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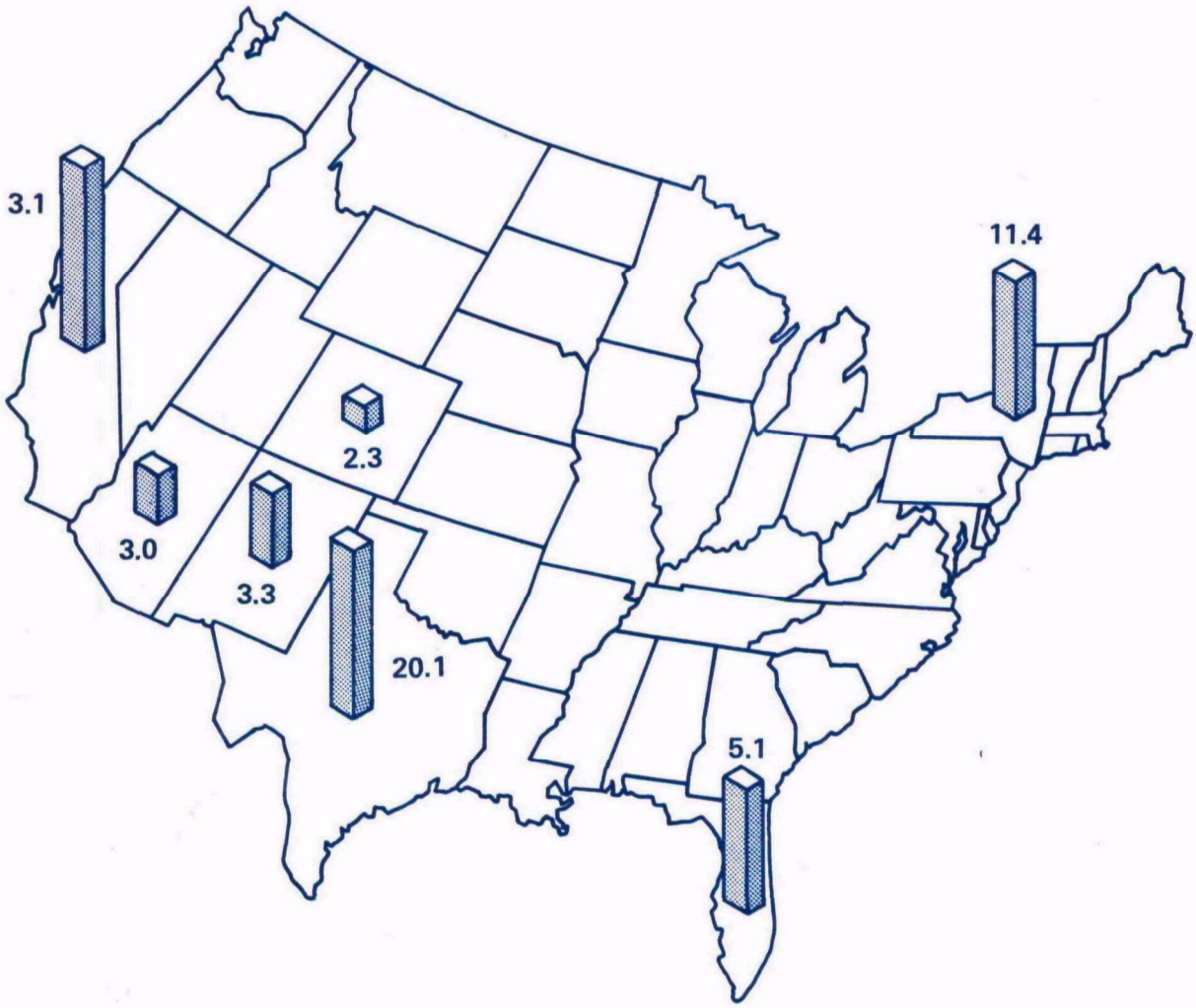
Hispanic HANES Pilot Study

Measurement of Volatile and Semivolatile Organic Compounds in Blood and Urine Specimens



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HISPANIC HANES PILOT STUDY
Measurement of Volatile and Semivolatile Organic Compounds in Blood and Urine Specimens

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I. INTRODUCTION AND BACKGROUND

The Hispanic Health and Nutritional Examination Survey (HANES) is one in a series of related studies carried out over the past 20 years by the National Center for Health Statistics (NCHS). These studies, authorized by Congress under the National Health Survey Act of 1956, are characteristically national in scope, based on probability sampling, and are used to collect a broad range of morbidity data and related health and nutrition information. In this latest study, NCHS will focus on the U.S. Hispanic population, conducting medical examinations and administering health-related questionnaires over a 2-year period to a probability sample of Hispanic residents in the United States. The study sites will include the southwestern States, portions of Florida, and the New York/New Jersey metropolitan area. The data collection period will extend from July 1982 through December 1984. During that time period, data will be collected from approximately 12,000 Hispanic participants in approximately 30 county sites.

EPA's objective in this study is to assess the Hispanic population's exposure to environmental pollutants (primarily pesticides) by measuring the concentrations of selected pesticides and toxic substances in body fluids, and evaluating the approximate amount and type of exposure as reported by the respondents. As part of a cooperative agreement, NCHS will provide EPA with blood and urine specimens and interview results from a subsample of study participants. EPA will chemically analyze the specimens, statistically analyze the interview and chemical analysis results, and provide estimates of body fluid residue levels and environmental exposure for the Hispanic participants living in the study sites.

In preparation for data collection, NCHS conducted a pilot study in El Paso, Texas, during January through March, 1982. During the pilot study, EPA received 171 blood, serum, and urine specimens from a subsample of study participants. These specimens were shipped to (1) the Chemical Epidemiology Division, Department of Epidemiology and Public Health, University of Miami, and (2) the EPA Toxicant Analysis Center (TAC), for volatile and semivolatile analyses, respectively. A total of 161 specimens were analyzed; 51 blood specimens were analyzed for volatiles, and 59 serum and 51 urine specimens were analyzed for semivolatiles. Tables 1 and 2 present lists of the pilot study target compounds. During the follow-on national study, the list of urine compounds will be expanded to include malathion metabolites and carbamates.

EPA's primary objective in participating in the pilot study was to assess the quality of the measurement procedures and data, and to set data quality objectives for the national study. To

Table 1. Hispanic HANES (EPA Component) Pilot Study Target Volatile Compounds

Bromodichloromethane
Bromoform
Carbon tetrachloride
Chlorobenzene
Chloroform
Dibromochloromethane
1,2-Dichloroethane
Tetrachloroethylene
1,1,1-Trichloroethane
Trichloroethylene

Table 2. Hispanic HANES (EPA Component) Pilot Study
Target Semivolatile Compounds

Serum	Urine
α-BHC	2,4-D (2,4-dichlorophenoxy acetic acid)
β-BHC	Dicamba
δ-BHC	p-Nitrophenol
γ-BHC	PCP (pentachlorophenol)
op'-DDD	Silvex
pp'-DDD	2,4,5-T (2,4,5-Trichlorophenoxy acetic acid)
op'-DDE	2,4,5-Trichlorophenol
pp'-DDE	3,5,6-Trichloro-2-pyridinol
op'-DDT	
pp'-DDT	
Dieldrin	
Endrin	
Heptachlor	
Heptachlor epoxide	
Hexachlorobenzene	
Mirex	
Oxychlordane	
PCB's (Polychlorinated biphenyl)(Arochlor 1254)	
trans-Nonachlor	

this end, RTI was engaged to develop a quality assurance plan for the pilot study and to assess the quality of the analytical results. This report describes EPA's participation in and results from the pilot study, including the QA procedures and their results. Section II contains a summary of the results. Sections III through IV provide overviews of the sample selection, data collection, and quality assurance procedures. In sections V through VII, the analytical methodologies, the analytical results, and the quality assessment data are presented in detail.

Because of the uniqueness of the quality assurance procedures implemented in this study, a unique terminology was created to facilitate distinguishing among the types of specimens and specimen groups. A glossary of selected terms is included as appendix D to assist the reader in understanding the terminology used in this report.

II. SUMMARY OF RESULTS

A. Introduction

The results of EPA's participation in the pilot study are summarized in the following subsections. In all, over 300 specimens from 171 subjects ("sample persons") were chemically analyzed over a 6-month period. A quality assurance plan was developed and implemented in order to assess data quality. This plan involved field controls to assess field contamination and degradation, replicate chemical analyses to assess precision, and spiked-split duplicate chemical analyses to assess chemical analysis bias through compound percent recovery.

A note of caution is in order regarding the interpretation of the pilot study results. Estimates were calculated for a number of parameters relating to these: percent detected; percent recovery; averages, percentiles, and variances for detected values; and variances of the measurement process. These parameters were all calculated from relatively small sample sizes of fewer than 60 specimens, mainly because field problems resulted in EPA being provided with a fewer number of specimens than was originally planned. A second, and perhaps more critical problem was the lack of data from which to base data quality estimates; of the 37 total target compounds, only 5 compounds were detected in a significant number of specimens. To estimate data quality for the compounds which were seldom endogenous* in the specimens, it was necessary to use substitute measurements such as field-spiked and spiked-split duplicate specimens for chemical analysis precision. These sample sizes were even fewer (e.g., only three to nine field spikes per matrix). Using field spikes and spiked-split specimens for making estimates for which they were not intended is inappropriate and yields data of unknown validity.

The estimates in this report are the best that could be made based on the available data; however, because of the few specimens available and lack of specimens with endogenous compounds, most of these results should be considered preliminary. Analysis of these data does point to potential problems with the analytical methodologies that warrant further investigation.

B. Analysis of Blood for Volatiles

Blood specimens from 51 sample persons were analyzed for the presence of volatile compounds at the Chemical Epidemiology Division, Department of Epidemiology and Public Health, University of Miami ("Miami lab"), using a purge/trap/desorb

*The word "endogenous" as used in this report refers to compounds that are found in the specimens naturally, as opposed to compounds that are spiked in the specimens.

procedure based on that of Bellar and Lichtenberg (see appendix A). A subsample of 20 specimens was also analyzed by an external reference laboratory, the Research Triangle Institute (RTI), using the identical procedure. Of the 10 target compounds, only chloroform was detected in a significant number of specimens (100 percent) at the primary laboratory. The median chloroform value was 8.3 ppb, and the highest value was 4,000 ppb. At the external reference laboratory, chloroform also had the highest percent detected and highest mean concentration. Of the 10 target compounds, positive detections were obtained for 6 compounds at the external reference laboratory, while interferences prevented analysis of the other 4 compounds. Different instrumentation allowed RTI to set lower minimum reporting levels than could the Miami lab. An interlaboratory chemical analysis comparison showed that the Miami lab obtained mean chloroform values approximately 183 percent higher than did RTI.

An assessment of total error for chemical analysis based upon a combination of percent recovery and precision showed that only chloroform had a total error estimate (36 percent) within the EPA guidelines (USEPA 1979) for acceptability (below 50 percent). The other 9 compounds (excluding chloroform) had unacceptable total errors, ranging up to 256 percent for dibromochloromethane. The estimates for these 9 compounds were wholly obtained, however, from a small sample of field spikes, due to the lack of endogenous-compounds data. The high total errors and the large interlaboratory differences suggest possible problems with the analytical method.

Replicate analyses over time from a specimen pool showed that no degradation of chloroform levels occurred over a 2½-month time period.

C. Analysis of Serum for Semivolatiles

Specimens from a total of 59 sample persons were analyzed for the presence of 19 semivolatile compounds at the EPA Toxicant Analysis Center (TAC) using the EPA standard electron capture gas chromatography method. Positive detections occurred for only 6 of the 19 tested compounds: trans-nonachlor; pp'-DDT; pp'-DDE; β-BHC; dieldrin, and δ-BHC. Of these, only 3 were detected in a significant number of specimens: in 44 percent of specimens, pp'-DDT was detected with a mean positive value* of 3.2 ppb; in 100 percent of specimens, pp'-DDE was detected with a mean positive value of 34.2 ppb; and in nearly 85 percent of specimens, β-BHC was detected with a mean positive value of 2.4 ppb.

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*When the adjective "positive" is used, it means that zero values were excluded from the calculation.

An assessment of chemical analysis total error was made for 6 of the 19 target compounds. Due to the lack of endogenous-compounds data, substitute measurements for precision were used for 3 of these 6 compounds, while estimates could not be made at all for the other 13 compounds. The total error estimates for each of the 6 compounds were within the EPA guidelines for acceptability (below 50 percent). Based on limited data, it appears that data of acceptable quality can be produced with the analytical procedures used for the compounds for which estimates could be made.

D. Analysis of Urine for Semivolatiles

Urine specimens from a total of 51 sample persons were analyzed at TAC for the presence of eight phenol compounds using the electron capture gas chromatography method of T. M. Shafik (see appendix C). Four of the eight tested compounds were detected. Only pentachlorophenol (PCP) was detected in a significant number of specimens; PCP had a 75-percent detection rate and a mean positive value of 4.3 ppb. Three other compounds (3,5,6-trichloro-2-pyridinol; p-nitrophenol; and 2,4,5-trichlorophenol) were detected, but in less than 4 percent of the specimens.

An assessment of chemical analysis total error (defined in section V.E.5) showed that of the eight target compounds, only two compounds, 3,5,6-trichloro-2-pyridinol and 2,4,5-trichorophenoxy acetic acid (2,4,5-T) were within the EPA guidelines criterion of below 50-percent total error. Because of the lack of endogenous-compounds data, chemical analysis precision had to be estimated from the percent coefficient of variation of the spiked-split duplicates for every compound except PCP. The total error for PCP was 83 percent, based upon a 9.1 percent coefficient of variation of duplicate measurements and a 45-percent chemical analysis percent recovery.

E. Conclusion

The great majority of target compounds (32 of 37) were not detected. For volatiles, only chloroform was consistently detected, and only chloroform showed an acceptable total error rate. For semivolatiles in serum, only three compounds were consistently detected: pp'-DDT; pp'-DDE; and trans-nonachlor. Total error rates for these three compounds were acceptable, as were the rates for three additional compounds for which substitute data quality estimates were made. Only one semivolatile compound, PCP, was consistently detected in urine. Substitute measurements permitted the estimation of total error for all eight compounds; however, of these, only two compounds had total error estimates within acceptable limits. The total error estimates for the PCP analytical data were not within the acceptable limits.

Judging from the high total error estimates made, it appears that problems may exist with the analytical methods for volatiles in blood and for semivolatiles in urine. The method for semivolatiles in serum appears to produce data of acceptable quality. Again, it should be stressed that because of the small sample sizes and lack of endogenous-compounds data, these results should be considered preliminary. Further study is needed to more accurately assess data quality from these methods.

III. SAMPLE SELECTION AND DATA COLLECTION OVERVIEW

The pilot study site, El Paso, Texas, was selected by NCHS because of its high proportion of Hispanic residents. An area probability sample of El Paso households was selected, and study participants were chosen from eligible households within the sample. Households were considered eligible if at least one member was of Hispanic descent. Selected participants were administered a health history questionnaire in the home and then scheduled for a detailed physical examination at a mobile examination center.

