38	0.0001
Total	30		
State	7	16.11	0.0001
Race	1	0.23	NS
Age	2	0.91	NS
Sex	1	2.72	0.109

*Calculated at RTI.

**Probability that this might occur by chance under the assumption of no difference among categories.

Table 5.4 Estimated Summary Statistics for Quantifiable Positive Residue Values: Weighted Geometric Means and Standard Errors*

Factor	Factor Levels	Estimate of N**	LOGY Geometric Mean (ppm)	Standard Error
All		1,919,220	0.286	0.048
AGE	0-14	88,474	0.172	0.037
	15-44	504,045	0.283	0.039
	≥45	1,326,701	0.297	0.077
CENSUS	East South Central	621,846	0.292	0.018
	South Atlantic	924,120	0.266	0.083
	West South Central	373,254	0.331	0.049
RACE	Caucasian	1,427,135	0.252	0.030
	Non-Caucasian	492,085	0.406	0.102
SEX	Male	1,033,219	0.322	0.085
	Female	886,001	0.249	0.023
STATE	Alabama	23,193	0.340	***
	Florida	132,962	0.298	0.105
	Georgia	764,235	0.250	0.085
	Louisiana	373,254	0.331	0.049
	Mississippi	598,654	0.290	0.019
	North Carolina	***	***	***
	South Carolina	26,923	0.897	***
	Texas	***	***	***

*Calculated at RTI.

**Estimate of total population for the region of interest = 23,371,372.

***No information in sample on which to base estimate.

Table 5.5 Estimated Summary Statistics for Quantifiable Positive Residue Values: Weighted Geometric Means and Standard Errors*

Factor	Factor Levels	Estimate of N**	Log_{10} Residue Amount Geometric Mean (ppm)	Standard Error
All		1,919,220	0.197	0.010
AGE	0-14	88,474	0.095	0.009
	15-44	504,045	0.192	0.012
	≥45	1,326,701	0.209	0.017
CENSUS	East South Central	621,846	0.189	0.003
	South Atlantic	924,120	0.187	0.017
	West South Central	373,254	0.244	0.014
RACE	Caucasian	1,427,135	0.184	0.011
	Non-Caucasian	492,085	0.240	0.020
SEX	Male	1,033,219	0.208	0.018
	Female	886,001	0.186	0.006
STATE	Alabama	23,193	0.290	***
	Florida	132,962	0.190	0.030
	Georgia	764,235	0.181	0.018
	Louisiana	373,254	0.244	0.014
	Mississippi	598,654	0.185	0.003
	North Carolina	***	***	***
	South Carolina	26,923	0.420	***
	Texas	***	***	***

*Calculated at RTI.

**Estimate of total population for the region of interest = 23,371,372.

***No information in sample on which to base estimate.

The variable of most interest is the LEM adjusted residue amount; three models involving this variable are listed in Table 5.6. The first two models in the table are main effects models, one with State as the location factor and one with Census Division. Neither model has any significant main effects. The third model in Table 5.6 contains all possible second order interactions. There are marginally significant interactions for Census Division by age group ($p<0.069$) and for Census Division by race ($p<0.0106$). This indicates that there may be differences in levels of mirex concentraction among subgroups of the target population. However, these differences are not straight-forward in the sense that it can be asssumed that the same subgroup had the highest mirex levels in every Census Division. For example, Caucasians in the East South Central and West South Central Census Divisions have higher levels of mirex concentrations than do Non-Caucasians. In the South Atlantic Census Division Non-Caucasians have higher mirex residue levels than do Caucasians.

The same three models were analyzed for the unadjusted residue amount values. These results are in Table 5.7. There is a significant effect for State ($p<0.0027$) and for age group ($p<0.0138$) when State is the location tested. In the second order interaction model, with Census Division as the location factor, the interactions are similar to those obtained when the LEM adjusted residue amount is the dependent variable in the same model.

5.5 Weighted Analysis Conclusions

The two weighted analyses are conducted so that inferences can be made about the population of the mirex treated areas. The use of two different analyses provides a check on the test results.

The chi-square statistic is relatively assumption-free, but it is less sensitive to differences than regression analysis. Regression analysis, in

Table 5.6 Weighted Regression Model for Location, Age, Race, and Sex: Quantitative Positive Residue Amounts*

Source	Degrees of freedom	F Value	Pr>F** Significance
Overall Model***	9	7.42	0.0001
State	5	1.34	NS
Age	1	0.66	NS
Race	1	1.65	NS
Sex	2	2.22	0.1322
Overall Model	6	6.25	0.006
Census Division	2	0.85	NS
Age	2	2.02	0.1561
Race	1	2.25	0.1481
Sex	1	0.77	NS
Overall Model***	18	129409	0.0001
Census Division	2	Not Testable	
Age	2	Not Testable	
Race	1	Not Testable	
Sex	1	Not Testable	
CD X A	3	2.72	0.0690
CD X R	2	5.63	0.0106
CD X S	2	0.43	NS
A X R	2	0.55	NS
A X S	2	0.50	NS
R X S	1	2.17	0.155

*Calculated at RTI: dependent variable in model is the LEM adjusted residue amount.

**Probability that this might occur by chance under the assumption of no difference among categories.

***Full higher order interaction models not estimable.

Table 5.7 Weighted Regression of Quantifiable Positive Residue Amounts: Location, Age, Race, and Sex*

Source	Degrees of freedom	F Value	Pr>F** Significance
Overall Model***	9	34.18	0.0001
State	5	5.20	0.0027
Age	1	5.24	0.0138
Race	1	1.34	NS
Sex	2	0.01	NS
Overall Model	6	13.70	0.001
Census Division	2	1.31	NS
Age	2	4.83	0.0182
Race	1	1.70	NS
Sex	1	0.02	NS
Overall Model***			
Census Division		Not Testable	
Age		Not Testable	
Race		Not Testable	
Sex		Not Testable	
CD X A	3	4.03	0.020
CD X R	2	6.59	0.0057
CD X S	2	0.84	NS
A X R	2	1.34	NS
A X S	2	0.43	NS
R X S	1	1.70	NS

*Calculated at RTI. Variable $\log_{10}$ residue amount.

**Probability that this might occur by chance under the assumption of no difference among categories.

***Models containing higher level interactions not computed.

turn, requires more assumptions to be met about the underlying structure of the data before it can be considered an appropriate test.

It appears that location is an important variable in the presence or absence of mirex in human adipose tissue. Mississippi is the State which shows the largest proportion of presence of mirex in human adipose tissue. There is less clear cut evidence that there are any large differences in level of residue amount in any particular category studied, although marginally statistically significant results are found for several categories.

REFERENCES

[1] Kutz, F. W., and Strassman, S.C. National Human Monitoring Program Study Plan for Mirex Special Project. EPA internal document, not dated.

[2] Commercial Atlas and Marketing Guide, Rand McNally and Company, New York, New York. 1974.

[3] Hartwell, T. et. al (1979). Analysis of EPA Pesticides Monitoring Networks. EPA, Office of Toxic Substances, Washington, D.C., 560/13-79-014.

[4] U. S. Department of Commerce (1972). 1970 Census of Population. Characteristics of the Population (Volume I, Part A, Section 1 and 2).

[5] Analysis of Pesticide Residues in Human and Environmental Samples, J. F., Thompson, ed., U. S. Environmental Protection Agency, Research Triangle Park, NC, 1974, Section 5 A [1].

[6] U. S. Environmental Protection Agency (1979). Analysis of EPA Pesticides Monitoring Networks. ADDENDA. EPA. Office of Toxic Substances, D.C. 5601/13-79-014. December, 1979.

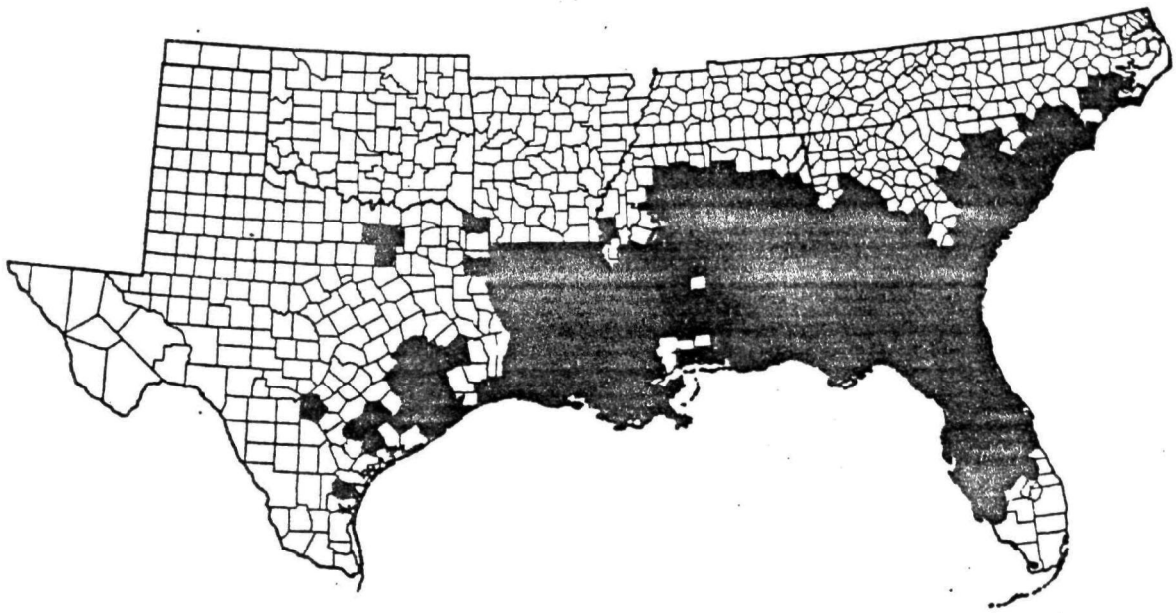
[7] Shah, B. V. (June, 1979). SESUDAAN: Standard Errors Program for Computing of Standardized Rates from Sample Survey Data. Research Triangle Institute, Research Triangle Park, North Carolina. RTI/1789/00-01F.

[8] Holt, M. M. (May 1977). SURREGR: Standard Errors of Regression Coefficients From Sample Survey Data. Research Triangle Institute, Research Triangle Park, North Carolina.