As part of the physical examination process, blood and urine specimens were collected from each participant. NCHS collected specimens for EPA from a systematic subsample of study participants who were 12-74 years of age. Whole blood was collected for volatile analysis, and serum and urine were collected for semi-volatile analysis. Table 3 depicts the number of specimens that EPA received within each age group for each matrix. The response rate for the EPA component was significantly lower than expected within each category, in most cases lower than 50 percent.

Table 3. Number of Specimens Collected by Type in the El Paso, Texas, Pilot Study Site

Matrix	Age group	Number of EPA sample persons from whom specimens were collected
Serum	20-74 years	59
Urine	12-74 years	54
Blood	12-19 years	58

IV. QUALITY ASSURANCE PLAN OVERVIEW

A quality assurance plan was implemented by EPA during the pilot study in order to assess and set objectives for data quality and provide a method for identifying specific problems or factors affecting data quality. The plan was designed to assess overall measurement error and chemical analysis precision and accuracy, and to identify specific biases such as specimen contamination or degradation. The quality assurance procedures that were implemented in the pilot are summarized below. Specific procedural details and the data assessment results are presented in sections V through VII.

A. Field Controls

Field controls were used to monitor specimen contamination and degradation occurring in the field and during shipping. The controls, which consisted of both spiked and blank (unspiked) specimens, were prepared from a large homogenous matrix pool on a weekly basis at RTI and shipped overnight to the collection site. At the collection site, the controls were stored with the survey specimens and then included in the shipment of specimens to the laboratories. The arrival of controls at the collection site was timed so that each set of controls would remain there for the same length of time as the longest holding time for survey specimens, in order to provide data on a worst case storage-time basis.

Two blanks and two spikes (spiked specimens) of each matrix were prepared to accompany each specimen shipment to the laboratories. RTI also prepared matching laboratory controls for each field control set and shipped these directly to the laboratories.

Upon receipt at the laboratories, one-half of the field controls--one spike and one blank from each shipment--were chemically analyzed. The remaining field controls and the matching laboratory controls were held in reserve for analysis in case problems were detected in the analysis of the initial field controls. The identity of field controls was not known to the chemists at TAC; however, at the Miami lab, it was not feasible to prevent the chemist from knowing the identity of the contents to be analyzed because of limited staff.

B. Replicate Specimen Analysis

In order to obtain estimates of measurement error, a subsample of specimens was independently analyzed in duplicate. Various types of duplicates were used in order to provide different levels of precision estimates. Due to the differences in procedures among blood, serum, and urine collection and processing, the procedures for collecting and analyzing duplicates were not identical for each matrix. The general types and purposes of

replicate analyses are listed below, and the specific procedures for each matrix are described in sections V-VII.

- Field Duplicates - Blood was drawn into separate vacuum tubes and processed and analyzed independently. This type of replicate analysis provided the best estimates of overall study precision. It was not possible to collect this type of duplicate for urine due to the nature of urine collection.

- Field Splits - For serum semivolatile specimens, duplicate aliquots were prepared. This type of duplicate provided an estimate of precision from the point of specimen splitting through chemical analysis.

- Lab-Split Duplicates - Specimens, either single or duplicate halves, were split in the laboratory prior to chemical analysis. This provided the best estimate of chemical analysis precision.

- External Reference Duplicate Analyses - A subsample of blood specimens was sent to an external reference laboratory, RTI, for volatile analysis. These specimens were the field duplicates of specimens analyzed at the Miami lab. The results of the two analyses were compared to provide an estimate of interlaboratory chemical analysis precision.

All chemical analyses were performed within "sets" consisting of groups of other specimens analyzed at the same time. In order to estimate the chemical analysis precision both within and among sets, some duplicates had both halves analyzed in the same set, and other duplicates had the halves analyzed in separate sets.

C. Spiked-Split Duplicate Analysis

Spiked-split specimens were used in order to estimate the compound percent recovery being obtained in the semivolatile chemical analyses. Spiked-split specimens were created by spiking only one of the two duplicate specimens. Upon chemical analysis, the measured difference between the spiked and unspiked specimens was divided by the known spiking amount to obtain the fractional recovery.

V. ANALYSIS OF BLOOD FOR VOLATILE COMPOUNDS

Blood specimens were shipped to the Miami Lab and chemically analyzed for the presence of selected volatile compounds. A quality assurance plan involving replicate analyses was developed and followed. As an external quality assurance procedure, a subsample of 20 duplicate specimens was analyzed by RTI. In order to determine if compound levels were changing, samples from two specimen pools were repeatedly analyzed over a 2½-month time period. The analytical and quality assurance procedures and results are discussed below.

A. Summary of Results

The results at the primary laboratory show only chloroform being detected consistently in levels above the minimum reporting level of 1 ppb. The median chloroform detection was 8.3 ppb. Bromoform and dibromochloromethane were detected by the primary laboratory in fewer than 10 percent of the specimens, with median positive values of 6.0 and 6.5 ppb, respectively. In the external reference laboratory (RTI), six of the ten target compounds were detected. Interferences and a resolution problem prevented data from being obtained for the remaining four compounds. RTI also found that the highest concentrations measured were those of chloroform. An interlaboratory chemical analysis comparison, however, showed that the Miami lab obtained mean chloroform values that were approximately 183 percent higher than did RTI.

In order to assess the quality of the chemical analysis data, a total error estimate was calculated from a combination of the percent recovery and precision estimates of the chemical analyses. Due to the infeasibility of using spiked-split duplicates, the field spikes were used to estimate chemical analysis percent recovery. Since there were no endogenous-compounds precision data, field spikes were also used to estimate precision for every compound except chloroform, for which duplicate specimens were used. Of the ten target compounds, only the chloroform total error (36 percent) was within the EPA guidelines (USEPA 1979) for acceptability (below 50 percent). The remaining nine compounds all had unacceptable total error estimates, ranging up to 256 percent for dibromochloromethane. The small number of specimens and limited endogenous-compounds data limit the conclusions that may be drawn from the data. The high total error rates and large interlaboratory differences suggest that problems exist with the analytical method.

Analyses of the field blanks showed that chloroform was present at a mean concentration of 11.3 ppb. No other compounds were detected. The analysis of the field spikes showed a percent recovery for chloroform of 101 percent; however, the percent

recoveries for the other compounds ranged from 0 to 290 percent, indicating that there were problems either with contamination and degradation in the field, or, more likely, with the analytical method. Field contamination seems unlikely since basically only chloroform was detected in the field blanks and survey specimens.

Results from the degradation study showed that no changes in levels occurred over the 2½-month time period.

B. Specimen Collection, Storage, and Shipping

Blood specimens for volatile analysis were collected via brachial venipuncture into Becton-Dickinson green-top vacuum tubes. After the specimens were drawn, the containers were inverted several times in order to mix the anticoagulant present in the container. Specimens were labeled and immediately stored in the refrigerator in order to minimize the loss of volatile compounds.

Specimens were collected from a total of 58 sample persons. Duplicate specimens were obtained from 39 of these sample persons by drawing blood into separate vacuum tubes.

Specimens were shipped from the collection site to the Miami lab by overnight mail service. Insulated boxes with cold packs were used for shipping in order to maintain a cool temperature; however, it is known that the first several shipments of specimens arrived at the Miami lab at an ambient temperature.

C. Analytical Methodology

The method of Peoples and coworkers (appendix A), a purge/trap/desorb method based on that of Bellar and Lichtenberg, was used by both the Miami lab and RTI to determine the concentration of purgeable halogenated hydrocarbons in blood plasma. The procedure involves heating the specimen while purging the volatiles from the solution with a flow of an inert gas. The purged compounds are directed to an absorbent trap. After the purge/trap period is completed, the volatile analytes are thermally desorbed from the absorbent trap to a gas chromatograph programmed to provide complete resolution of all the compounds of interest. Other incidental compounds may cause interference problems.

The Miami lab analyzed each specimen on two GC columns: N-octane and SP-1000. Peak heights were manually measured, and quantitations were based on the response of a single standard-analyte mixture.

RTI analyzed each of 20 specimens once on an N-octane GC column. An integrator was used to measure peak area, and quantitation was based on a five-point standard calibration curve.

D. Analytical Results

1. Limits for Detection and Reporting Level

The minimum reporting level set by the Miami lab was 1 ppb. Compounds detected at levels between 0 and 1 ppb were reported as not detected (zero).

RTI reported all compounds detected in amounts above the calculated quantitation limits:

	RTI minimum reporting levels	
Compound	Quantitation limit (ng)	PPB for 1 mL analysis volume
carbon tetrachloride	4.3×10^{-2}	.043
chloroform	3.4×10^{-2}	.034
1,1,1-trichloroethane	5.5×10^{-2}	.055
trichloroethylene	4.6×10^{-2}	.046
bromodichloromethane	5.9×10^{-2}	.059
tetrachloroethylene	3.8×10^{-2}	.038

RTI also reported trace values that occurred.

2. Primary Laboratory Results

Specimens from a total of 51* sample persons were analyzed by the primary laboratory. Since the majority of specimens were analyzed in duplicate, a total of 90 independent analyses were performed.

Of the ten target compounds, only three were detected in levels above 1 ppb: chloroform, bromoform, and dibromochloromethane. Of these, only chloroform was consistently detected in levels above 1 ppb, with 100 percent of the specimens containing concentrations between 2 and 4,000 ppb. The speciment with the highest detected chloroform value, 4,000 ppb, was substantially higher than any amounts found in the other specimens, the next highest detected value was 165 ppb. Due to the great influence that this extreme value has on the mean and variance, these sta-

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*The remaining 7 specimens were either not analyzable or were used in the degradation study pool.

†RTI analyzed this specimen also and reported a concentration of 120 ppb.

tistics were calculated both with and without this value. Table 4 presents a summary of the chloroform data.

The proportions above the 1 ppb detection level for bromoform and dibromochloromethane were 5.9 and 7.8 percent, respectively. Table 5 presents a summary of the results for both compounds. Because the detection rate was so low, only the nonzero values were used in calculating statistics.

3. External Reference Laboratory Results

No data were obtained by RTI for four of the ten target compounds. Due to unidentified late eluting general interferences, data were not obtained for three compounds: chlorobenzene, bromoform, and dibromochloromethane. The fourth compound, 1,2-dichloroethane, could not be quantitated because of a resolution problem with tetrachloroethylene under the N-octane column. Like the Miami lab, RTI obtained the highest concentrations for chloroform. Of the six compounds for which results were obtained, three compounds other than chloroform were detected in amounts exceeding 1 ppb. These were 1,1,1-trichloroethane, tetrachloroethylene, and carbon tetrachloride. The values reported for these three compounds ranged up to 4.6 ppb. A summary of the RTI results is presented in Table 6.

E. Quality Assurance Procedures and Results

1. Field Controls

Field controls were prepared at RTI, shipped to and stored at the collection site, and then included in the specimen shipments to the Miami lab (sec. IV.C). Matching laboratory controls were prepared at RTI and shipped directly to the Miami lab. Upon receipt at the Miami lab, approximately one-half of the field controls and several of the matching lab controls were chemically analyzed.

A total of eight blanks were analyzed: six field blanks and two matching lab blanks. Both types of blanks were found to contain similar levels of chloroform. The two lab blanks contained 11 and 9 ppb, and the six field blanks contained a mean chloroform concentration of 11.3 ppb. No other compounds were detected in either type of blank.

Table 7 presents a summary of overall mean percent recoveries, and percent recoveries for field and matching lab spikes. Seven field spikes and two matching lab spikes were analyzed, each spiked at varying levels. Because the spiking levels varied among the specimens, they are not included in the table. The detected levels were adjusted for the inherent presence of chloroform in blood by subtracting out the mean detected level of chloroform found in the field and matching lab blanks.

Table 4. Analysis of Blood for Volatiles: Summary of Chloroform Results*

Number of specimens analyzed	51
Number of positive detections	51
Percent positive detections	100%
Mean (ppb)[a]	18.7
Mean (ppb)[b]	96.8
Variance[a]	929
Variance[b]	3.12×10^5
Percent coefficient of variation[a]	163%
Percent coefficient of variation[b]	577%
Minimum value (ppb)	2
Maximum value (ppb)	4,000
Second highest value (ppb)	165
Median (ppb)[a]	8.3
Twenty-fifth percentile (ppb)[a]	6.0
Seventy-fifth percentile (ppb)[a]	17.0

*Based on results reported by the Chemical Epidemiology Division, Department of Epidemiology and Public Health, University of Miami.

[a]Excludes the high value of 4,000 ppb.

[b]Includes the high value of 4,000 ppb.

Table 5. Analysis of Blood for Volatiles:
Summary of Bromoform and Dibromochloromethane Results*

	Bromoform	Dibromochloromethane
Number of specimens analyzed	51	51
Number of positive detections[a]	3	4
Percent positive detections[a]	5.9%	7.8%
Mean positive values (ppb)	5.8	6.5
Median positive values (ppb)	6	6.5
Minimum reported value (ppb)	4.5	3
Maximum value (ppb)	7	10
Variance of the positive values	1.6	16.3
Coefficient of variation of the positive values	21.6%	62.2%

*Based on results reported by the Chemical Epidemiology Division, Department of Epidemiology and Public Health, University of Miami.

[a]Above the minimum reporting level of 1 ppb.

Table 6. Analysis of Blood for Volatiles:
Summary of External Reference Laboratory Results*

	Compound[a]					
	Bromo-dichloro-methane	Trichloro-ethylene	1,1,1-Trichloro-ethane	Tetra-chloro-ethylene	Chloro-form	Carbon tetra-chloride
Number and percent positive detections (excluding trace[b] detections)	2(10%)	7(35%)	14(70%)	14(70%)	19(95%)	2(10%)
Number and percent trace[b] detections	2(10%)	3(15%)	0 (0%)	0 (0%)	0 (0%)	2(10%)
Number and percent of values > 1 ppb	0 (0%)	0 (0%)	3(15%)	4(20%)	18(90%)	1 (5%)
Mean (ppb)	.02	.1	.6	.9	14.5	.1
Mean positive value (ppb)	.2	.3	.9	1.2	15.2	.8
Median positive value (ppb)	.2	.3	.6	.7	2.7	.2
Maximum value (ppb)	.3	.5	4.6	4.4	120	1.5

*Based on analyses of 20 specimen analyses reported by the Analytical Sciences Division, Research Triangle Institute.

[a]Due to interferences, no data were obtained for chlorobenzene, bromoform, dibromo-chloromethane, and 1,2-dichloroethane.

[b]Trace is defined as below the minimum quantitation levels defined in section V.C.1.

Table 7. Analysis of Blood for Volatiles: Results of Analyzing Field and Matching Laboratory-Spiked Specimens*

Compounds	Mean percent recovery[a]		
	Overall	Field spikes	Matching lab spikes
Bromodichloromethane	155	177	80
Bromoform	100	95	119
Carbon tetrachloride	87	93	67
Chlorobenzene	56	72	0
Chloroform	101	102	98
Dibromochloromethane	134	130	149
1,2-Dichloroethylene	121	121	119
Tetrachloroethylene	86	77	90
1,1,1-Trichloroethane	156	118	290
Trichloroethylene	115	108	141

Note: The mean positive values have been adjusted by subtracting the mean concentration found in the blanks; chloroform was the only compound affected.

*Based on results of analyzing 7 field spikes and 2 matching laboratory spikes at the Chemical Epidemiology Division, Department of Epidemiology and Public Health, University of Miami.

[a]The relative percent bias is estimated by the difference of the mean percent recovery and 100 percent.

The overall mean percent recovery for chloroform was excellent, approximately 101 percent. The mean percent recoveries for the other compounds ranged from 0 to 290 percent, indicating either problems with contamination and degradation in the field, or, more likely, problems with the analytical method. Field contamination seems unlikely, however, since only chloroform was detected in any frequency in the field blanks and survey specimens.

2. Overall Study Precision

The majority of specimens (39 of 58) were collected in duplicate in the field (blood drawn in separate vacutainers) and independently analyzed. In addition to the field duplicates, ten specimens were split and independently analyzed at the Miami lab. Approximately one-half of all duplicates were analyzed in the same set, and approximately one-half were analyzed in separate sets.* Due to a logistical problem at the primary laboratory that prevented specimen renumbering and relabeling, the identities of duplicate specimens was known to the chemist performing the analyses.

An estimate of overall study precision was obtained by comparing the results obtained from the analyses of both field duplicates (blood collected and processed in separate vacutainers), and laboratory-split duplicates at the primary laboratory. Both types of duplicates were analyzed both within and among sets. A total of 39 duplicate specimen pairs were analyzed at the Miami lab; 10 pairs were laboratory splits and the remaining pairs were field duplicates.

A pooled estimate of variance, s^2, was calculated using

$$s^2 = \frac{\sum_{i=1}^{n} \sum_{j=1}^{2} (X_{ij} - \bar{X}_i)^2}{n}$$

where s^2 denotes estimate of variance, X_{ij} represents the jth measurement on the ith pair, $\bar{X}_i$ is the mean of the ith pair, and n is total number of duplicate pairs (39). For chloroform, s^2 was equal to 5.76. The coefficient of variation, 17.7 percent, was obtained by dividing s by $\bar{X}$ where $\bar{X}$ is the overall mean of the 39 pairs.

The variance was not calculated for bromoform or dibromochloromethane because of the low frequency of detection. Bromoform was detected in only one duplicate pair; the values obtained

*A set consisted of all specimens analyzed on the same day, usually 1-3 specimens.

were 4 and 5 ppb. Dibromochloromethane was detected in two duplicate pairs; in one pair the values were the same, and in the other pair the values were 6 and 14 ppb.

3. Chemical Analysis Precision Estimates Using Field Spikes

As noted in the preceding sections, not much data were obtained on compounds endogenous to the blood. Only chloroform was detected with sufficient frequency to obtain reliable estimates of the chemical analysis precision. In order to supplement the limited endogenous-compounds data available, the field spikes and matching lab spikes were used to provide estimates of the chemical analysis precision for all ten compounds. The coefficient of variation was calculated separately for field and matching lab spikes and overall. The overall coefficients of variation ranged from 23 percent for 1,2-dichloroethylene to 111 percent for dibromochloromethane. Only dibromochloromethane was higher than 41 percent. Table 8 presents these estimates.

4. Comparison of Interlaboratory Chemical Analysis

An estimate of the chemical analysis precision between the primary and reference laboratories was calculated from the results of the 20 specimens that were analyzed by both laboratories. Chloroform is the only compound for which both laboratories obtained positive results, and it is the only compound for which precision was estimated.

The mean and variance of the differences in detected values between laboratories were calculated both including and excluding the specimen for which the Miami lab obtained a value of 4,000 ppb, since RTI obtained a much lower value (120 ppb) for the same specimen. The Miami lab obtained higher chloroform values than did RTI for each of the 20 specimens. When the 4,000 ppb specimen was excluded, the mean difference was 16.37 and the variance of the differences was 623.45; the mean was found to be significant at the .01 level. When the 4,000 ppb specimen was excluded, the Miami lab was 183 percent higher than was RTI. After including the 4,000 ppb specimen, the mean difference was 209, and the variance of the differences was 746,974. After including the 4,000 ppb specimen, the Miami lab was 1,447 percent higher than was RTI.

5. Total Accuracy Estimates for Chemical Analysis

Chemical analysis total error was estimated for each compound by using both the chemical analysis bias (percent recovery) and precision information. Due to the infeasibility of using spiked-split duplicates, the percent recovery of the field spikes was used to estimate chemical analysis percent recovery.

Table 8. Analysis of Blood for Volatiles: Estimates of Chemical Analysis Precision From Field and Laboratory Spikes*

Compounds	Coefficient of variation (percent)		
	Field spikes	Lab spikes	Overall
Bromodichloromethane	39	0	30
Bromoform	28	21	26
Carbon tetrachloride	19	40	24
Chlorobenzene	51	0	40
Chloroform	36	11	30
Dibromochloromethane	110	113	111
1,2-Dichloroethylene	24	21	23
Tetrachloroethylene	31	0	24
1,1,1-Trichloroethane	50	8	41
Trichloroethylene	36	32	35

*Based on results from the analysis of 7 field spikes and 2 matching laboratory spikes at the Chemical Epidemiology Division, Department of Epidemiology and Public Health, University of Miami.

Since there were no endogenous precision data, the coefficient of variation of the field spikes was used to estimate precision for every compound except chloroform, for which the coefficient of variation of duplicate analyses was used. Two estimates were calculated for each compound. The Total Error was calculated by:

$$|\text{percent recovery} - 100| + 2\ (\text{percent coefficient of variation}) .$$

The twice Root Mean Square Error was calculated by:

$$2\ [(\text{percent recovery} - 100)^2 + (\text{percent coefficient of variation})^2]^{\frac{1}{2}} .$$

The estimates for both Total Error and twice the Root Mean Square Error are presented in Table 9. Only one compound, chloroform, had either Total Error or Root Mean Square Error less than 50 percent, the EPA guidelines acceptance criterion. The Total Error for chloroform was 36 percent. The remaining nine compounds had Total Errors or Root Mean Square Errors of unacceptable proportions, ranging up to 256 percent for dibromochloromethane.

6. Compound Degradation

In order to determine if changes in compound levels were occurring in specimens over time, samples from two specimen pools were repeatedly analyzed over a 2½-month time period. The plasma pools were created by combining several study specimens, each pool containing a different composite.

One specimen from each pool was analyzed approximately every 2 weeks over the 2½-month period. Chloroform was the only compound detected in either pool. The results, depicted in the graph in Figure 1, show no apparent trends in the levels of chloroform over time.

Table 9. Analysis of Blood for Volatiles: Estimates of Chemical Analysis Total Error Using Field Spikes*

Compound	2 RMSE (percent)[a]	Total error (percent)[b]
Bromodichloromethane	125	115
Bromoform	52	52
Carbon tetrachloride	55	61
Chlorobenzene	119	124
Chloroform	36[c]	36[c]
Dibromochloromethane	232	256
1,2-Dichloroethylene	62	67
Tetrachloroethylene	62	68
1,1,1-Trichloroethane	139	138
Trichloroethylene	76	85

*Based on results from the Chemical Epidemiology Division, Department of Epidemiology and Public Health, University of Miami.

[a]Two Root Mean Square Error (RMSE)

$$= 2\,[(\text{percent recovery} - 100)^2 + (\text{percent coefficient of variation})^2]^{\frac{1}{2}}.$$

[b]Total Error

$$= |\text{percent recovery} - 100| + 2\,(\text{percent coefficient of variation}).$$

[c]The percent coefficient of variation of the duplicate analyses was used for chloroform.

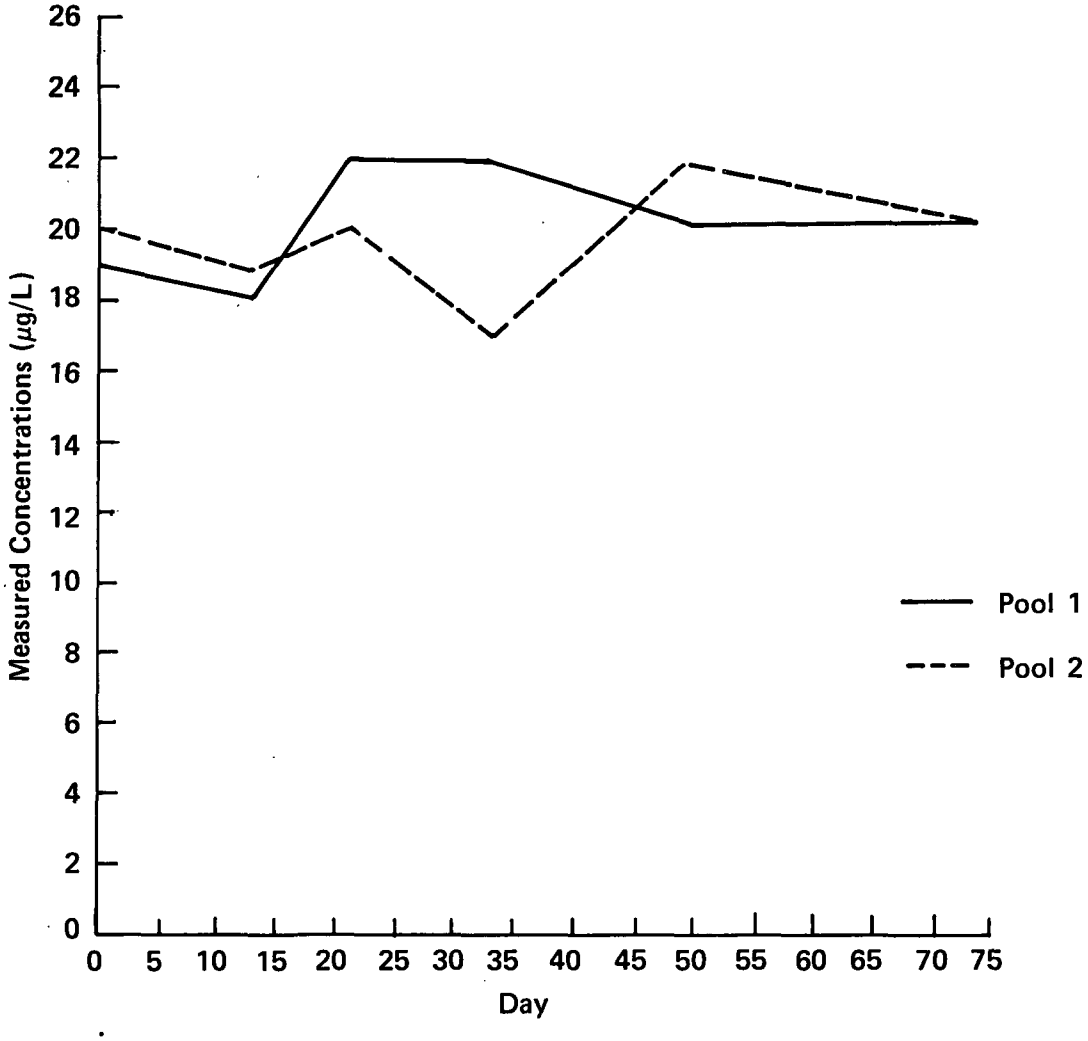

Figure 1. Stability of Chloroform Levels in Pooled Specimens Over Time.

VI. ANALYSIS OF SERUM FOR SEMIVOLATILES

Serum specimens were shipped to the EPA Toxicant Analysis Center (TAC) and analyzed for the presence of selected semivolatile compounds (Table 2). A quality assurance plan involving replicate analyses and spiked-split duplicate analyses was developed and followed. The analytical and quality assurance procedures and results are discussed below.

A. Summary of Results

Only 6 of the 19 tested compounds were detected: trans-nonachlor; pp'-DDT; pp'-DDE; β-BHC; dieldrin; and δ-BHC. Of these, only three yielded reporting levels in a significant number of specimens: in 44 percent of the specimens, pp'-DDT was measured with a mean positive value of 3.2 ppb; in 100 percent of the specimens, pp'-DDE was measured with a mean value of 34.2 ppb; and in nearly 85 percent of the specimens, β-BHC was measured with a mean positive value of 2.4 ppb.

In order to assess the quality of the chemical analytical data, total error estimates were calculated based upon a combination of the chemical analysis percent recovery and analytical precision estimates. Chemical analysis percent recovery was estimated from the spiked-split duplicates. The percent coefficient of variation of the lab-split duplicates was used to estimate precision for the three compounds frequently detected: pp'-DDE, pp'-DDT, and β-BHC. Precision for trans-nonachlor, dieldrin, and oxychlordane was estimated from the percent coefficient of variation of the spiked-split duplicates. Due to the lack of endogenous-compounds data, total error estimates could be made for only 6 of the 19 target compounds. The accuracy estimates were within the EPA guidelines (USEPA 1979) for acceptability (below 50 percent) for each of the six compounds. It therefore appears that the analytical procedures resulted in data of acceptable quality for the six compounds, although the small sample sizes and the use of substitute precision measurements limit the conclusions that can be drawn from the data.

After comparing duplicates analyzed within the same set to duplicates analyzed in separate sets, much better precision was obtained from within-set analyses, suggesting large measurement differences from set to set.

The analysis of the field blanks showed moderate levels of pp'-DDE and hexachlorobenzene. Analysis of the field spikes showed that the majority of percent recoveries fell within the 75 to 125 percent range. Two compounds, heptachlor and oxychlordane, had very high percent recovery estimates of 161 and 130 percent, respectively. Field contamination seems unlikely, however, since the two compounds were not detected in either

field blanks or survey specimens. Three compounds were detected that were not present in the spiking solution: op'-DDT; δ-BHC; and dieldrin. This suggests that false positives may be produced using the analytical method since none of the three compounds were detected in the field blanks.

B. Specimen Collection, Storage, and Shipping

Blood specimens were drawn via brachial venipuncture into red top Becton-Dickinson vacuum tubes. Several specimens were drawn from each participant. After collection, the tubes were stored upright at room temperature until clots formed, and then centrifuged. After centrifuging, the serum from each tube was drawn off by suction into a temporary storage container, and EPA's subspecimens were removed using a disposable pipette. Specimens were labeled and stored in the freezer until shipment to the laboratory. Specimens were shipped to the laboratory in insulated containers packed with dry ice in order to maintain the frozen state.

C. Analytical Methodology

Serum specimens were extracted and the analytes of interest were determined by electron capture gas chromotography. The procedure in the EPA reference manual (EPA-600/8-80-030, June 1980, Analysis of Human Blood or Serum in Analysis of Pesticide Residues in Human and Environmental Samples, RTP, NC) was followed exactly.