[9] Barr, Antony J., et al. (1979). A User's Guide to SAS-79, SAS Institute, Inc., North Carolina.

APPENDIX A

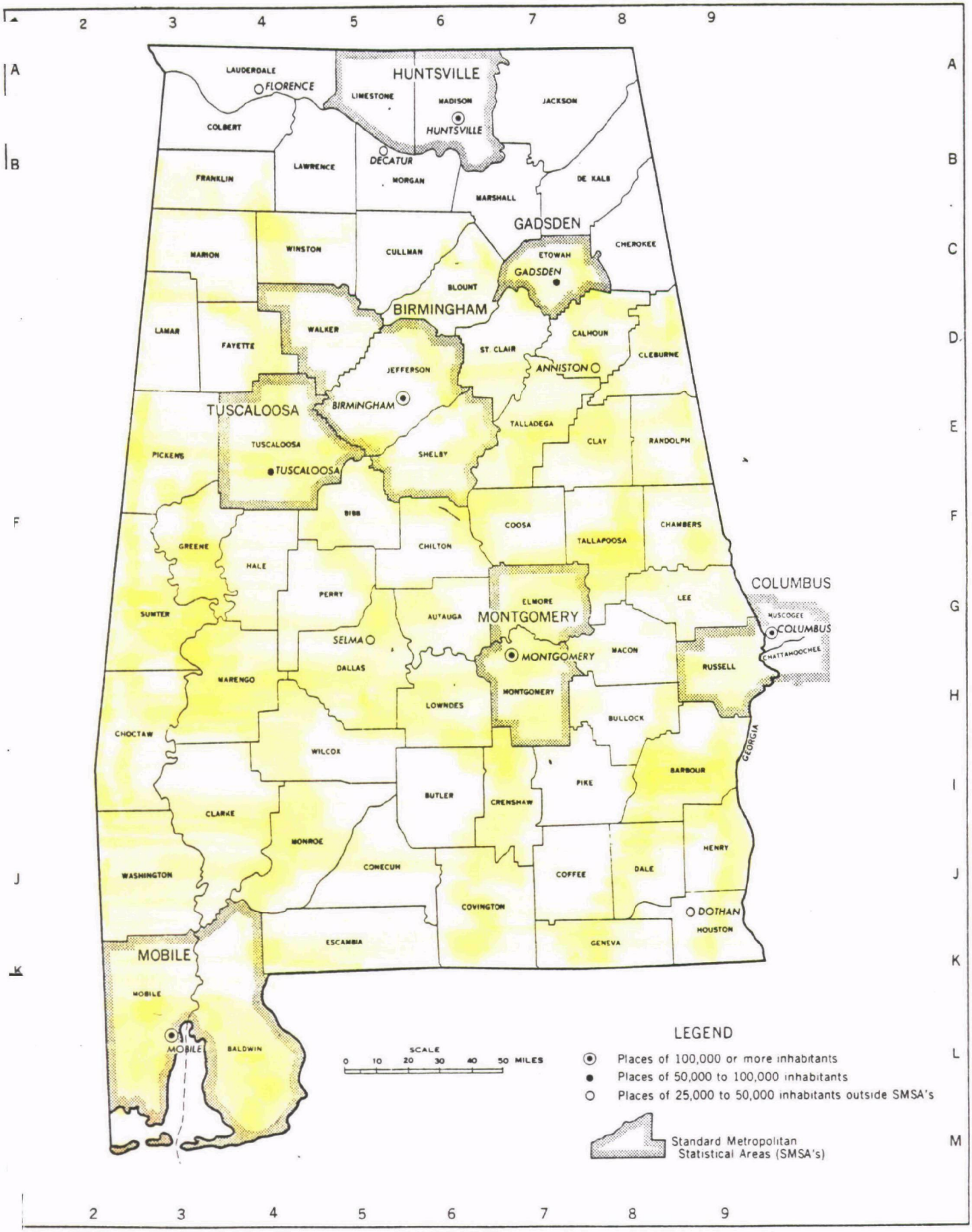
State Maps Showing Counties Where Mirex Was Applied At Some Time During 1965-1974



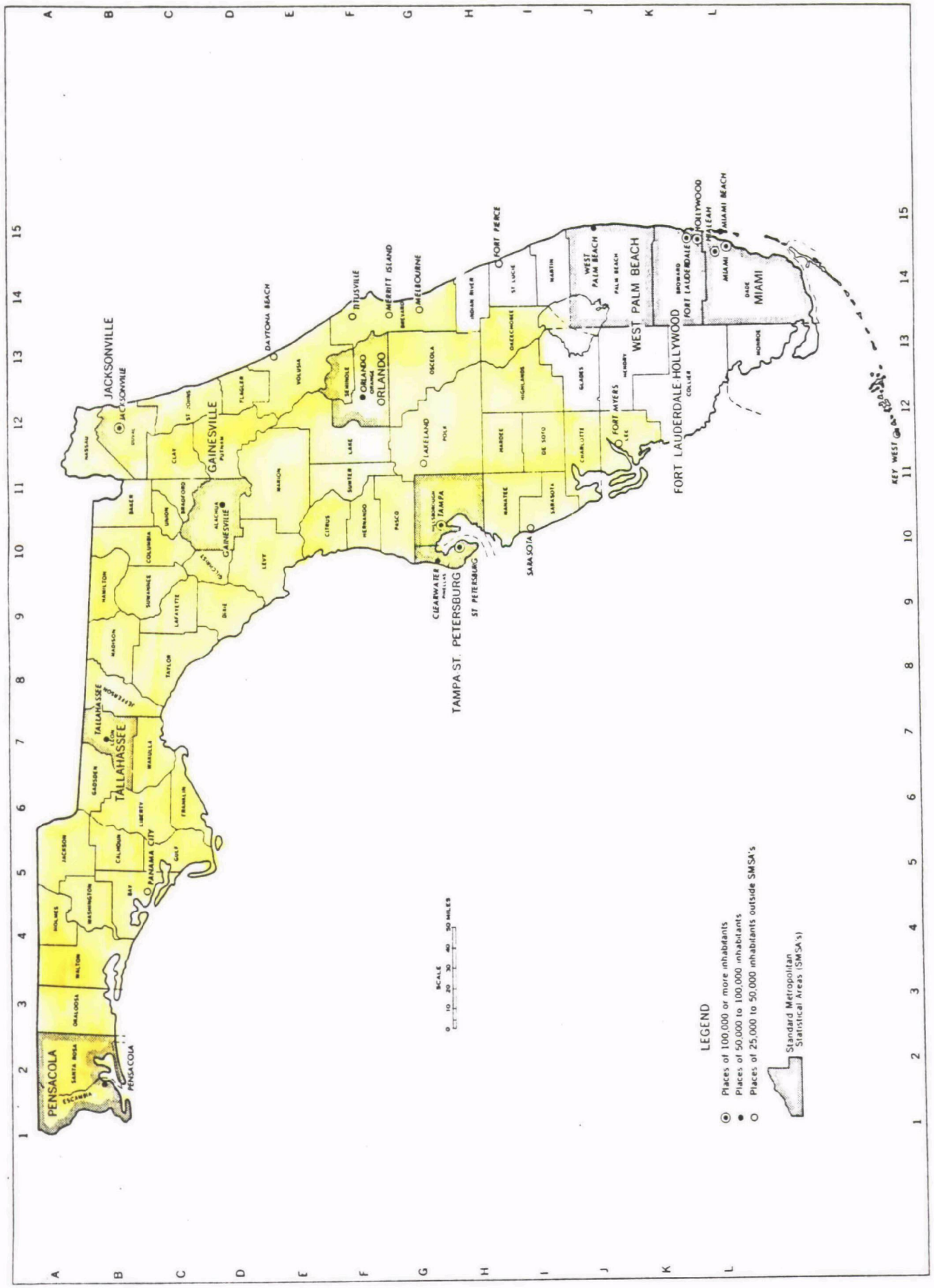
Shaded areas had some application of mirex during the period 1965-1974

ALABAMA

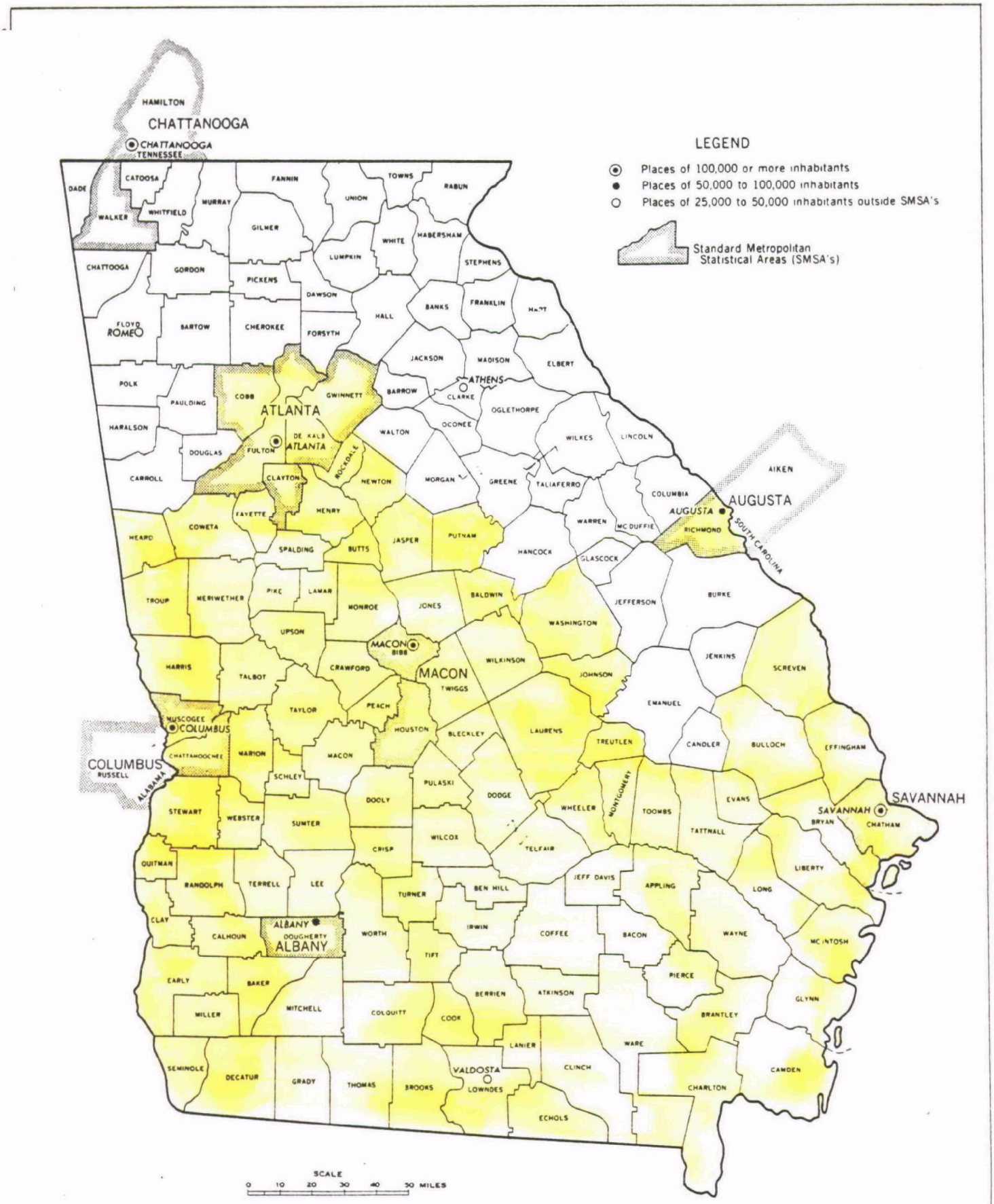
Counties, Standard Metropolitan Statistical Areas, and Selected Places