D. Analytical Results

1. Detection and Reporting Level Limits

The minimum reporting levels set by TAC for this study are listed below. Compounds detected in amounts below these levels were reported as zero, except in the case of spiked-split specimens, where trace values were reported.

Compound	Minimum reporting level (ppb)
α-BHC	1
β-BHC	2
δ-BHC	1
γ-BHC	1
op'-DDD	2
pp'-DDD	2
op'-DDE	2
pp'-DDE	2

Compound	Minimum reporting level (ppb)
op'-DDT	2
pp'-DDT	2
dieldrin	2
endrin	2
heptachlor epoxide	1
heptachlor	1
hexachlorobenzene	1
mirex	4
oxychlordane	2
PCB's (Arochlor 1254)	20
trans-nonachlor	1

2. Results

Specimens from a total of 59 sample persons were analyzed for the presence of 19 separate compounds. Six of the 19 target compounds had levels measured above the minimum reporting level:

β-BHC;
δ-BHC;
pp'-DDE;
pp'-DDT;
dieldrin; and
trans-nonachlor.

Of these, only three yielded reporting levels in a significant number of specimens. Table 10 presents a summary of these results: in 44 percent of the specimens, pp'-DDT was measured with a mean positive value of 3.2 ppb; in 100 percent of the specimens, pp'-DDE was measured with a mean positive value of 34.2 ppb and the values ranged from 105 to 10 ppb; in nearly 85 percent of the specimens, β-BHC was measured with a mean positive value of 2.4 and the median positive value was 2 ppb; in two specimens, trans-nonachlor was measured with a mean positive value of 1.2 and the median positive value was 0.7 ppb; in only one specimen, dieldrin was measured at 1 ppb; and in one specimen, δ-BHC was also only measured at 0.9 ppb.

Table 10. Analysis of Serum for Semivolatiles: Summary of pp'-DDT, pp'-DDE, and β-BHC Results*

	pp'-DDT	pp'-DDE	β-BHC
Number of specimens analyzed	59	59	59
Number of positive detections[a]	26	59	50
Percent positive detections[a]	44.1	100	84.8
Mean of the positive values (ppb)	3.2	34.2	2.4
Mean overall value (ppb)	1.4	34.2	2
Median of the detected value (ppb)	2.8	29.7	2
Median overall value (ppb)	0	29.7	1.9
Minimum reported positive value (ppb)	1.5	9.5	.6
Maximum value (ppb)	8	105	5.8
Variance of the positive values	2.4	414	1.6
Coefficient of variation of the positive values (Percent)	47.8	59.5	52.9

*Based on results reported by the EPA Toxicant Analysis Center.

[a]Above the minimum reporting level of 2 ppb.

E. Quality Assurance Procedures and Results

1. Field Controls

Field Controls were prepared at RTI, shipped to and stored at the collection site, and then included in the specimen shipments to the primary analysis laboratory (see sec. IV.C). Matching laboratory controls were prepared at RTI and shipped directly to the laboratory.

Upon receipt at the primary laboratory, four field spikes and four field blanks were analyzed. All four field blanks showed levels of pp'-DDE, with a mean level of 7 ppb. Two blanks showed levels of hexachlorobenzene with a mean positive value of 0.6 ppb.

The results of the field-spike analyses are presented in Table 11. Mean percent recoveries ranged from 0 for PCB's (Arochlor 1254) and mirex to 161 percent for heptachlor. Most compounds fell within the 75 to 125 percent recovery range. Endrin's mean percent recovery was 64 percent, while heptachlor and oxychlordane had recoveries of 161 and 130 percent, respectively. PCB's and mirex were spiked but not detected.

It would be appropriate to compare the field-spike percent recoveries to the spiked-split duplicate percent recoveries; however, data are only available for five compounds. Of these five compounds, the pp'-DDT, pp'-DDE, and β-BHC data are comparable. Trans-nonachlor had a field-spike recovery of 115 percent and a lab-spike recovery of 80 percent, and oxychlordane had a field-spike recovery of 130 percent and a lab-spike recovery of 90 percent. Field contamination of heptachlor and oxychlordane seems unlikely since the two compounds were not detected in either field blanks or survey specimens.

2. Plan for Set Structure and Analysis

All specimens, including field spikes and blanks, were analyzed in analytical sets, a set consisting of a group of specimens analyzed at the same time under the same conditions. A randomization scheme was used to assign specimens to sets and to distribute duplicates both within and among sets. Specimens were renumbered so that the identity of duplicates would not be known to the chemist.

In addition to the specimens, three standards, a method blank, and a standard reference material (SRM) were run with every set. Table 12 outlines the set structure and composition. Prior to analyzing any field specimens, TAC analyzed a number of SRM's and calculated the standard deviation from the resulting data. A control chart was constructed using these data, with the

Table 11. Analysis of Serum for Semivolatiles: Results of Field-Spiked Specimens*

Compound	Spiking level (ppb)	Mean positive value (ppb)	Standard deviation	Mean percent recovery	Coefficient of variation (percent)
Hexachloro-benzene	0.9	0.9	0.3	106	29
trans-Nonachlor	1.7	2	0.1	115	7
pp'-DDT	5.8	5.8	0.6	99	10
op'-DDE	0	0.5	0.5	--	116
pp'-DDE	2.6	0.6	1.3	21	238
pp'-DDD	3.3	3.2	0.4	96	13
α-BHC	1	1.2	0.4	123	28
β-BHC	1.2	1.2	0.2	98	16
γ-BHC	1.3	1.1	0.1	91	12
δ-BHC	0	0.8	0.2	--	26
Aldrin	1.2	0.9	0.2	77	27
Dieldrin	0	0.7	0.1	--	14
Endrin	1	0.7	0.4	64	68
Heptachlor	1.1	1.7	0.3	161	20
Heptachlor epoxide	1.5	1.5	0.4	101	24
PCB's	3.5	NR†	--	--	--
Oxychlordane	1.2	1.5	0.2	130	11
Mirex	1.1	NR	--	--	--

Note: The mean positive values have been adjusted by subtracting the mean compound level detection in the field blanks.

*Based on the results of analyzing 4 field-spiked specimens at the EPA Toxicant Analysis Center.

†NR denotes not reported.

Table 12. Analysis of Serum for Semivolatiles: Set Structure and Composition

Order	Type
1	Standard[a]
2	Method Blank
3	SRM
4	Field Specimen[b]
5	Field Specimen
6	Field Specimen
7	Field Specimen
8	Standard
9	Field Specimen
10	Field Specimen
11	Field Specimen
12	Field Specimen
13	Standard

[a]Additional standards were run at the discretion of the analyst.

[b]Field specimens included serum specimens collected in the field and field spikes and blanks.

upper and lower control limit lines being set at ± 2 standard deviations. An SRM was run with every set and plotted on the control chart in order to provide information on both precision and accuracy. The SRM results are contained in appendix B.

3. Procedures for Replicate Specimen Analysis

Various types of serum duplicates were analyzed in order to provide estimates of overall study and chemical analysis precision. In the original plans for the pilot, EPA was to receive two types of serum field duplicates: true field duplicates, created by collecting and processing blood in separate containers; and field-split duplicates, obtained by taking two specimens from a common serum pool after centrifuging (sec. IV.C). In addition to the two types of field duplicates, a third type of duplicate was to be created by splitting specimens in the laboratory just prior to chemical analysis.

The three types of duplicates, created at three separate stages in the collection/processing/analysis process, were to provide some comparison as to the different levels of precision obtained at different stages in the study. Problems at the collection site, however, prevented EPA from obtaining as many field duplicates as originally planned. Additionally, no records were kept in the field to distinguish the two types of field duplicates, and many of the specimens that were received at TAC were of too low a volume to split again, thereby creating a shortage of lab-split duplicates. In light of these problems, it was decided (1) to treat all field duplicates as of the same type and (2) to create substitute lab-split duplicates (pseudo-duplicates) by compositing two single specimens from separate sample persons and then resplitting these specimens for independent duplicate analyses.

In all, four types of duplicates were analyzed. Field duplicates were analyzed either within the same set (internal duplicate), or in different sets (external duplicate). Lab-split duplicates that were created from pseudo-duplicates were also analyzed either within the same set (internal-split specimen), or in different sets (external-split specimen). These duplicates were analyzed both within and among sets in order to determine if precision was greater for specimens analyzed at the same time than for specimens analyzed during different time periods.

4. Analytical Procedures for Spiked-Split Specimens

A subsample of lab-split duplicates was used to create spiked-split duplicate specimens in order to provide an estimate of the chemical analysis percent recovery. Spiked-split specimens were created by spiking one-half of a duplicate, and not spiking the other half. Both halves were independently ana-

lyzed, either within the same set (internal spiked-split duplicate) or in different sets (external spiked-split duplicate).

5. Precision Estimates

a. Overall Measurement Precision

Overall measurement precision was estimated for each compound by calculating a pooled estimate of variance for all field duplicates with a mean value greater than zero. Table 13 presents these estimates. The variance was calculated as follows:

$$s^2 = \frac{\sum_{i=1}^{n} \sum_{j=1}^{2} (X_{ij} - \bar{X}_i)^2}{n} \qquad (1)$$

where s^2 denotes estimate of variance, X_{ij} represents the jth measurement on the ith pair, $\bar{X}_i$ is the mean of the ith pair, and n is the total number of duplicate pairs with a mean value greater than zero.

Table 14 presents precision estimates by compound for each of the four duplicate types: internal duplicate, external duplicate, internal split specimen, and external split specimen. The poorest precision was obtained with external field duplicates.

b. Precision of Chemical Analysis

Chemical analysis precision was estimated by two different methods. In the first method, a pooled estimate of variance for all lab-split duplicates with a mean greater than zero was calculated for each compound using eq. (1). The coefficient of variation was obtained by dividing the square root of this variance by the overall mean for lab-split duplicates. In the second method, spiked-split duplicates were used to estimate precision by taking the coefficient of variation of the difference between the spiked and nonspiked halves. Table 15 presents the two chemical analysis precision estimates for each compound.

The difference between the chemical analysis precision obtained in within-set analyses and that obtained in among-set analyses was estimated by comparing internal and external field and lab-split duplicates. Again, precision was estimated from the coefficient of variation, obtained by dividing the square root of the pooled estimate of variance for internal and external duplicates with a mean greater than zero by the overall mean for each type of duplicate. As can be seen from Table 16, internal field and lab-split duplicates had better precision than external field and lab-split duplicates, indicating potential large set-to-set differences in the measurement process.

Table 13. Analysis of Serum for Semivolatiles: Estimates of Overall Measurement Precision*

Compound	Coefficient of variation (C.V.) (percent)[a]
trans-Nonachlor	141[b]
pp'-DDT	60.9[c]
pp'-DDE	20.2
β-BHC	77.2
δ-BHC	141[b]

*Based on the analyses of 19 field duplicate pairs at the EPA Toxicant Analysis Center.

[a]The coefficient of variation was calculated by dividing the square root of the pooled estimate of variance for all field duplicates (internal and external) with a mean > 0 by the overall mean for these specimens.

[b]Measured in only one specimen from one duplicate pair; because of this, the percent C.V. always equals the square root of 2 times 100.

[c]Measured in specimens from 44 percent of the sample persons overall.

Table 14. Analysis of Serum for Semivolatiles: Precision Estimate by Duplicate Type*

Compound	Coefficient of variation (C.V.) (percent)[a]			
	Internal field duplicate	Internal lab split	External field duplicate	External lab split
trans-Nonachlor	ND[e]	11.1[b]	141[c]	ND[e]
pp'-DDT	2.2[d]	8.8[d]	62.8[d]	1.0[d]
pp'-DDE	14.7	16.8	22.8	9
β-BHC	21.8	13.9	92	22.8
δ-BHC	141[c]	ND[e]	ND[e]	ND[e]

*Based on the analyses of 9 internal field duplicate specimens, 4 internal lab splits, 10 external field duplicates, and 4 external laboratory splits at the EPA Toxicant Analysis Center.

[a]The coefficient of variation was calculated by dividing the square root of the pooled estimate of variance for all duplicates (internal and external) with a mean > 0 by the overall mean for these specimens.

[b]Measured in only one duplicate pair (in both specimens).

[c]Measured in only one specimen from one duplicate pair; because of this, the percent C.V. always equals the square root of 2 times 100.

[d]Measured in specimens from 44 percent of the sample persons overall.

[e]Not detected.

Table 15. Analysis of Serum for Semivolatiles: Estimates of Chemical Analysis Precision*

	Coefficient of variation (C.V.) (percent)	
	C.V. for all lab split duplicates[a]	C.V. for differences between spiked and nonspiked[b]
trans-Nonachlor	11.1[c]	11
pp'-DDT	6.8[d]	15.3
pp'-DDE	14.8	67.5
β-BHC	17.3	8.2
Dieldrin	ND[e]	8.9
Oxychlordane	ND[e]	9.1

*Based on the analyses of 8 lab-split duplicate pairs and 12 spiked-split duplicate pairs at the EPA Toxicant Analysis Center.

[a]The coefficient of variation was calculated by dividing the square root of the pooled estimate of variance for all lab-split duplicates with a mean > 0 (internal and external) by the overall mean for these specimens. The results from 8 specimen pairs (16 analyses) were used in the calculations.

[b]The coefficient of variation of the difference between the spiked and unspiked halves of spiked-split specimens. Results from 12 specimen pairs (24 analyses) were used in the calculations.

[c]Measured in only one duplicate pair (in both specimens).

[d]Measured in specimens from only 44 percent of the sample persons overall.

[e]Not detected.

Table 16. Analysis of Serum for Semivolatiles: Estimates of Within- and Among-Set Chemical Analysis Precision*

Compound	Coefficient of variation (C.V.) (percent)[a]	
	Internal duplicates[b]	External duplicates[c]
trans-Nonachlor	11.1[d]	141[e]
pp'-DDT	8.1[f]	55.8[f]
pp'-DDE	15.9	20.7
β-BHC	18.5	85.2
δ-BHC	141[e]	ND[g]

*Based on the analyses of 14 external duplicate pairs and 13 internal duplicate pairs at the EPA Toxicant Analysis Center.

[a]The coefficient of variation was calculated by dividing the square root of the pooled estimate of variance for all specimens with a mean > 0 by the overall mean for these specimens.

[b]Field duplicates and lab-split duplicates with both specimens analyzed in the same set.

[c]Field duplicates and lab-split duplicates with both specimens analyzed in separate sets.

[d]Measured in only one duplicate pair (in both specimens).

[e]Measured in only one specimen from one duplicate pair; because of this, the percent C.V. always equals the square root of 2 times 100.

[f]Measured in specimens from 44 percent of sample persons overall.

[g]Not detected.

6. Chemical Analysis Percent Recovery

Accuracy of the analytical method was estimated by calculating the chemical analysis percent recovery for each compound. Spiked-split duplicate analyses were used for this purpose. Percent recovery was estimated by dividing the mean difference between the spiked and unspiked halves by the spiked amount. Table 17 presents the estimated percent recoveries for each spiked compound. The percent recoveries ranged from 83 percent for pp'-DDT to 99 percent for dieldrin.