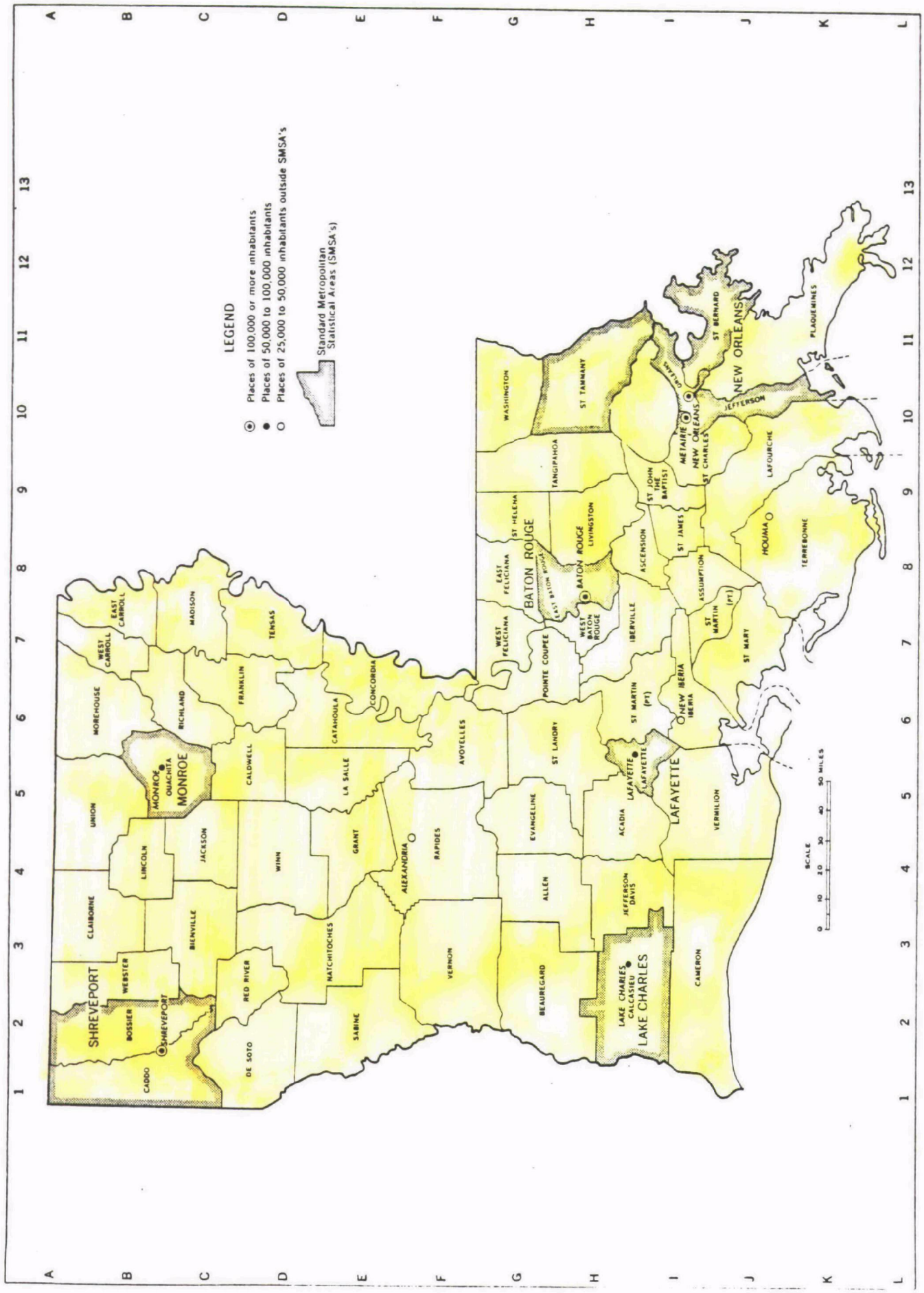
Counties, Standard Metropolitan Statistical Areas, and Selected Places



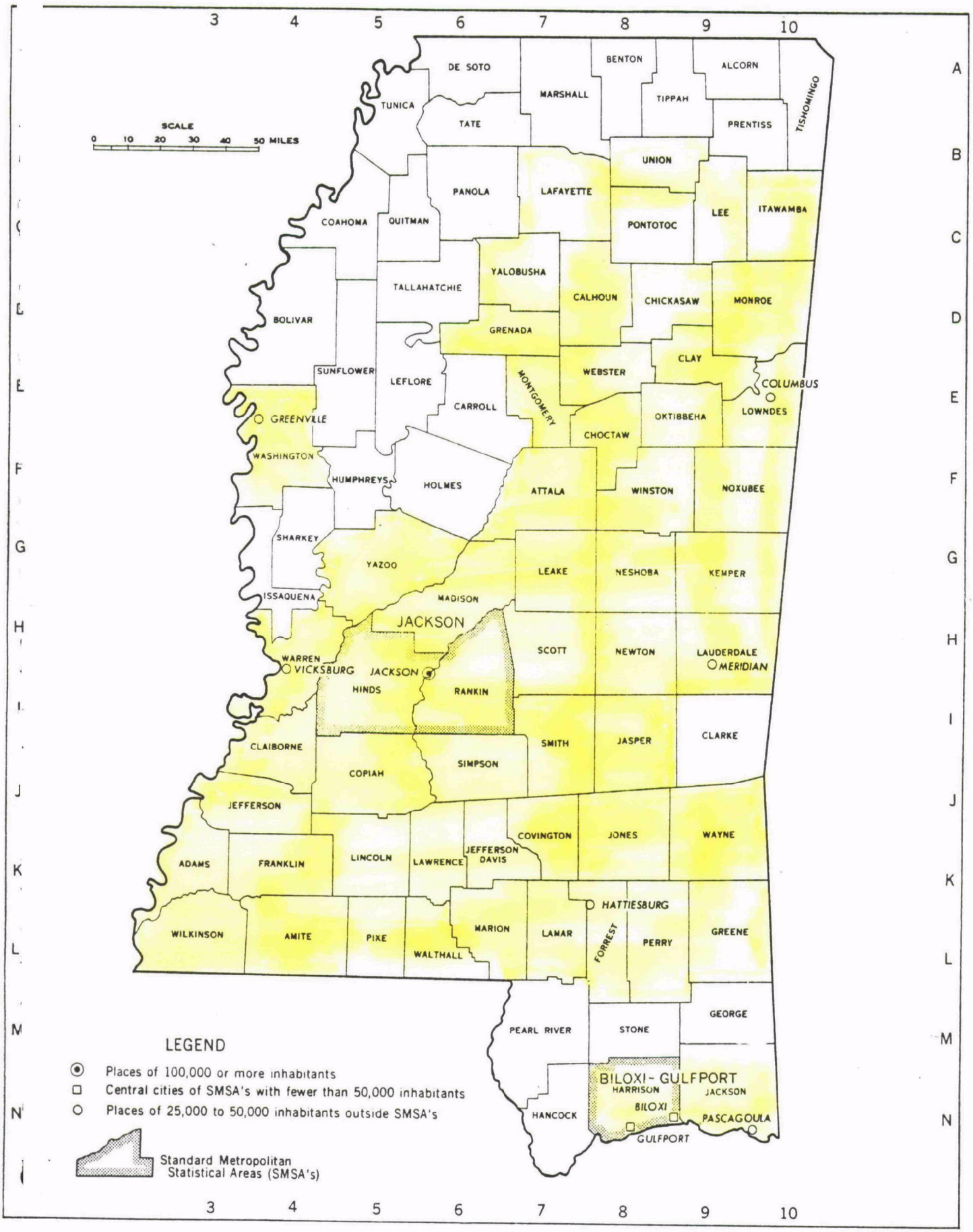
Counties, Standard Metropolitan Statistical Areas, and Selected Places