7. Total Error Estimates

Total analytical error was estimated from six compounds using both the chemical analysis bias (percent recovery) and precision information. Chemical analysis percent recovery was estimated from the spiked-split duplicates, as discussed in section VI.D.4. The percent coefficient of variation of the lab-split duplicates was used to estimate precision for the three compounds frequently detected: pp'-DDE (100 percent); pp'-DDT (44 percent); and β-BHC (85 percent). Due to the lack of endogenous-compounds data, precision estimates for trans-nonachlor, dieldrin, and oxychlordane was estimated from the percent coefficient of variation of the difference between the spiked and nonspiked halves of spiked-split duplicates. Table 15 presents both types of precision estimates.

Two estimates of chemical analysis total error were calculated for each of the six compounds. The Total Error was calculated by:

$$\left|\text{percent recovery} - 100\right| + 2\,(\text{percent coefficient of variation}) .$$

The twice Root Mean Square Error was calculated by:

$$2\,[(\text{percent recovery} - 100)^2 + (\text{percent coefficient of variation})^2]^{\frac{1}{2}} .$$

The estimates for both Total Error and twice Root Mean Square Error are presented in Table 18. With the analytical procedures, data of acceptable quality were produced for the six compounds; the total error estimates are all under 50 percent, which is the EPA guidelines acceptability level.

Table 17. Analysis of Serum for Semivolatiles: Mean Percent Recovery by Compound*

Compound	Spiked amount (ppb)	Mean difference	Mean percent recovery
trans-Nonachlor	20	17.1	86
pp'-DDT	40	33.0	83
pp'-DDE	20	18.5	93
β-BHC	20	17.5	88
Dieldrin	20	19.8	99
Oxychlordane	20	17.9	90

*Based on the analysis of 12 spiked-split duplicate specimen pairs at the EPA Toxicant Analysis Center.

Table 18. Analysis of Serum for Semivolatiles: Estimates of Chemical Analysis Total Error*

Compound	Percent 2 RMSE[a]	Percent total error[b]
trans-Nonachlor	36	36
pp'-DDT	37	31
pp'-DDE	33	37
β-BHC	42	47
Dieldrin	18	19
Oxychlordane	27	28

*Based on the analyses of 12 spiked-split specimens at the EPA Toxicant Analysis Center.

[a]Two Root Mean Square Error (RMSE)

$$= 2\,[(\text{percent recovery} - 100)^2 + (\text{percent coefficient of variation})^2]^{\frac{1}{2}}.$$

[b]Total Error

$$= |\text{percent recovery} - 100| + 2\,(\text{percent coefficient of variation}).$$

VII. ANALYSIS OF URINE FOR SEMIVOLATILES

Urine specimens were shipped to the EPA Toxicant Analysis Center (TAC) and chemically analyzed for the presence of selected semivolatile compounds (phenols). A quality assurance plan involving field controls, replicate analyses, and spiked-split duplicate analyses was developed and followed. Table 2 presents the list of target compounds. The analytical results and quality assurance procedures are discussed below.

A. Summary of Results

Four of the eight target compounds were detected; however, only one compound, pentachlorophenol (PCP), was measured in a significant number of sample persons (74.5 percent positive). The mean positive value was 4.3 ppb and the variance was 5.7. The compounds 3,5,6-trichloro-2-pyridinol, p-nitrophenol, and 2,4,5-trichlorophenol were measured in less than 4 percent of the specimens.

In order to assess the quality of the chemical analysis data, a total error estimate was calculated from the chemical analysis percent recovery and precision estimates. Percent recovery was estimated from the spiked-split duplicates. Because of the lack of endogenous-compounds data for every compound except PCP, precision was estimated from the percent coefficient of variation of the spiked-split duplicates, except for PCP, where the percent coefficient of variation of all duplicates was used. The total error estimates show that only two of the target compounds, 3,5,6-trichloro-2-pyridinol and trichlorophenoxy acetic acid (2,4,5-T), were within the EPA guidelines criterion (USEPA 1979) of less than 50 percent total error. The remaining six compounds, including PCP, had unacceptable error rates. The chemical analysis precision for PCP was fairly good, 9.1 percent; however, the mean percent recovery was only 45 percent. The total error estimates suggest that problems may exist with the analytical method for six of the eight target compounds, including PCP; however, the small sample sizes and lack of endogenous-compounds data limits the conclusions that may be drawn from the data.

The analysis of the three field blanks showed the presence of PCP, the mean value being 5.5 ppb. Of the three field spikes analyzed, two compounds, p-nitrophenol and 2,4,5-trichlorophenol, appeared to have abnormal recoveries, indicating that degradation or contamination may have occurred in the field. However, no firm conclusions can be drawn from a sample size of only three field spikes.

B. Specimen Collection, Storage, and Shipping

EPA received urine specimens from a subsample of study participants. Each EPA specimen was an aliquot drawn from a larger urine sample. Upon collection, specimens were labeled and stored in the freezer until shipment to the laboratory. Specimens were then shipped to the laboratory in insulated containers packed with dry ice in order to maintain the frozen state.

C. Analytical Methodology

Urine specimens were extracted and the analytes of interest determined by electron capture gas chromotography. The procedural details are described in "Multiresidue Procedure for Halo- and Nitrophenols. Measurement of Exposure to Biodegradable Pesticides Yielding These Compounds as Metabolites," (T. M. Shafik et al.). A copy of the procedure is contained in appendix C.

D. Analytical Results

1. Detection and Reporting Level Limits

The minimum reporting levels set by TAC for this study are listed below. Compounds detected in amounts less than these levels were reported as zero, except in the case of spiked-split specimens, for which the actual values were reported.

Compound	Minimum Reporting Level (ppb)
pyridinol	3
2,4,5-trichlorophenol	5
pentachlorophenol (PCP)	2
p-nitrophenol	10
dicamba	2
dichlorophenoxy acetic acid (2,4-D)	10
silvex	5
trichlorophenoxy acetic acid (2,4,5-T)	5

2. Results

Specimens from a total of 51* sample persons were analyzed for the presence of 8 phenol compounds. Only four of

*Three urine specimens were not analyzed successfully due to laboratory technical problems.

the tested compounds were measured:

3,5,6-trichloro-2-pyridinol;

pentachlorophenol (PCP);

p-nitrophenol; and

2,4,5-trichlorophenol.

Table 19 presents the percent positive measurements for each compound.

Only one compound, pentachlorophenol (PCP), was detected in a significant number of sample persons (75 percent). The mean positive value was 4.3 ppb, and the variance was 5.7. Table 20 presents a summary of PCP results.

The compound 3,5,6-trichloro-2-pyridinol was measured in specimens from one sample person, in both halves of duplicate analyses. The mean of the two duplicate halves was 5.1 ppb. P-nitrophenol was measured in two specimens, one with a value of 12.4 ppb and another with a value of 27.1 ppb. In one specimen, 2,4,5-trichlorophenol was measured with a value of 15.4 ppb.

E. Quality Assurance Procedures and Results

1. Field Controls

Field controls were prepared at RTI, shipped to and stored at the collection site, and then included in the specimen shipments to the laboratory (sec. IV.C). Matching laboratory controls were prepared at RTI and shipped directly to the laboratory.

Upon receipt at the laboratory, three field blanks and three field spikes were analyzed. All three field blanks showed levels of PCP, the mean value being 5.5 ppb. No other compounds were measured in the blanks analyzed.

Table 21 presents the results from the field spikes: Two compounds had unusual recovery levels. The percent recovery for p-nitrophenol was only 39 percent, and the percent recovery for 2,4,5-trichlorophenol was at 135 percent. The percent recoveries obtained from the spiked-split specimens, by comparison, were 78 and 97 percent respectively, well within the expected range.

2. Plan for Set Structure and Analysis

All specimens, including field spikes and blanks, were analyzed in analytical sets, a set consisting of a group of specimens analyzed at the same time under the same conditions. A randomization scheme was used to assign specimens to sets and to distribute duplicates both within and among sets. Specimens were

Table 19. Analysis of Urine for Semivolatiles: Frequency of Detection by Compound*

Compound	Sample persons tested	Number with positive detection	Percent with positive detection
3,5,6-Trichloro-2-pyridinol	51	1	2.0
Dicamba	51	0	0
2,4-dichlorophenoxy acetic acid (2,4-D)	51	0	0
Pentachlorophenol (PCP)	51	38	74.5
p-Nitrophenol	51	2	3.9
2,4,5-trichlorophenoxy acetic acid (2,4,5-T)	51	0	0
Silvex	51	0	0
2,4,5-Trichlorophenol	51	1	2.0

*Based on results reported by the EPA Toxicant Analysis Center.

Table 20. Analysis of Urine Semivolatiles: Summary of PCP Results*

Number of specimens analyzed	51
Number of positive detections	38
Percent positive detections	74.5
Mean of the positive values (ppb)	4.3
Mean overall value (ppb)	3.2
Median of the positive values (ppb)	3.5
Minimum reported positive value (ppb)	2.0
Maximum value (ppb)	14.6
Variance of the positive values	5.7
Coefficient of variation of the positive values	56%

*Based on results reported by the EPA Toxicant Analysis Center.

Table 21. Analysis of Urine for Semivolatiles: Results of Analyzing Field-Spiked Specimens*

Compound	Spiking level (ppb)	Mean detected level (ppb)	Measurement standard deviation	Mean percent recovery	Percent coefficient of variation
3,5,6-Trichloro-2-pyridinol	26.1	23.1	4.0	87	17
Dicamba	10.9	10	2.3	92	23
2,4-dichlorophenoxy acetic acid (2,4-D)	19.7	15.7	2.2	80	14
Pentachlorophenol (PCP)	129	117	1.7	90	11
p-Nitrophenol	11.5	4.5	7.7	39	173
2,4,5-trichlorophenoxy acetic acid (2,4,5-T)	11.7	10	0.2	85	2
Silvex	13.7	14	4.2	102	30
2,4,5-Trichlorophenol	16.8	22.8	2.8	135	12

Note: The mean positive levels have been adjusted by subtracting the mean levels measured in the field blanks.

*Based on the analyses of 3 field-spiked specimens at the EPA Toxicant Analysis Center.

renumbered so that the identity of duplicate specimens would not be known to the chemist.

In addition to the specimens, three standards, a method blank, and a lab spike were run with every set. Table 22 outlines the set structure and composition. Prior to analysis of any field specimens, TAC analyzed a number of lab spikes and calculated the standard deviation. A control chart was constructed using these data, with the upper and lower control limits set at the mean ±2 standard deviations. A lab spike was run with every set and plotted on the control chart to provide information on both precision and accuracy.

3. Procedures for Replicate Specimen Analysis

In order to provide estimates of chemical analysis precision, urine specimens were split in the laboratory and independently analyzed. Duplicates were analyzed both within the same set (internal duplicates) and in separate sets (external duplicates).

4. Procedures for Spiked-Split Specimen Analysis

A subsample of duplicates was used to create spiked-split specimens in order to estimate chemical analysis percent recovery. Spiked-split specimens were created by spiking one-half of a duplicate; the other half was not spiked. Both halves were independently analyzed, either within the same set (internal spiked-split specimen) or in different sets (external spiked-split specimens).

5. Chemical Analysis Precision Estimates

a. Overall Estimate

Overall chemical analysis precision was estimated for each compound by two different methods. In the first method, a pooled estimate of variance for all duplicates with a mean greater than zero was calculated using

$$s^2 = \frac{\sum_{i=1}^{n} \sum_{j=1}^{2} (X_{ij} - \bar{X}_i)^2}{n}$$

where s^2 denotes estimate of variance, X_{ij} represents the jth measurement on the ith pair, $\bar{X}_i$ is the mean of the ith pair, and n is the total number of duplicate pairs with mean value greater than zero. The coefficient of variation was obtained by dividing the square root of this variance by the overall mean for all duplicates with mean greater than zero.

Table 22. Analysis of Urine for Semivolatiles: Set Structure and Composition

Order	Type
1	Standard[a]
2	Method Blank
3	Lab Control (Spike)
4	Field Specimen[b]
5	Field Specimen
6	Field Specimen
7	Field Specimen
8	Standard
9	Field Specimen
10	Field Specimen
11	Field Specimen
12	Field Specimen
13	Standard

[a]Additional standards were run at the discretion of the analyst.

[b]Field specimens included serum specimens collected in the field and field spikes and blanks.

In the second method, spiked-split specimens were used to estimate precision by taking the coefficient of variation of the differences between the spiked and nonspiked halves. Table 23 presents precision estimates for each compound by each method.

b. Within- and Among-Set Precision

The precision obtained between specimens analyzed in the same set and the precision obtained when specimens are analyzed in separate sets was estimated by the same methods as was used to estimate overall chemical analysis precision. These estimates are given in Table 24.

6. Chemical Analysis Percent Recovery

The chemical analysis percent recovery of each compound from spiked-split duplicates was estimated by dividing the mean difference between the spiked and unspiked halves by the spiked amount. Table 25 contains the estimated percent recoveries for each spiked compound. The recoveries range from a low of 45 percent for pentachlorophenol (PCP) to 97 percent for 2,4,5-trichlorophenol.

7. Total Error Estimates

Total error was estimated for each compound using both the bias (percent recovery) and precision information. Percent recovery was estimated from the spiked-split duplicates, as presented in Table 25. Because of the lack of endogenous-compounds data for every compound except for PCP, precision was estimated from the percent coefficient of variation of the spiked-split duplicates except for PCP, where the percent coefficient of variation of all duplicates was used.

Two estimates were calculated for each compound. The Total Error was calculated by:

$$\left|\text{percent recovery} - 100\right| + 2\,(\text{percent coefficient of variation}) .$$

The twice Root Mean Square Error was calculated by:

$$2\,[(\text{percent recovery} - 100)^2 + (\text{percent coefficient of variation})^2]^{\frac{1}{2}} .$$

The estimates for both Total Error and twice Root Mean Square Error are presented in Table 26. Of the eight target compounds, only two compounds, 3,5,6-trichloro-2-pyridinol and trichlorophenoxy acetic acid (2,4,5-T) had Total Error or twice Root Mean Square Errors within the EPA guidelines criterion (below 50 percent total error). The remaining six compounds,

Table 23. Analysis of Urine Semivolatiles: Chemical Analysis Precision Estimates*

Compound	Coefficient of variation (C.V.) (percent)	
	C.V. for[a] all duplicates	C.V. for[b] all spiked-nonspiked differences
3,5,6-Trichloro-2-pyridinol	.4[c]	10.3
Dicamba	ND	28.5
Dichlorophenoxy acetic acid (2,4-D)	ND	13.1
Pentachlorophenol (PCP)	9.1	26.8
p-Nitrophenol	141[d]	26.5
Trichlorophenoxy acetic acid (2,4,5-T)	ND	10.9
Silvex	ND	27.3
2,4,5-Trichlorophenol	141[d]	84.9

*Based on analyses of analyzing 24 duplicate pairs and 25 spiked-split duplicate pairs at the EPA Toxicant Analysis Center.