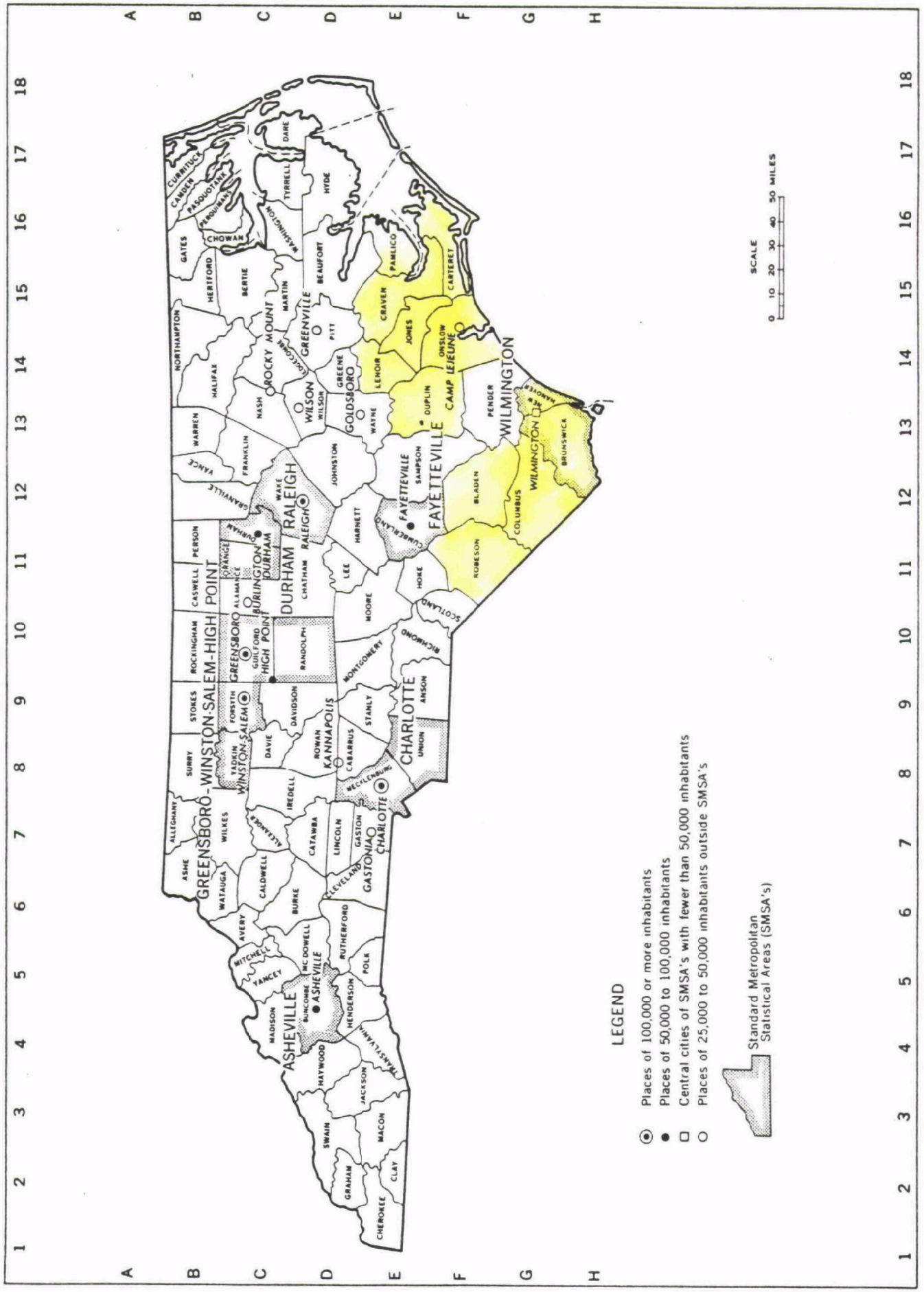
Parishes, Standard Metropolitan Statistical Areas, and Selected Places



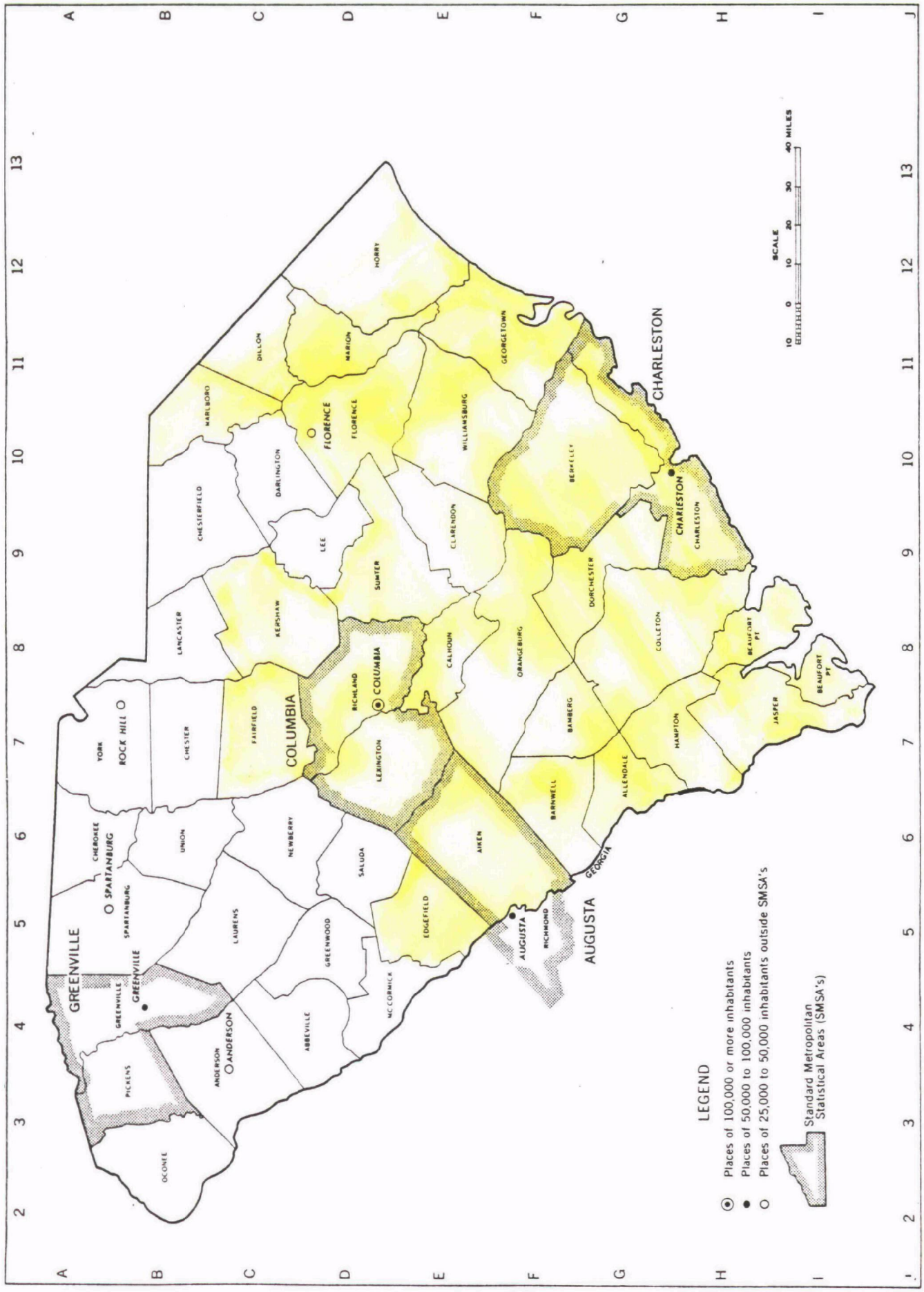
Counties, Standard Metropolitan Statistical Areas, and Selected Places



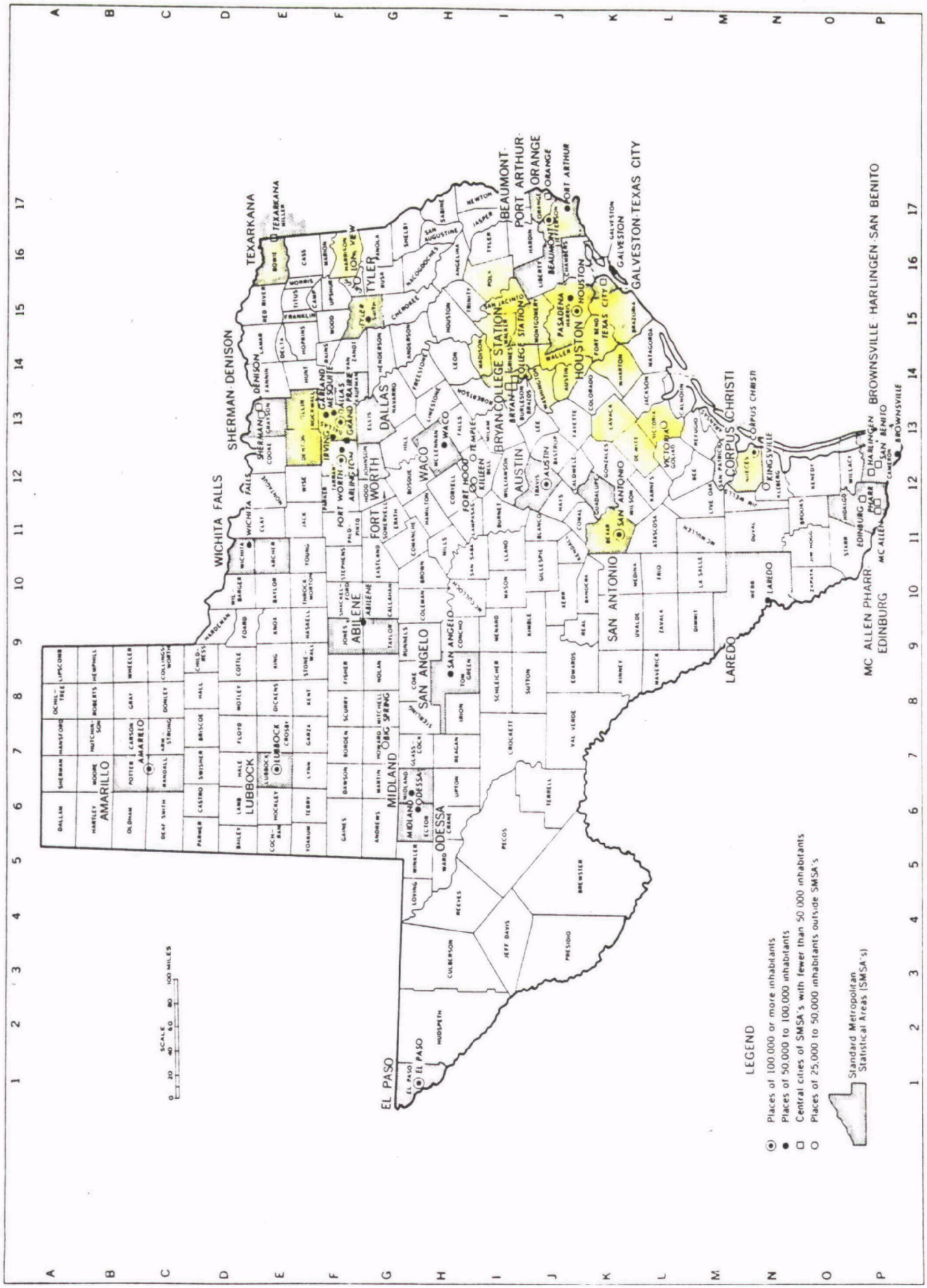
Counties, Standard Metropolitan Statistical Areas, and Selected Places



Counties, Standard Metropolitan Statistical Areas, and Selected Places



Counties, Standard Metropolitan Statistical Areas, and Selected Places



APPENDIX B

Counties Where Mirex was Applied at Some Time During 1965-1974

Note: Population values based on 1970 Census Data

Alabama — Total Population 3,444,165

All counties except	Population
Colbert	49,632
Lauderdale	68,111
Lawrence	27,281
Limestone	41,699
Madison	186,540
Morgan	77,306
Marshall	54,211
Jackson	39,202
DeKalb	41,981
Cherokee	15,606
	601,569
Mirex Area Popultation	2,842,596

Florida — Total Population 6,789,443

All counties except	Population
Monroe	52,586
Dade	1,267,792
Collier	38,040
Broward	620,100
Hendry	11,859
Palm Beach	348,753
Glades	3,669
Martin	28,035
St. Lucie	50,836
Indian River	35,992
	2,457,662
Mirex Area Population	4,331,781

Georgia

Total Population 4,589,575

All counties except	Population
Dade	9,910
Walker	50,691
Catoosa	28,271
Whitfield	55,108
Murray	12,986
Fannin	13,257
Gilmer	8,956
Union	6,811
Towns	4,565
Rabun	8,327
Habersham	20,691
White	7,742
Lumpkin	8,728
Dawson	3,639
Pickens	9,620
Gordon	23,570
Chattooga	20,541
Floyd	73,742
Polk	29,656
Haralson	15,927
Carroll	45,404
Douglas	28,659
Paulding	17,520
Bartow	32,663
Cherokee	31,059
Forsyth	16,928
Hall	59,405
Banks	6,833
Stephens	20,331
Franklin	12,784
Hart	15,814
Elbert	17,262
Madison	13,517
Jackson	21,093
Barrow	16,859
Walton	23,404
Morgan	9,904
Oconee	7,915
Clarke	61,177
Oglethorpe	7,598
Wilkes	10,184
Lincoln	5,895
Columbia	22,327
McDuffie	15,276
Warren	6,669
Taliaferro	2,423
Greene	10,212
Hancock	9,019
Glascock	2,280

	Population
Jefferson	17,174
Burke	18,255
Jenkins	8,332
Emanuel	18,189
Candler	6,412
	1,031,614
Mirex Area Population	3,557,961

Louisiana — Total Population 3,641,306

	Population
All counties mirex treated	3,641,306

Mississippi — Total Population 2,216,912

All counties except	Population
Sunflower	37,047
Leflore	42,111
Humphreys	14,601
Sharkey	8,937
Issaquena	2,737
Bolivar	49,409
Coahoma	40,447
Quitman	15,888
Tallahatchie	19,338
Tunica	11,854
Panola	26,829
Tate	18,544
DeSoto	35,885
Marshall	24,027
Benton	7,505
Tippah	15,852
Alcorn	27,179
Prentiss	20,133
Tishomingo	14,940
Carroll	9,397
Holmes	23,120
Attala	19,570
Clarke	15,049
George	12,459
Stone	8,101
Pearl River	27,802
Hancock	17,387
	566,148
Mirex Area Population	1,650,764

North Carolina — Total Population 5,082,059

	Population
New Hanover	82,996
Brunswick	24,223
Columbus	46,937
Robeson	84,842
Bladen	26,477
Duplin	38,015
Onslow	103,126
Lenoir	55,204
Jones	9,779
Craven	65,554
Pamlico	9,467
Cateret	31,603
Mirex Area Population	578,223

South Carolina — Total Population 2,590,516

	Population
Marlboro	27,151
Dillon	28,838
Marion	30,270
Horry	69,992
Georgetown	33,500
Williamsburg	34,243
Florence	89,636
Clarendon	25,604
Sumter	79,425
Kershaw	34,727
Fairfield	19,999
Richland	233,868
Calhoun	10,780
Orangeburg	69,789
Berkeley	56,199
Charleston	247,650
Dorchester	32,276
Colleton	27,622
Bamberg	15,950
Barnwell	17,176
Aiken	91,023
Allendale	9,692
Edgefield	15,692
Lexington	89,012
Hampton	15,878
Jasper	11,885
Beaufort Point	51,136
Mirex Area Population	1,469,023

Texas Total Population 11,196,730

	Population
Denton	75,633
Collin	66,920
Dallas	1,327,321
Bowie	67,813
Harrison	44,841
Smith	97,096
Orange	71,170
Jefferson	244,733
Polk	14,457
San Jacinto	6,702
Walker	27,680
Madison	7,693
Grimes	11,855
Harris	1,741;912
Galveston	169,812
Brazoria	108,312
Fort Bend	52,314
Waller	14,285
Austin	13,831
Washington	18,842
Wharton	36,729
Lavaca	17,903
DeWitt	18,660
Victoria	53,766
Bexar	830,460
Nueces	237,544
Montgomery	49,479
Brazos	57,978
Mirex Area Population	5,485,781

APPENDIX C

Definition of Mirex Treated Areas

Definition of Mirex-Treated Areas

The definition of mirex-treated areas for the MSS is global; a mirex treated county is a county including an area having any application of mirex during a 10-year period. This definition does not take into consideration factors of repeated applications, amount of active substance displaced, or land area covered.

Before being banned from sale and use in 1977, mirex was sold over the counter to private individuals and applied by both State action agencies and the States and Federal Government working together. It should be possible to obtain mirex use information from individual State action agencies and from the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA, APHIS) which would help answer some of the above questions about the frequency and intensity of mirex application. For example, this kind of historical information about mirex application in the State of North Carolina could be gathered from existing records in the North Carolina Department of Agriculture's Pest Control Division.[1/] RTI has information from the APHIS of USDA concerning the number of mirex-treated acres by State; this only includes those acres treated from 1964 to 1973 by the Federal Government in connection with the individual States.[2/] Additional mirex treatment must have varied from State to State, dependent upon the magnitude of the fire ant problem and the resources of the State.

[1/] Howard M. Singletary, North Carolina State Department of Agriculture, Pesticide Division. Personal communication, May 8, 1980. C. Leininger.

[2/] Table of Aggregate Acres Treated, provided to EPA by APHIS. Revised date 8/1/73.

In an epidemiological study such as this, it is still more important to obtain specific residence information about the individuals from whom specimens are collected than a better definition of the area in which the individuals are assumed to reside. At this time it would be inconclusive to attempt to correlate more detailed information about mirex use with this human adipose tissue data.

APPENDIX D

MSS Sample Selection Method

MSS Sample Selection Method

D.1 Introduction

In order to calculate weights that can be used in making population estimates from sample data, the method used to select the sample must be known. This appendix is a discussion of the method used to select the sample RMA's for the MSS. For the NHMP, the method used for selection of sites was probability proportional to the size of the site's population (PPS). There was some question about the method used to select sites for the MSS. The selection procedure was checked by calculating the expected outcome of selecting 32 out of 60 RMA's by both PPS and simple random sampling (SRS). It was concluded that SRS was the method used.

D.2 The NHMP and Probability Proportional to Size

The method of sample selection used in the NHMP was selection with probability proportional to size (PPS). This means that the population of a city was a factor in the possibility of the city being selected in a sample. PPS sampling with equal-size final stage samples results in a self-weighting sample. When n areas are to be selected with PPS, the selection probability for a given area is calculated by dividing n times the population of that area by the total population of all the areas in the sampling frame. This is expressed as

$$\text{selection probability (PPS) of area } i' = \frac{n \text{ population of area } i'}{\sum_{i=1}^{N} \text{population of area } i},$$

where N is the number of areas in the sampling frame.

NHMP cities were selected by PPS, within strata defined by Census Divisions. As discussed in Chapter 2, the 8 NHMP cities located in the mirex area were included in the MSS. This was an effort to use the existing tissue collection network. The MSS supplementary sample was selected from 60 RMA's not in the NHMP. The majority of the existing documentation of the MSS indicates that SRS was used to select the 32 MSS sites.

D.3 Simple Random Sampling

In SRS, any RMA has the same chance of being selected as any other RMA in the list of 60 RMA's in the mirex area. A listing of the eligible sites might be made and a consecutive number assigned to each site. A sample of sites can then be selected by drawing numbers (sites) from the list using a table of random numbers. This is the method documented on page 3 of the NHMP study plan for the MSS[1/]. There was one instance of conflicting MSS documentation, suggesting PPS selection for the MSS supplementary sites. This was sufficient reason to investigate the sample selection method.

If SRS was used to choose the 32 RMA's, then any one RMA was as likely as any other to be chosen. To test this, the 60 RMA's were first ordered by population size, and then divided into 4 groups, each with 15 RMA's. There were 32 RMA's actually selected. Under SRS it would be expected that approximately the same number of RMA's would be selected from each of the 4 divisions that have been arbitrarily made in the ordered listing. This can be seen more easily in the line chart below, where the 4 segments or groups are indicated by Roman numerals, with a subscript "s' or 'f' to indicate membership in the sample or in the frame.

1	I_s	8	II_s	16	III_s	24	IV_s	32 = sample RMA's
1	I_f	15	II_f	30	III_f	45	IV_f	60 = frame RMA's

In repeated sampling, the average proportion of selected RMA's from a segment of the frame will be one-fourth. Since it is a random process, there will be some variation in the number of selected RMA's from a segment of the frame. A 95 percent confidence interval (C.I.) will give the range of values that will be encountered 95 out of 100 times a given sample selection procedure is used. The expected number of sites selected by SRS in any of the 4 segments of the frame is one-fourth of 32, or 8. To find a 95 percent confidence interval for the number of sites chosen from each segment, computations were based on the variance of the number of sites selected per segment and the properties of the normal curve. These calculations are given in detail in section D.6. For this case, where 8 is the expected number of sites selected from a segment, the 95 percent C.I. is $8 \pm 1.96\sqrt{6} = 8 \pm 4.8$. The number of sites selected under SRS can range from 3.2 to 12.8 sites within a 95 percent C.I. This agrees with the actual distribution of sites among the arbitrary segments, as indicated in the chart below.