[a]The coefficient of variation was calculated by dividing the square root of the pooled estimate of variance for all duplicates with a mean > 0 by the overall mean for these specimens.

[b]The coefficient of variation of the difference between the spiked and unspiked halves of the spiked-split specimens.

[c]Measured in specimens from only one sample person, in both halves of duplicate analyses.

[d]Measured in only one specimen from one duplicate pair; because of this, the percent C.V. always equals the square root of 2 times 100.

Table 24. Analysis of Urine Semivolatiles: Within- and Among-Set Chemical Analysis Precision Estimates*

Compound	Coefficient of variation (C.V.) (percent)			
	Internal (within-set)[a]		External (among-set)[b]	
	C.V.(%)[c]	C.V.(%)[d]	C.V.(%)[c]	C.V.(%)[d]
3,5,6-Trichloro-2-pyridinol	ND[g]	9	.4[e]	11.8
Dicamba	ND[g]	18.6	ND[g]	36.2
Dichlorophenoxy acetic acid (2,4-D)	ND[g]	10.7	ND[g]	15.2
Pentachlorophenol (PCP)	10.6	26.1	7.9	28.4
p-nitrophenol	ND[g]	36.2	141[f]	18
Trichlorophenoxy acetic acid (2,4,5-T)	ND[g]	13.4	ND[g]	9
Silvex	ND[g]	15.2	ND[g]	11.4
2,4,5-Trichlorophenol	ND[g]	29.4	141[f]	11

*Based on results reported by the EPA Toxicant Analysis Center.

[a]Duplicates with both specimens analyzed in the same set; 11 internal spiked-split duplicates and 13 internal duplicates were analyzed.

[b]Duplicates with specimen halves analyzed in separate sets; 14 external spiked-split duplicates and 15 external duplicates were analyzed.

[c]The coefficient of variation was calculated by dividing the square root of the pooled estimate of variance for all duplicates with a mean > 0 by the overall mean for these specimens.

[d]The coefficient of variation of the difference between the spiked and unspiked halves of the spiked-split specimens.

[e]Measured in only one sample person, in both halves of duplicate analyses.

[f]Measured in only one specimen from one duplicate pair; because of this, the percent C.V. always equals the square root of 2 times 100.

[g]Not detected.

Table 25. Analysis of Urine Semivolatiles: Mean Percent Recovery by Compound*

Compound	Spiked amount (ppb)	Mean difference	Mean percent recovery
3,5,6-Trichloro-2-pyridinol	10	8.2	82
Dicamba	10	6.7	67
dichlorophenoxy acetic acid (2,4-D)	60	45.1	75
pentachlorophenol (PCP)	10	4.5	45
p-Nitrophenol	50	39.2	78
Trichlorophenoxy acetic acid (2,4,5-T)	20	15.8	79
Silvex	20	16.4	82
2,4,5-Trichlorophenol	20	19.4	97

*Based on the analysis of 25 spiked-split duplicates at the EPA Toxicant Analysis Center.

Table 26. Analysis of Urine Semivolatiles: Estimates of Chemical Analysis Total Error*

Compound	Percent[a] 2 RMSE	Percent[b] total error
3,5,6-Trichloro-2-pyridinol	42	39
Dicamba	87	90
Dichlorophenoxy acetic acid (2,4-D)	56	51
Pentachlorophenol (PCP)	66[c]	83[c]
p-Nitrophenol	69	75
Trichlorophenoxy acetic acid (2,4,5-T)	47	43
Silvex	65	73
2,4,5-Trichlorophenol	170	173

*Based on results reported by the EPA Toxicant Analysis Center. Chemical analysis percent recovery and precision was estimated from spiked-split duplicates.

[a]Two Root Mean Square Error (RMSE)

$$= 2\,[(\text{percent recovery} - 100)^2 + (\text{percent coefficient of variation})^2]^{\frac{1}{2}}.$$

[b]Total Error

$$= |\text{percent recovery} - 100| + 2\,(\text{percent coefficient of variation}).$$

[c]Using field-spike percent recoveries, the RMSE drops to 14 percent and the Total Error drops to 28 percent; however, the spiked-split duplicates most likely provide a better estimate of chemical analysis percent recovery.

including PCP, had unacceptable error levels. The chemical analysis precision for PCP was fairly good, 9.1 percent; however, the spiked-split duplicate mean percent recovery was only 45 percent. As an alternative percent recovery estimate, the field-spike percent recovery for PCP, 90 percent, could be used, yielding a Total Error of 28 percent and Root Mean Square Error of only 14 percent. As previously stressed, however, field spikes are not ideal for estimating chemical analysis precision because of possible biases introduced in the field, and only three field spikes were analyzed, compared to 24 spiked-split duplicates analyzed. Spiked-split duplicates provide a better estimate of chemical analysis percent recovery. Judging from the limited data available, it seems appropriate to suggest that problems may exist with the analytical method for six of the eight target compounds, including PCP.

REFERENCES

"Multiresidue Procedure for Halo- and Nitrophenols. Measurement of Exposure to Biodegradable Pesticides Yielding these Compounds as Metabolites," J. Agric. and Food Chemistry. 21(2):295-298. 1973.

USEPA. 1979. U.S. Environmental Protection Agency. Manual for Analyzing Quality Control for Pesticides and Related Compounds in Human and Environmental Samples. Section 2K. First Revision. Washington, DC: USEPA. EPA-600/1-79-008.

APPENDIX A

VOLATILES ANALYTICAL METHODOLOGY

Bull. Environm. Contam. Toxicol. 23,244-249 (1979)

Determination of Volatile Purgeable Halogenated Hydrocarbons in Human Adipose Tissue and Blood Serum

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Organohalogens have been detected in virtually all chlorinated drinking waters (ROOK 1974, BELLAR et al. 1974, SYMONS et al. 1975, THOMASON et al. 1978). During a nationwide study (SYMONS et al. 1975), concentration levels in the finished drinking water of 79 cities of the United States were established for six volatile purgeable halogenated hydrocarbons (VPHH's): chloroform ($CHCl_3$), bromodichloromethane (BDCM), dibromochloromethane (DBCM), bromoform ($CHBr_3$), carbon tetrachloride (CCl_4) and 1,2-dichloroethane (DCE). The health implications of these findings stimulated the development of a project to determine if any of these six substances or trichloroethylene (TCE) could be detected in relatively small samples of human adipose tissue (250 mg) or blood serum (0.5 ml) obtained from residents of Dade County, Florida, an area in which chloroform levels in excess of 300 µg/l (ppb) have been reported. Accordingly, the purge/trap/desorb method of BELLAR and LICHTENBERG (1974) was modified to accomplish these objectives. The procedure reported here requires no extraction or clean-up step and is relatively inexpensive to perform. Each analysis is completed in about 30 minutes.

MATERIALS AND METHODS

Apparatus. A Tekmar Model LSC-1 liquid sample concentrator was interfaced to a Tracor Model 222 gas chromatograph (GC) equipped with a Hall electrolytic conductivity detector which was operated in the halide specific mode. The chromatographic column was a 6-ft x 0.25-in I.D. glass U-tube containing n-octane on 100-120 mesh Porasil C packing. The GC operating conditions included: a nitrogen carrier gas flow-rate of 30 ml/min, an inlet temperature of 140^0, and a transfer line temperature of 210^0. The Hall detector furnace was maintained at 900^0 with a hydrogen flow-rate of 40 ml/min and a solvent (1:1 n-propanol: distilled water) flow of 0.4 ml/min.

A Finnigan Model 4000 gas chromatograph/mass spectrometer (GC/MS) analytical system interfaced to a Tekmar liquid sample concentrator was used to confirm the identities of the compounds quantified by the gas chromatographic procedure.

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0007-4861/79/0023-0244 $01.20

Both the GC and the GC/MS systems utilized a hot plate stirrer and a glycerol bath to heat the sample in the Tekmar purging device.

Solvents and Reagents. Chloroform, carbon tetrachloride and hexane were Pesticide Grade from Fisher Scientific Co. Trichloroethylene, 1,2-dichloroethane and bromoform were from Aldrich Chemical Co.; bromodichloromethane and dibromochloromethane, from Columbia Organic Chemical Co. Dow Corning antifoam emulsion B was from Fisher Scientific Co. and the n-octane on 100-120 mesh Porasil C chromatographic packing was purchased from Supelco, Inc.

Preparation of Standards. Two ml each of carbon tetrachloride, dibromochloromethane and bromoform and 1 ml each of chloroform, trichloroethylene, bromodichloromethane and 1,2-dichloroethane are diluted with hexane to a final volume of 100 ml (solution 1). The concentration of each component is calculated by using the respective specific gravities. One ml of solution 1 is quantitatively diluted to 100 ml with hexane (solution 2), and a convenient working standard is prepared by diluting 0.1 ml of solution 2 to 25 ml with hexane. Use of 5 µl of this solution leads to acceptable peak heights when the Hall detector attenuation is 10 x 8.

A standard curve may be obtained by using three hexane dilutions of solution 2: the working standard, 0.1 ml diluted to 50 ml (for 10 x 4 attenuation) and 0.2 ml diluted to 25 ml (for 10 x 16 attenuation). (Although solutions 1 and 2 are stable at room temperature, fresh working standards must be made daily.)

Procedure for Blood Serum. One ml of 1% aqueous antifoam is added to a 5-ml purging device and the sample concentrator is operated in the Trap Bake Mode for 20 min while the trap temperature is 200°. (This procedure purges the system of all potentially interfering volatile compounds; however, a blank run may be made at this time to be certain that the system is uncontaminated.) The trap is then cooled to the ambient temperature. By means of a gas tight syringe, 0.5 ml of serum is introduced into the purging device and a purge flow-rate of 10 ml/min is started. The lower portion of the purging device is immersed in a 115° stirred glycerol bath for 30 min.* To prevent steam contamination of the Tenax/silica gel trap, a small glass vapor trap or interceptor is placed between the purging device and the adsorbent trap. After the purge/trap period is complete, the adsorbed compounds are desorbed and transferred to the analytical column (60°) by heating the trap at 150° for 6 min. The GC column is then temperature programmed 7°/min to 140°.

*Both serum and fat appear to have an inherent binding capacity for chloroform which can be overcome by purging at elevated temperatures.

Procedure for Adipose Tissue. Between 200 and 500 mg of frozen adipose tissue is cut into thin strips and pushed to the bottom of a pre-purged, 5-ml Tekmar purging device. The purge flow-rate is adjusted to 10 ml/min, and the lower portion of the device is immersed in a 115° stirred glycerol bath for 20 min. After the volatile components have been purged from the liquefied fat, the analytical procedure given above for serum analysis is followed. Hexane is added to the purging device containing the purged adipose tissue, and the residue is quantitatively extracted to separate the fat component from residual connective tissue. Values are reported in ng VPHC/g of hexane extractable fat.

GC/MS Component Confirmation. The identities of the components quantified by the LSC/GC method are confirmed by using an LSC/GC/MS analytical system. Any confirmation is based on both relative retention values (GC data) and mass fragmentation data (m/e values and isotopic ratios).

RESULTS AND DISCUSSION

Chloroform is the major volatile purgeable halogenated hydrocarbon identified during the analysis of human adipose tissue and blood serum when this methodology is used.

Reproducibility. One serum sample was analyzed ten times over a two-day period. The chloroform concentration ranged from 23 to 36 µg/l with a mean value of 27 µg/l and a standard deviation of 4.

One fat sample was analyzed ten times over a three-day period. The chloroform values ranged from 104 to 140 ng per gram of hexane extractable fat with a mean value of 122 ng/g and a standard deviation of 6.

Recovery. Because of the volatility of the compounds in this study, recovery studies were performed within the purging device. Serum was pre-purged, then analyzed to insure that no halogenated compounds were present. For each replication, a 5-µl aliquot of the working standard was added to the serum in the purging device. The components of the standard were allowed to mix with the serum for several minutes; then the serum was treated like an unknown sample. Table 1 indicates how much of each VPHH was added, the average per cent recovered and the recovery range for ten replications.

TABLE 1
Recovery of VPHH's from Human Blood Serum

	Amount Added (µg/l)	Average Recovery (%)	Recovery Range (%)
CCl_4	1.3	112	108-124
$CHCl_3$	0.6	100	100
TCE	0.6	98	83-100
BDCM	0.8	92	88-100
DCE	1.0	100	93-110
DBCM	2.1	87	78-100
$CHBr_3$	2.3	90	79-100

A heated sample of human adipose tissue was purged for 30 minutes to remove all volatile purgeable halogenated compounds. For each replication, a 5-µl aliquot of the working standard was added to the fat in the purging device and allowed to mix for several minutes. Then the fat was treated as an unknown sample. Table 2 indicates how much of each VPHH was added, the average per cent recovered and the recovery range for ten replications.

TABLE 2
Recovery of VPHH's from Human Adipose Tissue

	Amount Added (µg/l)	Average Recovery (%)	Recovery Range (%)
CCl_4	1.3	96	90-100
$CHCl_3$	0.6	92	88-100
TCE	0.6	101	100-110
BDCM	0.8	109	100-125
DCE	1.0	98	93-100
DBCM	2.1	105	90-118
$CHBr_3$	2.3	110	83-137

Blood Serum Analyses. Ten serum samples were collected from healthy human subjects and analyzed within 24 hours of collection. The chloroform values ranged from 13 to 49 µg/l as indicated in Table 3.

Sample S-1 was analyzed several times over a period of two months; each result was within 2 µg/l of the initial value.

Adipose Tissue Analyses. Ten fat samples, taken from near the anterior abdominal wall at autopsy, were analyzed. Chloroform concentrations ranged from 20 to 460 ng per gram of hexane extractable fat as indicated in Table 4.

TABLE 3
Human Blood Serum
Chloroform Levels

Sample	µg/l	Sample	µg/l
S-1	13	S-6	25
S-2	13	S-7	26
S-3	49	S-8	30
S-4	13	S-9	30
S-5	45	S-10	13

TABLE 4
Human Adipose Tissue
Chloroform Levels

Sample	ng/g*	Sample	ng/g*
F-1	80	F-6	20
F-2	230	F-7	28
F-3	460	F-8	140
F-4	65	F-9	95
F-5	240	F-10	240

*hexane extractable fat

The identities of the reference compounds and the chloroform in human serum and fat were confirmed by LSC/GC/MS methods. When coupled with the appropriate GC retention data, the cluster having m/e = 83, 85 and 87 corresponding to the respective positively charged fragments $CHCl_2^{35}$, $CHCl^{35}Cl^{37}$ and $CHCl_2^{37}$ was particularly useful to confirm the presence of chloroform in the biological samples.

The exact source of the chloroform detected in human fat and serum by this procedure is presently unknown. Possible sources include municipal drinking water and chronic exposure to trichloroethylene (TCE) and/or tetrachloroethylene (perchloroethylene, PCE).