	I_s	II_s	III_s	IV_s
Expected number of sites (SRS) and the 95% C.I.	8 ± 4.8	8 ± 4.8	8 ± 4.8	8 ± 4.8
Number of sites actually in the MSS	8	5	8	11

D.4 Probability Proportional to Size

The same arbitrary segments were created, and 95 percent C.I.'s for the number of sites selected from each segment were calculated, assuming selection PPS. The selection rates varied from segment to segment, because

the combined population of the areas within a segment determined the sampling rate for that segment. The combined population for each of the four divisions varied considerably: I_F = 730,600, II_F = 1,159,800, III_F = 2,124,100, and IV_F = 6,779,200, for a frame total of 10,793,700. The selection probabilities also varied accordingly, from $Pr(I_s) = 0.07$ to $Pr(IV_s) = 0.63$. C.I.'s for the number of selected sites from each segment under PPS were computed and are displayed in the chart below.

	I_s	II_s	III_s	IV_s
Expected number of sites (PPS) and the 95% C.I.	2.2 ± 2.8	3.4 ± 3.5	6.3 ± 4.4	20.1 ± 5.4
Actually selected for MSS	8	5	8	11

The number of sites actually selected in 2 of the 4 segments falls outside the 95% C.I. for number of selected sites from the segment when selection is PPS.

D.5 Conclusions

Based on the computations described in Section D.3 and D.4, it appears that the MSS sample sites were selected by simple random sampling. Therefore, the selection rates used in computing the quasi selection weights were calculated under the assumption of SRS. This agrees with the majority of the documentation for the MSS.

D.6 Calculations

This section contains a detailed description of the calculation of confidence intervals for the number of sites selected per segment when selecting 32 of 60 RMA's by SRS. The method used to determine C.I.'s when

using PPS sampling is similar to that used in SRS, and will be described briefly.

Confidence intervals are calculated using the properties of the normal curve and the variance of a random variable. In this case, the random variable, which can be called y, is the number of sites that will be selected from a given segment. The distribution of y is binomial. Variances are calculated for binomial random variables (in this case, the Bernoulli event is that a site is selected in a given segment or it is not selected in that segment) by multiplying the total number of sites selected by the probability (p) that the selected site is in the segment by the probability (q) that it is not. Thus, the variance of y can be expressed as $V(y) = npq$.

The probability that a selected site falls in a given segment is 8/32 = 1/4 = p. The value of q is then 24/32 or 3/4. The variance will be the same for the number of sites selected in each segment: $32(1/4)(3/4)=6$. To find the 95 percent C.I. for y (where y is the number of sites selected for a segment) requires multiplying the square root of the variance (this is the standard deviation) by 1.96. The constant 1.96 is a value (called a z score) which corresponds to 95 percent of the area under the normal curve. Other constants, which correspond to different percentages of the area under the normal curve, can be found in a table of z scores in any standard statistical text. In this case, where the probability that a selected site falls in the given segment is the same for all segments, the 95 percent

C.I. is $8 \pm 1.96\sqrt{6} = 8 \pm 4.8$ for each segment.

To find the 95% confidence interval for number of selected sites falling in a given segment, assuming PPS, the same procedure is followed as for SRS, except that the probability of a selected site falling in a given segment is different for each segment. This reflects the difference in the population size of the sites in the frame. To calculate the expected number of sites in a segment, the probability of selection for a segment is multiplied by the number of sites desired in the final sample. As an example, for segment I_f, with Pr(selection) = 730,600/ 10,793,700 = 0.07, the expected value of y = (0.07)(32) = 2.2. The variance of y was calculated separately for each segment, and the C.I.'s computed for each segment as was done when assuming SRS.

1/ Kutz, F. W. and Strassman, S. C. (not dated). National Human Monitoring Program. Study Plan for Mirex Special Project. EPA internal document.

APPENDIX E

List of MSS Collection Sites

COLLECTION SITES

Alabama	RMA Population[1]
Anniston	93,100
° Birmingham	679,000
Dothan	51,200
°*Mobile	312,500
° Montgomery	201,000
* Tuscaloosa	106,300
Florida	
Fort Myers	83,500
Fort Walton Beach	76,500
Lakeland	97,000
Orlando	473,000
°*Panama City	72,000
Sarasota	222,000
° St. Petersburg	625,000
Tallahassee	121,500
°*Tampa	495,000
Georgia	
Atlanta	1,720,000
° Brunswick	54,300
Columbus	226,700
Macon	219,500
Savannah	184,000
x Valdosta	50,100
Louisiana	
Alexandria	97,500
° Lafayette	128,800
Lake Charles	128,500
Monroe	110,800
* New Orleans	1,126,000
Shreveport	282,000

Mississippi	RMA Population[1]
Columbus	43,200
° Greenville	51,100
Hattiesburg	57,200
Jackson	258,000
Laurel	41,000
Meridian	56,200
Pascagoula	74,900
North Carolina	
Wilmington	91,300
South Carolina	
Charleston	290,000
Columbia	315,000
Texas	
* Dallas	2,455,000
* Houston	2,085,000
* San Antonio	890,000

ALTERNATE COLLECTION SITES [2]

Albany, Georgia	94,800	Gadsden, Alabama	79,000
Opelika, Alabama	46,700	Jacksonville, Florida	585,000
Melbourne, Florida	100,000	Baton Rouge, Louisiana	361,000
Bryan, Texas	60,700	Pensacola, Florida	217,000
		Gulfport, Mississippi	173,500

* National Human Monitoring Site
x Replaced by Albany, Georgia, in survey
° Nonresponding Site
1 1974 Rand McNally Population Estimates
2 Listed in Order of Selection

RMA Remainder Frame* After Selection of Collection Sites and Alternates

Florida	RMA Population 1/
Cocoa	87,000
Daytona Beach	117,000
Gainesville	97,000
Titusville	39,000
Winter Haven	64,800
Georgia	
Augusta	215,500
Louisiana	
Houma	53,000
New Iberia	40,500
North Carolina	
Jacksonville	71,000
South Carolina	
Florence	49,600
Sumter	69,600

Texas	RMA Population 1/
Beaumont-Port Arthur	226,000
Corpus Christi	257,000
Freeport-Lake Jackson	58,700
Galveston-Texas City	135,000
Orange	51,600
Texarkana	75,000
Tyler	79,200
Victoria	45,400

1 1974 Rand McNally Population Estimates

* Remainder Frame: The sites remaining after sample selection has been made from the frame of target sites.

APPENDIX F

Instructions to Pathologists

Dear _____:

_____ has been selected as one of 40 representative cities in which the U. S. Environmental Protection Agency would like to conduct a special research study to determine the frequency of finding residues of the insecticide, mirex in humans. This letter is an invitation to you to participate in this study. This project is an activity of the National Human Monitoring Program for Pesticides and will be ongoing for the next 6 to 9 months.

Mirex is an organochlorine insecticide which has been used for the control of the imported fire ant in large areas of the southern United States. Our information indicates that over 14 million acres have been treated to date. Mirex is extremely resistant to chemical degradation and thus its persistence enables it to accumulate in certain organisms in the food chain. As you will note from the enclosed reprint, mirex has been found in humans. We now need to make a more precise estimate of the epidemiology of this chemical.

As a mechanism for estimating exposure to mirex, samples of human adipose tissue will be chemically analyzed in our laboratories. Reports of findings will be furnished to each participating physician. Data from these analyses are useful in evaluating various factors and conditions pertaining to human health and pesticide regulation.

We will need samples of human adipose tissue from previously excised surgical specimens and/or postmortem examinations. The samples should be collected in accordance with the enclosed guidelines. Each participant is being asked to collect specimens according to the enclosed age/sex/race quota form. We furnish all supplies (mailers, forms, bottles, postage-paid address cards, etc.) and are able to remunerate you or your designee for professional services at the rate of $10.00 per acceptable sample.

We hope that you will help in this study by providing selected adipose tissues obtained during routine pathology. We will telephone you about this in a few days.

Sincerely yours,

Frederick W. Kutz, Ph.D.
Project Officer
National Human Monitoring Program (WH-569)

Enclosures

INSTRUCTIONS FOR THE COLLECTION OF HUMAN ADIPOSE TISSUE FOR MIREX ANALYSIS

Mirex is an organochlorine insecticide used for the control of the imported fire ant in large areas of southern United States. The chemical is extremely resistant to degradation and this persistence and its chemical characteristics enable it to accumulate in certain organisms in the food chain.

The objective of this special environmental study is to determine the incidence and levels of mirex in human adipose tissue collected from areas which have been treated with this chemical. The results from the program are used in evaluating various factors and conditions pertaining to human health and effective pesticide regulation.

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

In order to develop precise epidemiologic data, collections must be made according to an experimental design which dictates the number of samples required according to the demographic distribution of the population of your area. You should have a copy of the quota of samples expected to be collected from your location on a fiscal year basis. All collections should be made according to this age/sex/race distribution. You should be able to collect the number of samples required in each category in the next 6 months. If you feel that you will be unable to collect the number of samples required, please let us know.

Criteria for Selection of Patients to be Sampled

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

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

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

Collection of Surgical Adipose Tissue

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

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

Collection of Postmortem Adipose Tissue

Adipose tissue samples must be obtained only from unembalmed cadavers. The interval between death and the collection of tissue should be as short as possible and must not exceed 24 hours, assuming refrigeration during the interval. Samples of adipose tissue must weigh at least five grams and should be placed in the

supplied chemically-clean container with a completed label affixed. Specimens should be stored at -4°F (-20°C) without any fixative or preservative until shipment. Submit only good quality fat; do not submit omentum as it contains too much connective tissue for satisfactory analysis.

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

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

Completion of the Patient Summary Report

A Patient Summary Report should be completed for each patient from whom a sample was taken. Special attention should be given to the completeness of the information. First and last initials, in that order, should be used instead of the complete name to insure that confidentiality is maintained. The initials, along with the date of birth, sex, and race, are used in this office to compose the AMA identification number. The patient's identification number and/or the pathology department's accession number are for your information in referring back to the individual patient when you receive the results of the pesticide analysis.