Municipal drinking water contains both residual chlorine and chloroform generated during the chlorination process from reactions between humic substances in raw water and either dissolved chlorine or hypochlorous acid (MORRIS 1978). It has also been proposed that "intermediate bonding states" between halogen and various organic molecules can exist (GLAZE et al. 1977; NICHOLSON et al. 1977). If this latter situation occurs to any great extent, ingestion of municipal drinking water could conceivably lead to in vivo generation of chloroform from the precursors. Moreover, the residual chlorine in finished drinking water could also form chloroform precursors after the water has been consumed. A clear explanation of the origin of chloroform in human tissue is thus not currently possible.

A satisfactory explanation is even more difficult if exposure to TCE and/or PCE is considered. During an earlier study and from periodic water analysis reports, neither of these compounds has been detected in amounts comparable to the chloroform levels usually found in Miami drinking water. However, both TCE and PCE are metabolic precursors of chloroform (BUTLER 1949, IKEDA 1977), and FISHBEIN (1976) has reviewed two additional routes of exposure, air and food (McCONNELL et al. 1975, CAMISA 1975). McCONNELL et al. (1975) reported tissue ranges of TCE and PCE for humans between less than 0.5 µg/kg and 29 µg/kg wet tissue. These values are far below the chloroform levels reported here for adipose tissue. Although it is impossible to rule out the possibility that some of our experimental subjects had been exposed to these two organochlorides, it is reasonable to assume that they were not. None of

the subjects were involved in either dry cleaning or degreasing occupations nor did any of them work near these types of establishments. If a portion of the serum chloroform reported in this investigation is traceable to metabolized TCE or PCE, the intact chlorinated ethylene(s) will be present in corresponding samples of adipose tissue from the individuals. Such a study is in progress.

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REFERENCES

BELLAR, T.A., and J.J. LICHTENBERG: J. Amer. Water Works Assoc. 66, 739 (1974).

BELLAR, T.A., J.J. LICHTENBERG, and R.C. KRONER: J. Amer. Water Works Assoc. 66, 703 (1974).

BUTLER, T.: J. Pharmacol. Exp. Ther. 97, 84 (1949).

CAMISA, A.G.: J. Water Pollut. Control Fed. 47, 1021 (1975).

FISHBEIN, LAWRENCE: Mutation Res. 32, 267 (1976).

GLAZE, W.H., G.R. PEYTON, and R. RAWLEY: Environ. Sci. Techn. 11, 685 (1977).

IKEDA, MASAYUKI: Environ. Health Perspect. 21, 239 (1977).

McCONNELL, G., O.M. FERGUSON, and C.R. PEARSON: Endeavor 34, 13 (1975).

MORRIS, J.C.: Water chlorination, environmental impact and health effects, 1 ed. Michigan: Ann Arbor Science Publishers 1978.

NICHOLSON, A.A., O. MERESZ, and B. LEMYK: Anal. Chem. 49, 814 (1977).

ROOK, J.J.: Water Treatm. Examin. 23, 234 (1974).

SYMONS, J.M., T.M. BELLAR, J.K. CARSWELL, J. DEMARCO, K.L. KROPP, G.G. ROBECK, D.R. SEEGER, C.J. SLOCUM, B.L. SMITH, and A.A. STEVENS: J. Amer. Water Works Assoc. 11, 634 (1975).

THOMASON, M., M. SHOULTS, W. BERTSCH, and G. HOLZER: J. Chromatogr. 158, 437 (1978).

APPENDIX B

TAC-SPIKED BLOOD SERUM RECOVERIES

Appendix B. TAC-Spiked Blood Serum Recoveries

Compound	SPRM 107* fort.-level	PPB Recovered--TAC-spiked blood serum															
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	$\bar{X}$	SD	RSD (%)
Hexachloro-benzene	2.4	2.22	2.25	2.33	2.23	2.15	2.13	2.31	2.14	2.25	2.16	2.22	2.18	2.31	2.22	0.07	3.04
β-BHC	4.6	3.94	3.86	4.03	4.00	4.08	3.92	4.08	4.00	4.08	3.95	4.11	3.87	4.11	4.00	.09	2.21
Oxychlordane	6.5	6.11	5.90	5.90	5.30	5.20	4.90	5.29	5.48	5.86	5.35	5.25	5.63	5.53	5.52	.35	6.31
Heptachlor epoxide	5.7	5.07	5.18	5.07	5.21	5.21	5.10	5.43	5.12	4.91	5.10	5.30	5.00	5.30	5.15	0.14	2.69
trans-Nonachlor	9.7	9.28	9.16	9.03	6.97	6.63	6.63	7.78	7.56	7.67	7.53	7.42	7.97	7.86	7.81	0.88	11.27
pp'-DDE	28.7	28.60	28.30	28.00	20.81	20.41	19.66	23.42	22.97	23.27	22.85	22.42	23.86	23.29	23.68	2.92	12.34
Dieldrin	5.7	5.35	5.35	5.17	5.30	5.47	5.12	5.54	5.19	5.19	5.27	5.27	5.27	5.44	5.30	0.13	2.37
pp'-DDT	4.7	4.36	4.00	4.00	3.27	3.27	2.91	3.48	3.83	3.83	3.48	3.48	3.48	3.48	3.61	0.38	10.6
Hexachloro-benzene	2.4	1.94	2.17	2.00	1.99	1.82									1.98	0.13	6.38
β-BHC	4.6	3.60	4.20	4.13	4.00	3.64									3.91	0.28	7.12
Oxychlordane	6.5	5.37	6.92	6.61	6.36	5.97									6.25	0.60	9.58
Heptachlor epoxide	5.7	4.33	5.68	5.41	5.22	4.93									5.11	0.50	9.80
trans-Nonachlor	9.7	7.05	9.90	9.31	9.09	8.81									8.83	1.07	12.16
pp'-DDE	28.7	15.67	23.67	22.51	22.69	21.40									21.19	3.19	15.05
Dieldrin	5.7	4.55	5.86	5.68	5.68	5.25									5.40	0.53	9.77
pp'-DDT	4.7	3.08	4.00	4.55	4.80	4.30									4.15	0.67	16.04

*SPRM = standard pesticide reference material.

Fort. level = fortification level.

APPENDIX C

SEMIVOLATILE ANALYTICAL METHODOLOGY FOR URINE

Multiresidue Procedure for Halo- and Nitrophenols. Measurement of Exposure to Biodegradable Pesticides Yielding these Compounds as Metabolites

Talaat M. Shafik,* Hazel C. Sullivan, and Henry R. Enos

The urinary level of phenolic compounds may be the key for establishing an index of exposure to pesticides containing this moiety as an easily hydrolyzed or metabolized portion of the molecule. A method has been developed for quantitating ten halo- and nitrophenols in rat urine which could result from exposure to and subsequent metabolism and excretion of a broad spectrum of pesticides. The procedure involves acid hydrolysis, extraction, derivatization, silica gel chromatography, and electron-capture gas chromatography. Male rats fed pesticidal compounds containing halo- and nitrophenol moieties at levels varying from factors of 10^{-5} to 10^{-2} of the LD_{50} were used to establish the usefulness of this procedure for determining the extent of exposure to the biodegradable pesticides.

Determination of the urinary excretion of halo- and nitrophenol metabolites of biodegradable pesticides is of increasing interest to those involved in pesticide epidemiology. Metabolism studies (Menzie, 1969) of the biodegradable pesticides EPN, fenitrothion, dicapthon, methyl bromophos, C-9491, Dursban, DNOC, PCP, VC-13, and ronnel, which contain halo- or nitrophenol substituent groups in their molecular structures, indicate that these phenols are the major urinary metabolites. The purpose of this investigation was to develop a multiresidue method for the determination of low levels of halo- and nitrophenols in urine. The method is based on electron-capture gas chromatography of ethyl ether derivatives of the phenols (Bradway and Shafik, 1971; Shafik *et al.*, 1971a). Such a procedure may be of value in developing an exposure index to biodegradable pesticides based on levels of these urinary phenols.

EXPERIMENTAL SECTION

Apparatus and Equipment. A Micro-Tek 220 gas chromatograph equipped with tritium foil electron-capture detector was used. A glass U-shaped column, 6 ft × ¼ in., was packed with 4% SE-30/6% QF-1 on 80/100 mesh Chromosorb W (HP). The gas chromatographic column was operated under the following parameters: nitrogen carrier gas flow rate, 60 ml/min; column temperature, 175°; inlet, 210°; detector, 210°; transfer line, 240°. Chromatographic columns were size 22, Kontes K-420100. Concentrator tubes were 25 ml, Kontes K-570050. Condensers were Kontes K-286810. Nitrogen evaporator was equipped with a water bath maintained at 40°. N-Evap was obtained from Organomation Associates, Worcester, Mass.

Reagents. The following were used: *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wis.); ethylating reagent (Stanley, 1966; Shafik *et al.*, 1971a); and silica gel, Woelm, activity grade I (Waters Associates, Inc., Framingham, Mass.). Dry adsorbent for 48 hr at 170° and store in a desiccator. Prepare daily 2 g of deactivated silica gel for each chromatographic column by adding 40 μl of benzene-extracted deionized or distilled water for each 2 g of dried silica gel in a tightly stoppered container. Rotate container until the water is evenly distributed throughout the adsorbent. Allow to equilibrate for 2 to 3 hr with periodic shaking. Prepare the chromatographic columns just before use. Deionized or distilled water used for deactivating the silica gel must be extracted twice with benzene. Prepare each day the anticipated amount of deactivated silica gel to be used. Larger amounts may be prepared by using the same ratio of water to dried silica gel.

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Preparation of Standard Solutions. The phenolic compounds were 95+% pure and a mixture of the ten compounds was prepared at the final concentrations as follows: 2,4-dichlorophenol (2,4-DCP), 0.4 μg/ml; 2,4,5-trichlorophenol (2,4,5-TCP), 0.1 μg/ml; and 3,5,6-trichloro-2-pyridinol (3,5,6-TCPyridinol), 0.1 μg/ml, Dow Chemical Co., Midland, Mich.; 3,6-dichloro-4-iodophenol (3,6-DCIP), 0.05 μg/ml, Ciba Agrochemicals Co., Summit, N. J.; 2,5-dichloro-4-bromophenol (2,5-DCBrP), 0.02 μg/ml, Cela, Ingelheim, West Germany; pentachlorophenol (PCP), 0.01 μg/ml, and *p*-nitrophenol (PNP), 0.2 μg/ml, Aldrich Chemical Co., Milwaukee, Wis.; *p*-nitro-*m*-cresol (PNC), 1.2 μg/ml, Chemagro Corp., Kansas City, Mo.; 2-chloro-4-nitrophenol (2-C-4NP), 0.08 μg/ml, American Cyanamid Co., Princeton, N. J.; 2,4-dinitro-6-methylphenol (DNOC), 0.4 μg/ml, Chemical Insecticide Corp., Edison, N. J. These compounds are the corresponding phenolic metabolites of pesticides listed in Column 1, Table II.

Weigh 10 mg of each of the ten analytical standards into separate 10-ml volumetric flasks. Add 5 ml of benzene to each flask and swirl the flask until the compound dissolves. In a well-ventilated hood, wearing disposable gloves, add diazoethane dropwise with a disposable pipet until a definite yellow color persists. Allow the solution to stand 15 min, and then bubble dry nitrogen through the solution until the yellow color disappears (5–10 min). Dilute to volume with benzene. From these ethylated stock standards, prepare a mixture of the ten compounds at the final concentrations listed above.

Chromatographic Behavior of the Ten Ethyl Ethers on a Silica Gel Column. Pipet an aliquot of the mixture of the ten ethylated compounds into a 15-ml graduated centrifuge tube. Evaporate the benzene to a volume of 0.3 ml using the nitrogen evaporator, and add 1.7 ml of hexane. To a chromatographic column lightly plugged with glass wool, add the 2.00 g of the partially deactivated silica gel and top with 1.5 g of anhydrous sulfate. Prewash the column with 10 ml of hexane and discard the hexane eluate. Transfer the standard mixture quantitatively to the column using 8 ml of the 20% benzene-in-hexane solution and collect this fraction in a 15-ml centrifuge tube (fraction I). Elute the column with 10 ml of 40% benzene-in-hexane (fraction II), followed by 10 ml of 60% benzene-in-hexane and 10 ml of 80% benzene-in-hexane, collecting the 20 ml in a 25-ml concentrator tube (fraction III). Elute the column with 10 ml of benzene and collect this fraction in a 15-ml centrifuge tube (fraction IV). Inject 5–10 μl from each fraction into the gas chromatograph to establish the elution pattern of the ten ethyl ethers from the silica gel column.

Under our laboratory conditions, fraction I (20% benzene-in-hexane) contained the ethyl ethers of 2,4-DCP, 2,4,5-TCP, 3,5,6-TCPyridinol, 3,6-DCIP, 2,5-DCBrP, and

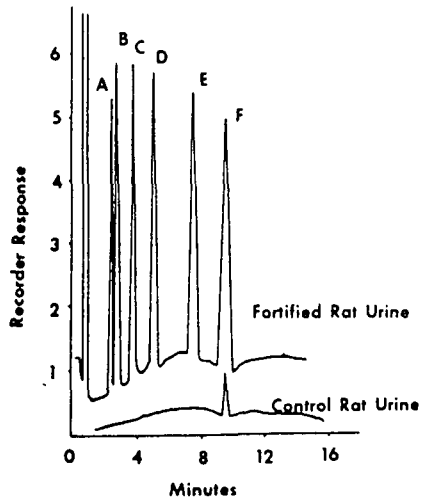

Figure 1. Chromatogram of the 20% benzene-in-hexane fraction of fortified and control rat urine. A. 2,4-DCP, 0.8 ppm; B. 3,5,6-TCPyridinol, 0.05 ppm; C. 2,4,5-TCP, 0.03 ppm; D. 2,5-DCBrP, 0.02 ppm; E. 3,6-DCIP, 0.03 ppm; F. PCP, 0.02 ppm.

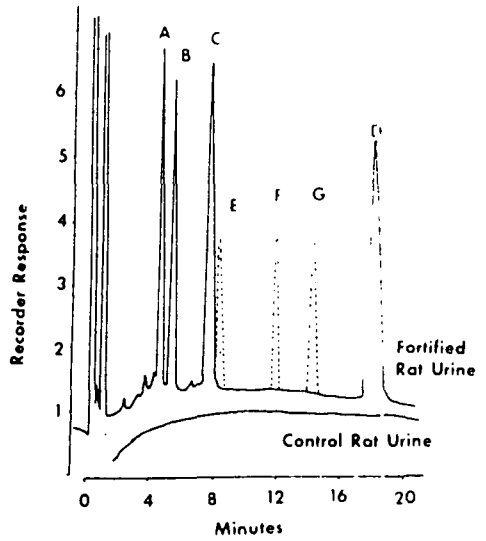

Figure 2. Chromatogram of the 60–80% benzene-in-hexane fraction of fortified and control rat urine: A. PNP, 0.1 ppm; B. PNC, 0.4 ppm; C. 2-C-4NP, 0.04 ppm, D. DNOC, 0.16 ppm; E. 2,4-D; F. 2,4,5-TP; G. 2,4,5-T.