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

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

Packing and Shipping

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

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

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

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

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

For Further Information

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

Frederick W. Kutz, Ph.D.
Sandra C. Strassman
National Human Monitoring Program

APPENDIX G

Quotas for Mirex Special Study Specimens

NATIONAL HUMAN MONITORING PROGRAM

U. S. ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D. C. 20460

QUOTA FOR MIREX SPECIAL PROJECT

Each collection site in the states of Alabama and Mississippi is requested to collect human adipose tissue from the following age/sex/race groups:

QUOTA	AGE GROUP	SEX
4	0-14	Male
4	0-14	Female
5	15-44	Male
6	15-44	Female
4	45 and above	Male
4	45 and above	Female

Quota of 27 samples should include 5 non-Caucasian specimens. Proportional distribution of these non-Caucasian specimens among the age/sex groups is preferable but not required.

NATIONAL HUMAN MONITORING PROGRAM

U. S. ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D. C. 20460

QUOTA FOR MIREX SPECIAL PROJECT

Each collection site in the states of Louisiana and Texas is requested to collect human adipose tissue from the following age/sex/race groups:

QUOTA	AGE GROUP	SEX
4	0-14	Male
4	0-14	Female
6	15-44	Male
6	15-44	Female
3	45 and above	Male
4	45 and above	Female

Quota of 27 samples should include 4 Non-Caucasian specimens. Proportional distribution of these non-Caucasian specimens among the age/sex groups is preferable but not required.

NATIONAL HUMAN MONITORING PROGRAM

U. S. ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D. C. 20460

QUOTA FOR MIREX SPECIAL PROJECT

Each collection site in the states of North Carolina, South Carolina, Georgia and Florida is requested to collect human adipose tissue from the following age/sex/race groups:

QUOTA	AGE GROUP	SEX
4	0-14	Male
4	0-14	Female
5	15-44	Male
6	15-44	Female
4	45 and above	Male
4	45 and above	Female

Quota of 27 samples should include 6 non-Caucasian specimens. Proportional distribution of these non-Caucasian specimens among the age/sex groups is preferable but not required.

APPENDIX H

Sample Mirex Chromatograms Made Available To RTI

SAMPLES AND DATES OF ANALYSIS FOR CHROMATOGRAMS MADE AVAILABLE TO RTI

Samples	Date of Analysis
MA 76-77	May 27, 1976
MA 76-78	May 27, 1976
MA 76-79	May 27, 1976
MA 76-80	May 27, 1976
MA 76-82	May 27, 1976
MA 76-83	May 27, 1976
MA 76-84	May 27, 1976
MA 76-85	May 27, 1976
MA 76-86	May 27, 1976
MA 76-98	June 4, 1976
MA 76-99	June 4, 1976
MA 76-100	June 4, 1976
MA 76-101	June 4, 1976
MA 76-71 Repeat	June 4, 1976
MA 76-102	June 4, 1976
MA 76-103	June 4, 1976
MA 76-104	June 4, 1976
MA 76-123	June 15, 1976
MA 76-124	June 15, 1976
MA 76-125	June 15, 1976
MA 76-126	June 15, 1976
MA 76-127	June 15, 1976
MA 76-124	June 16, 1976
MA 76-125	June 16, 1976
MA 76-126	June 16, 1976
MA 76-127	June 16, 1976
MA 76-128	June 16, 1976
MA 76-129	June 16, 1976
MA 76-130	June 16, 1976
MA 76-131	June 16, 1976
MA 76-153	June 24, 1976
MA 76-155	June 24, 1976
MA 76-157	June 24, 1976
MA 76-158	June 24, 1976
MA 76-159	June 24, 1976

SAMPLES AND DATES OF ANALYSIS FOR CHROMATOGRAMS MADE AVAILABLE TO RTI

(Cont'd)

Samples	Date of Analysis
MA 76-160	June 25, 1976
MA 76-161	June 25, 1976
MA 76-162	June 25, 1976
MA 76-163	June 25, 1976
MA 76-164	June 25, 1976
MA 76-165	June 25, 1976
MA 76-166	June 25, 1976
MA 76-167	June 25, 1976
MA 77-9	September 20, 1977
MA 77-10	September 20, 1977
MA 77-11	September 20, 1977
MA 77-12	September 20, 1977
MA 77-13	September 20, 1977
MA 77-14	September 20, 1977
MA 77-57	October 12, 1977
MA 77-58	October 12, 1977
MA 77-59	October 12, 1977
MA 77-60	October 12, 1977
MA 77-61	October 12, 1977
MA 77-62	October 12, 1977
MA 77-129	November 8, 1977
MA 77-130	November 8, 1977
MA 77-131	November 8, 1977
MA 77-132	November 8, 1977
MA 77-133	November 8, 1977
MA 77-134	November 8, 1977

APPENDIX I

Modification of Mirex Analysis Protocol - GC Column Temperature

ENVIRONMENTAL TOXICOLOGY DIVISION
HEALTH EFFECTS RESEARCH LABORATORY
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Research Triangle Park, North Carolina 27711

SUBJECT: Multiresidue Analysis of Adipose Tissue with Emphasis on Mirex and Chlordane/Heptachlor Derivatives

DATE: March 1, 1976

FROM: Chief, Quality Assurance Section, ETD, EPA, HERL, Research Triangle Park, N.C.

J. F. Thompson

TO: Michigan Project, Mr. R. L. Welch

After receiving a copy of Bob Welch's letter to Dr. Kutz (2/11/76) we cranked up a straight 5% OV-210 and a mixture column of 1.5/1.95, OV-17/OV-210 and ran some chromatograms in the appropriate concentration ranges incorporating the derivatives of heptachlor/chlordane, mirex, and Aroclor 1260.

Frankly, we were not overjoyed by the elution patterns produced by Aroclor 1260 and Mirex. As Bob commented in his letter, at 200°C Mirex overlaps nearly completely with the next to last Aroclor peak (a major one). However, at 195°, although the separation is slightly better, we could not agree that it was anything remarkable on our column. Our separation was not nearly as good as the separation in Bob's chromatogram. We dropped the column temp. to 190°, and while the mirex peak was clearly visible on the early side of the Aroclor, it was not a quantifiable separation.

Undoubtedly, the relative efficiency of his column vs. ours was a factor. We estimated his column to be yielding about 3,200 T.P. with his carrier flow obviously cranked down. To compensate for the excessive time consumption at 190°, we raised the flow to 80 ml with a resulting EFF. of about 2,700 T.P. Undoubtedly, our resolution characteristics suffered accordingly.

Just so long as Bob's column holds up and he can continue to obtain the separations shown in his sample chromatograms, we see no objection at all to making temperature and/or carrier flow adjustments in any way that it will best get the job done. There is nothing sanctified in the recommended parameters in the manual. They were written for a shotgun approach to general multiresidue work, but if some other combination is more suitable for special purposes, then by all means, appropriate modifications should be made.

Even with our poor separation, I believe the chromatographer would be alerted to the possible presence of mirex, then by further confirmation via ECD and GC/MS the final answers can be obtained.

I'm sure we will want to get together for further discussions of this topic the week of the chemists' meeting.

cc: Dr. F. W. Kutz, Washington
Dr. H. F. Enos, Athens

APPENDIX J

Confirmation Analyses of Mirex in Human Adipose Tissue Extracts

Analytical Results

Confirmational Analyses for

Mirex in Human Adipose Tissue Extracts

Analytical Chemistry Branch
Environmental Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

Date: 30 November 1976

J-3

Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE November 30, 1976

SUBJECT. Confirmational Analyses for Mirex in Human Adipose Tissue Extracts

FROM. Dr. Edward O. Oswald, Chief Edward O. Oswald.
Analytical Chemistry Branch, ETD (MD-69)

TO: Dr. Frederick W. Kutz
Ecological Monitoring Branch (WH-569)
OPP, Headquarters

THRU: Director, HERL (MD-51) R. Lee for

Attached you will find the results of confirmational analyses for Mirex as generated by members of the Analytical Chemistry Branch, ETD, HERL, RTP on extracts of human adipose tissue which were received from the Michigan OPP lab on the 2 September 1976. Three (3) separate mass spectrometry groups analyzed selected extracts by gas liquid chromatography interfaced either with low resolution or with high resolution mass spectrometry in the electron impact mode.

The results of the present report confirm both the qualitative and the comparable quantitative presence of Mirex in human adipose tissue as indicated by the electron capture results from the Michigan lab.

As indicated to you in a similar report of the 23 June 1976, the GC/EC results agree quite favorably with the confirmational analyses by GC/MS.

If additional GC/MS confirmational analyses are needed by OPP for Mirex in human adipose tissue, then I would suggest that only about 10% of the total extracts be analyzed.

If I or other members of the Branch can be of assistance on this or other matters, please feel free to contact me.

Enclosure

Mirex in Human Adipose Tissue Extracts

(*Concentrations expressed in parts per million--ppm)

Sample Identification	*GC/EC Analyses (Michigan)	Confirmational GC/MS Analyses Research Triangle Park, N. C.		
		**GC/LRMS Group A	**GC/LRMS Group B	***GC/HRMS
MA76-51	0.10	0.14	0.08	0.08
MA76-62	0.20	--	0.26	0.18
MA76-72	0.39	0.42	--	0.36
MA76-75	0.16	0.17	--	0.15
MA76-80	0.20	0.25	0.15	0.15
MA76-81	0.07	0.08	0.06	0.03
MA76-84	Negative	Negative	Negative	0.01
MA76-93	0.09	--	0.07	0.04
MA76-101	0.09	--	0.09	0.08
MA76-102	Negative	0.02	0.08	0.02
MA76-116	0.12	0.01	--	0.01
MA76-123	0.22	--	0.21	0.18
MA76-124	1.10	0.56	--	0.43
MA76-129	0.11	--	0.36	0.16
MA76-131	0.15	0.60	--	0.45
MA76-146	0.12	--	0.14	0.14
MA76-147	1.13	1.97	--	1.12
MA76-159	0.16	0.28	--	0.16
MA76-161	0.69	0.27	--	0.13
Control 41 #1	1.00	0.83	0.74	0.61
Control 41 #11	1.00	0.21	0.81	0.62

Note: A number of the analyses were hindered by the high fat content of the sample extract.

*GC/EC = Analyses by gas liquid chromatography using the electron capture detector.

**GC/LRMS = Analyses by gas liquid chromatography interfaced with low resolution (500-1000) electron impact mass spectrometry.

***GC/HRMS = Analyses by gas liquid chromatography interfaced with high resolution (7200-8500) electron impact mass spectrometry.

Analytical Methodology

Confirmational Analyses for Mirex in Human Adipose Tissue Extracts

Analytical Chemistry Branch
Environmental Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

Date: 30 November 1976

Methods

Extracts of human adipose tissue were received in sealed glass ampules. At the time that the sample vial was opened, aliquots of each selected sample extract were prepared for analysis by the various mass spectrometry groups. All samples were analyzed by at least two (2) of the analytical teams which generated multiple analyses. All quantitative results are expressed as mean concentrations in parts per million (ppm) for each analytical team. The three (3) mass spectrometry teams were coordinated by: (a) Mr. Robert Harless; (b) Dr. Wayne Sovocool; and (c) Dr. Lynn Wright.

Below are listed the detailed analytical conditions utilized for the GC/MS confirmational analyses of Mirex in extracts of human adipose tissue.

Group A.

Low Resolution GC/MS

Instrument: Hewlett Packard 5930A Mass Spectrometer equipped with a Hewlett Packard 5700 gas chromatograph

GC Column: 183 cm x 2 mm (i.d.) glass column packed with 3% OV-17 on 100/120 mesh Gas Chrom Q.

Column temperature: 235°C

Injector temperature: 235°C

Transfer line temperature: 250°C

Membrane separator temperature: 235°C

Carrier gas: Helium

Flow rate: 42 ml/min

Ionization voltage: 70 eV

Criteria for Analyses:

These low resolution mass spectrometric analyses (GC-LRMS) are based upon: (a) correct absolute retention time as compared to Mirex; (b) presence of intense fragments at m/e 270 with a 6-chlorine isotope cluster and at m/e 235 with a 5-chlorine cluster (corresponding to the main electron impact ion fragments of Mirex); and (c) the proper ratios of the m/e 270 and m/e 235 ion clusters.

Quantitative GC-LRMS measurements were accomplished by single ion monitoring of the m/e 272 ion in comparison to an external Mirex standard and to internal spiked samples. All samples were analyzed in at least duplicates.

Group B.

Low Resolution GC/MS

Instrument: Finnigan 3200 Mass Spectrometer equipped with a Finnigan 6100 data system and Finnigan 9500 gas chromatograph

GC Column: 122 cm x 2 mm (i.d.) glass column packed with 3% OV-17 on 100/120 mesh Gas Chrom Q

Column temperature: 240°C

Injector temperature: 240°C

Transfer line temperature: 240°C

Glass jet separator temperature: 240°C

Carrier gas: Helium

Flow rate: 20 ml/min

Ionization voltage: 70eV

Criteria for Analyses:

These low resolution mass spectrometric analyses (GC-LRMS) are based upon: (a) correct absolute retention time as compared to Mirex; and (b) presence of intense fragments in the m/e 270 region representing the most prominent ions of the $C_5Cl_6^+$ cluster.

The mass spectrometer was operated in the multiple ion detection mode monitoring the three (3) most prominent ions of the $C_5Cl_6^+$ cluster--m/e 270, m/e 272, and m/e 274. A fourth ion m/e 405--not present in the spectrum of Mirex--was monitored to provide a background. Any sample which gave a negative or trace response upon the initial dilution conditions was then further reduced in volume to approximately 1/10 of its original volume. The sample was then reanalyzed. Negative responses to the latter conditions were reported as negative.

Samples were quantitated by summating the areas the mass chromatograms for m/e 270, 272, and 274 and comparisons of these values with the average response obtained for an external Mirex standard which was injected either before or after each residue sample.

High Resolution GC/MS

Instrument: Varian MAT 311A Mass Spectrometer equipped with a Varian 2700 gas chromatograph and a Varian C-1024 time averaging computer (CAT).

GC Column: 91 cm x 2 mm (i.d.) glass column packed with 2% Dexil 410 on 90/100 mesh Anakrom Q.

Column temperature: 243°C

Injector port temperature: 255°C

Transfer line temperature: 255°C

Slit separator temperature: 255°C

Carrier gas: Helium

Flow rate: 10-12 ml/min

Ionization voltage: 70eV

Criteria for Analyses:

The high resolution mass spectrometric analyses (GC-HRMS) are based upon: (a) correct absolute retention time as compared to Mirex; (b) isotope ratios of two fragments of the molecular ion cluster--m/e 539.6262 ($C_{10}{}^{35}Cl_{12}$) and m/e 543.6203 ($C_{10}{}^{35}Cl_{10}{}^{37}Cl_2$); and (c) analysis with internal and external standards of Mirex.

The mass spectrometer was operated at a high sensitivity in order to observe the molecular ion m/e 543.6203 ($C_{10}{}^{35}CL_{10}{}^{37}Cl_2$) with a mass resolution capability of 7200-8500. Using the time averaging computer (CAT) system, the mass spectrometer was operated in a double ion monitoring mode for the molecular ion m/e 539.6262 and m/e 543.6203. The quantitative analyses were accomplished by the use of internal and external standards of Mirex combined with the double ion monitoring of two specific ion fragments in the molecular cluster of Mirex in the high resolution GC/MS mode.

Health Effects Research Laboratory
Environmental Research Center
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Research Triangle Park, North Carolina

SUBJECT: Confirmation of Mirex in Human Adipose Tissue Extracts

DATE: June 23, 1976

FROM: Dr. Robert G. Lewis, Chief *Robert C. Hamisch*
Chemical Characterization Section, ACB/ETD (MD-69)

TO: Dr. Frederick W. Kutz
Ecological Monitoring Branch (WH-569)
OPP, Headquarters

This is to confirm my telephone transmittals to you of the results of our confirmatory analysis of mirex in human adipose tissue extracts received from the Michigan project on June 15, 1976. Mirex was confirmed qualitatively and determined quantitatively in all five samples by each of three independent methods; i.e., photochemical, GC-low resolution mass spectrometry (GC-LRMS) and GC-high resolution mass spectrometry (GC-HRMS). Data are presented below:

		Mirex Concentration, ppm		
Sample I.D.	GC-ECD (Michigan)	Photochem. GC-ECD	GC-LRMS	GC-HRMS
A75-95	0.5	0.4	0.5	0.7
75-14673	1.3	0.8	0.7	0.9
A104-75	1.3	1.3	1.2	1.6
575-6796	0.4	0.3	0.2	0.4
575-6999	1.2	0.4	0.8	1.2

Samples were received in glass vials sealed with Teflon-lined caps. Volumes ranged from 3.6 to 14.6 ml. Aliquots of 1 to 4.5 ml were withdrawn for photochemical confirmation. In this procedure, the aliquot was added to 10% diethylamine in hexane sufficient to make a total volume of 5 ml. These solutions were analyzed for GC-ECD before and after 100 min. exposure in quartz tubes to a 275W sunlamp. Analytical conditions were as follows:

Instrument -- Tracor 222 - ^{63}Ni ECD

Column -- 183-cm x 4-mm (i.d.) glass, packed with 1.5% OV-17/1.95% OV-210 on 80/100 mesh Gas Chrom Q

Attenuation -- 10^2 x 8

Carrier Gas -- Ar/CH_4, 95:5

Carrier Flow -- 75 ml/min

Column temp -- 200°C

Detector temp -- 270°C

Inlet temp -- 225°C

2

B.G. Current -- 46% f.s.d. @ 10^2 x 128 D.C.

Voltage -- 50v

Pulse Width -- 5 µsec

Pulse Rate -- 150 µsec

Photochemical confirmation work was performed by R. C. Hanisch.

Portions of each sample were taken for GC-LRMS analysis. These were concentrated to 100 µl by evaporation under a gentle stream of nitrogen at room temperature. Portions corresponding to ca. 50 ng of mirex were injected into the GC-LRMS for determination of mass spectra over the 195 to 560 amu range. GC-LRMS confirmation was based on (1) correct absolute retention time; (2) presence of intense fragments at 270 m/e with 6-chlorine isotope cluster and at 235 m/e with 5-chlorine isotope cluster (corresponded to the main electron impact fragments of mirex); (3) presence of other fragments derivable from mirex; (4) absence of possible interferring compounds; and (5) proper ratios of the 270 and 235 m/e clusters. Quantitative GC-LRMS measurements were accomplished through single ion monitoring of the 272 m/e peak and the use of an external mirex standard. All samples were analyzed in duplicate or better. Precision with standards was ±10 to 15%. GC-LRMS parameters were as follows:

Instrument: Hewlett Packard 5930A quadrapole focusing mass spectrometer equipped with an HP 5700A gas chromatograph and an HP 5932A data system.

Column: 183-cm x 2-mm (i.d.) glass, packed with 3% OV-17 on 100/120 mesh Gas Chrom Q.

Carrier Gas: Helium

Flow rate: 41 ml/min

Injection port temperature: 200°C

Column temperature: 230°C

Transfer line temperature: 250°C

Membrane separator temperature: 230°C

Ion source temperature: 190°C

Mass filter temperature: 120°C

Ionization voltage: 70eV

Filament emission: 160 µamp

Ion source pressure: 2×10^{-6} mm Hg

Scan rate: 360 amu/sec

GC-LRMS measurements were performed by G. W. Sovocool and M. Simpson.

The same concentrated samples used for GC-LRMS were also used for GC-HRMS. The mass spectrometer was operated at extreme sensitivity in order to observe the molecular ion at 543.6203 m/e (corresponding to $C_{10}{}^{35}Cl_{10}{}^{37}Cl_2$). Qualitative and quantitative analyses were performed by double ion monitoring of the 539.6262 and molecular ions. GC retention times and internal standards were used for identification and quantification. GC-HRMS parameters are given below:

Instrument: Varian MAT 311A double focusing mass spectrometer equipped with a Varian gas chromatograph and a Varian data system.

Column: 91-cm x 2-mm (i.d.) glass, packed with 5% OV-101 on Gas Chrom Q.

Carrier gas: Helium

Flow rate: 15 ml/min

Column temperature: 235°C

Injection port, transfer line and separator temperatures: 250°C

Ion source temperature: 220°C

Ion source pressure: 5×10^{-6} mm Hg

GC-HRMS measurements were performed by R. L. Harless.

I trust that you will find these data useful. If we can be of further assistance, please call on us.