PCP. The ethyl ethers of PNP, PNC, 2-C-4NP, and DNOC eluted in fraction III (60 and 80% benzene-in-hexane). Occasionally, traces of DNOC are found in fraction IV (benzene). Fraction II (40% benzene-in-hexane) did not contain any of the derivatives.

Elution patterns may vary from one laboratory to another, depending on the temperature and relative humidity. It is therefore necessary to establish an elution pattern under local conditions before attempting to analyze samples.

Analysis of Urine. Pipet 1 to 5 ml (the actual volume to be determined by the anticipated residue level) of urine into a 25-ml concentrator tube. Add dropwise a volume of concentrated HCl equal to one-fifth the amount of urine, and mix well. Fit a stoppered reflux condenser to the tube and heat in a boiling water bath for 1 hr while cooling the condenser with circulating ice water. Remove from the bath, cool, and rinse the sides and tip of the condenser with a total of 2 ml 0.1 *N* NaOH. Add 3 ml of anhydrous ethyl ether to the tube and mix contents vigorously on a Vortex mixer for 2 min; then centrifuge and transfer ethyl ether layer to a 15-ml centrifuge tube with a disposable pipet. Repeat the extraction with an additional 3-ml volume of ethyl ether and add the second ethyl ether extract to the centrifuge tube.

Add diazoethane dropwise with a disposable pipet until the yellow color persists. Let the solution stand 15 min; then bubble clean dry nitrogen through the solution to remove excess reagent. Concentrate the ethylated urine extract to approximately 0.3 ml, using the nitrogen evaporator. Add 2 ml of hexane and continue evaporating the ether-hexane solution to 0.3 ml.

Prepare a silica gel chromatographic column as previously described. Prewash the column with 10 ml of hexane and discard the washing. Transfer the concentrated urine extract quantitatively to the column using 2 ml of the 20% benzene-in-hexane. As soon as the solvent sinks in the sodium sulfate, add 8 ml of the 20% benzene-in-hexane to the column and collect the total volume of the 20% benzene-in-hexane (10 ml). This fraction contains the halogenated phenols. Continue eluting with 10 ml of 40% benzene-hexane and discard this fraction. Add 10 ml of 60% benzene-hexane, followed by 10 ml of 80% benzene-hexane and collect these fractions in a single tube. The combined 60–80% benzene-hexane fractions will contain the nitrophenols. Add 10 ml of benzene to column and collect eluate. Frequently a small amount of the DNOC is found in the benzene fraction. The urinary impurities eluted by the benzene solvent do not interfere with the gas chromatographic determination of DNOC, which has a relatively long retention time. The elution pattern of spiked control urine extracts must be established before the analysis of actual samples is undertaken.

Table I. Electron-Capture Detector Sensitivities,[a] Limits of Detection and Recovery Data[b] for Ten Halo- and Nitrophenol Ethyl Ethers

Compound	Limits of		
	Sensitivity, ng	Detection, ppm	Recovery, %
2,4-DCP	0.2	0.1	87–96
2,4,5-TCP	0.02	0.01	85–95
3,5,6-TCPyridinol	0.05	0.01	91–97
3,6-DCIP	0.02	0.01	88–94
2,5-DCBrP	0.02	0.01	88–96
PCP	0.01	0.01	92–96
PNP	0.1	0.02	85–98
PNC	0.3	0.05	88–98
2-C-4NP	0.05	0.01	85–92
DNOC	0.1	0.05	86–96

[a] Based on 15% scale deflection [b] Based on use of the described method

RESULTS AND DISCUSSION

Control rat urine samples were fortified with the sodium salts of the phenols. The samples were analyzed and aliquots of the 20% B-H and 60 + 80% fractions were injected separately into the gas chromatograph. Figure 1 illustrates chromatograms of the 20% B-H fraction (halogenated phenols) of spiked and control rat urine samples, and Figure 2 shows chromatograms of the 60 + 80% fraction (nitrophenols) of control and fortified rat urine samples. An average of 0.01 ppm of pentachlorophenol was routinely found in all control urine samples. The percent recovery, limits of detection in ppm, and detector sensitivity in nanograms (based on 15% scale deflection) are shown in Table I.

Ethylation of the phenols proceeds rapidly at room temperature, producing ethyl ether derivatives which are gas chromatographable and stable to silica gel column chromatography. Better gas chromatographic resolution of the more volatile phenols was achieved by preparing the ethyl

Table II. The Relation between Total Dosage of Biodegradable Pesticides and Urinary Excretion of Halo- and Nitrophenols

Compound	Dose level LD_{50}	nmol fed	Excretion of phenolic-type metabolites: Metabolite	nmol excreted	% of dose excreted	Days for complete excretion
VC-13	10^{-2}	5140	2,4-DCP	3470	67	3
	10^{-3}	514		359	70	1
Ronnel	10^{-4}	234	2,4,5-TCP	124	53	2
	10^{-5}	23.4		7.4	32	1
Dursban	10^{-3}	232	3,5,6-TCPyridinol	157	68	4
	10^{-4}	23.2		29.5	100+	4
C-9491	10^{-4}	314	3,6-DCIP	7.6	2.4	2
	10^{-5}	31.4		0.834	2.7	1
Bromophos	10^{-4}	617	2,5-DCBrP	305	49	3
	10^{-5}	61.7		41.4	67	3
PCP	10^{-4}	40.5	PCP	9.03	22	2
	10^{-5}	4.06		1.05	26	1
EPN	10^{-2}	670	PNP	108	16	3
	10^{-3}	67.0		9.35	14	3
Fenitrothion	10^{-2}	5430	PNC	3610	66	2
	10^{-3}	543		472	87	1
Dicapthon	10^{-2}	8700	2-C-4NP	3570	41	1
	10^{-3}	810		20	2.5	1
DNOC	10^{-2}	910	DNOC	0	0	
	10^{-3}	91.0		0	0	

derivatives instead of the more commonly used methyl ethers.

Silica gel column chromatography serves two purposes: it provides a clean sample for gas chromatographic analysis and it conveniently separates the halogenated phenols, thus simplifying the gas chromatographic analysis.

A mixture of the ten phenols and three phenoxy acids, namely 2,4-D, 2,4,5-T, and silvex, can be determined in one sample. All of the halogenated phenols involved in this study are eluted with 20% benzene–hexane, while the nitrophenols and the phenoxy acids are eluted in the 60 and 80% benzene–hexane fractions. These herbicides are included in this report because they are detected as intact excreted residues in the urine using the present procedure. 2,4-DCP and 2,4,5-TCP, potential mammalian metabolites of 2,4-D and 2,4,5-T, are also determined in this procedure. The analysis of these herbicides and their metabolites has been described in detail in a previous report (Shafik *et al.*, 1971a). In the gas chromatographic step, the retention time of the ethyl ether derivative of 2-chloro-4-nitrophenol is almost identical to that of the ethyl ester of 2,4-D. In addition, these two derivatives are eluted in the 60 + 80% benzene–hexane fractions from silica gel. Confirmation of identity can be accomplished using the classical $NaHCO_3$ and acid extraction steps which separate carboxylic acids from phenols (Bakke and Scheline, 1969).

In order to determine if a correlation exists between exposure to intact pesticides and excretion of urinary phenolic metabolites, male Charles River rats weighing 190 to 220 g were dosed by gavage with peanut oil solutions of eight organophosphorus compounds, PCP, and DNOC in concentrations ranging from 10^{-2} through 10^{-5} of the LD_{50} (Kenaga and Allison, 1969), as indicated in Table II. The doses were administered daily for 3 days to two rats at each dose level. The animals were maintained in stainless steel metabolism cages with the two rats administered the same dose regimen maintained in the same cage. Urine samples were collected at 24-hr intervals and stored in a freezer until analysis was performed.

Urine samples were analyzed for several days following the third dose until no detectable levels of the phenolic type metabolites were observed. This established the number of days required for total excretion of the metabolites. The percent of the total dose excreted as the phenolic metabolite was calculated from the sum of the amount of phenolic metabolite excreted each day and the total amount of pesticide fed in the 3-day period.

As indicated in Table II, the amount of urinary metabolites excreted is proportional to the dose of parent compound administered. The percent of the dose excreted in the urine as a phenolic metabolite of the pesticide fed indicates that low level animal exposure to VC-13, ronnel, Dursban, Bromophos, PCP, EPN, and fenitrothion can be detected. The low excretion rates of the phenols of C-9491 and dicapthon in urine indicate that the method cannot detect low-level exposure to these compounds. DNOC was not detected in the urine of rats fed 10^{-2} and 10^{-3} of the LD_{50} of DNOC.

There are other pesticide chemicals not included in this investigation which may produce the same phenolic metabolites reported in this study. 2,4,5-TCP can be a urinary metabolite of ronnel, Gardona, lindane, 2,4,5-T, and Silvex. PNP in urine can result from exposure to EPN, ethyl parathion, and methyl parathion. The method is not capable of distinguishing between the sources of the urinary 2,4,5-TCP and PNP. When such a distinction is essential, the corresponding alkyl phosphate of the organophosphorous pesticide can be determined in the urine (Shafik *et al.*, 1971a). The free phenoxy acids of 2,4,5-T and Silvex can also be determined in the urine (Shafik *et al.*, 1971a). It must be emphasized that the rat-feeding experiment was designed only for the purpose of evaluating the method and not as a study of the metabolism of these compounds.

In conclusion, a multiresidue method has been developed for the determination of low levels of halo- and nitrophenols and phenoxy acids in rat urine which undoubtedly can be extended to environmental samples. Such a method should be useful, if properly evaluated on a monitoring and surveillance scale, in establishing human exposure to low levels of a large number of biodegradable pesticides.

LITERATURE CITED

Bakke, O. M., Scheline, R. R., *Anal. Biochem.* 27, 451 (1969).

Bradway, D., Shafik, M. T., "A Gas Chromatographic Method for the Determination of Low Levels of *p*-Nitrophenol in Human and Animal Urine," presented at the 162nd National Meeting of the American Chemical Society, Washington, D. C., September 12-17, 1971.

Kenaga, E. E., Allison, W. E., reprinted from *Bull. Entomol. Soc. Amer.* **15**, 85 (1969).
Menzie, C. M., Metabolism of Pesticides, Bureau of Sport Fisheries & Wildlife, Special Scientific Report, Wildlife No. 127, 1969.
Shafik, M. T., Sullivan, H. C., Enos, H. F., *J. Environ. Anal. Chem.* **1**, 23 (1971a).
Shafik, M. T., Bradway, D., Enos, H. F., *J. Agr. Food Chem.*, **19**, 885 (1971b).
Stanley, C. W., *J. Agr. Food Chem.* **14**, 321 (1966).

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MULTIRESIDUE PROCEDURE FOR HALO- AND NITROPHENOLS

1. Pipet 5 mL urine into a 50-mL culture tube.

2. Add 1.25 mL conc HCl, cap tube securely, and place in a 59° C oven overnight.

3. Remove from oven and allow to cool.

4. Transfer contents to a 12- to 15-mL centrifuge tube.

5. Rinse the 50-mL culture tube with 3 mL of anhydrous ethyl ether and add to the centrifuge tube.

6. Mix contents vigorously on a Vortex mixer for 2 min; then centrifuge and transfer ethyl ether layer to a 12- to 15-mL centrifuge tube with a disposable pipet.

7. Repeat the extraction with an additional 3-mL volume of ethyl ether and add the second ethyl ether extract to the centrifuge tube.

8. Add diazoethane dropwise with a disposable pipet until a yellow color persists, then mix gently (1 mL).

9. Let the solution stand 15 min; then bubble clean dry nitrogen through the solution to remove excess reagent.

10. Concentrate the ethylated urine extract to ca. 0.3 mL, using the nitrogen evaporator.

11. Add 2 mL of hexane, and continue evaporating the ether-hexane solutions to 0.3 mL.

12. Prepare a silica gel column using 2 grams of silica gel (Woelm) that has been dried for 48 hr at 170° C and deactivated by adding 40 microliters of benzene-extracted distilled H_2O and allowed to equilibrate for 2 to 3 hr with periodic shaking. Top with 1.5 g of anhydrous Na_2SO_4.

13. Prewash the column with 10 mL of hexane and discard.

14. Transfer the concentrated urine extract quantitatively to the column using 2 mL of the 20 percent-benzene-in-hexane.

15. As soon as the solvent sinks into the sodium sulfate, add 8 mL of the 20-percent-benzene-in-hexane to the column, and collect the total volume of the 20-percent-benzene-in-hexane (10).

16. Continue elution with 10 mL of 40-percent-benzene-in-hexane, and discard this fraction.

17. Add 10 mL of 60-percent-benzene-in-hexane, followed by 10 mL of 80-percent-benzene-in-hexane, and collect these fractions in a single tube.

18. Add 10 mL of benzene to column, and collect eluate.

19. Concentrate fractions to 5 mL.

MULTIRESIDUE PROCEDURE FOR HALO- AND NITROPHENOLS AND HERBICIDES

Color Code--Orange

1. Pipet 5 mL of urine into a 15-mL screw-top centrifuge tube.

2. Add 1.25 mL of conc. HCl, cap tube securely, mix, and place in a 59° C oven overnight.

3. Remove from oven and allow to cool.

4. Add 3 mL of anhydrous ethyl ether to the centrifuge tube.

5. Mix contents vigorously on a Vortex mixer for 2 min, then centrifuge and transfer ethyl ether layer to a 12- to 15-mL centrifuge tube with a disposable pipet.

6. Repeat the extraction with an additional 3-mL volume of ethyl ether and add the second ethyl ether extract to the first.

7. Using the glove box, add 1 mL of diazoethane to the combined extracts.

8. Let the solution stand for 15 min, then remove from glove box and place tubes on the concentrator and bubble nitrogen through the solution for 10 min to remove the excess reagent.

9. Raise the needles above the surface of the wolution, and concentrate the extract to ca. 0.3 mL.

10. Add 2 mL of hexane, mix, and continue evaporating the ether-hexane solution to 0.3 mL.

11. Prepare a silica gel column using 2 grams of silica gel (Woelm) that has been dried for 48 hr at 170° C and deactivated by adding 40 microliters of benzene-extracted distilled water and allowed to equilibrate for a minimum of 2 hours with periodic shaking. Top with 1.5 g of anhydrous Na_2SO_4.

12. Prewash the column with 10 mL of hexane and discard.

13. Transfer the concentrated urine extract quantitatively to the column using 2 mL of the 20-percent-benzene-in-hexane.

14. As soon as the extract sinks into the sodium sulfate, add 8 mL of the 20-percent-benzene-in-hexane to the column, and collect the total volume of the 20-percent-benzene-in-hexane (10 mL).

15. Continue eluting with 10 mL of 40-percent-benzene-in-hexane, and discard this fraction.

16. Add 10 mL of 60-percent-benzene-in-hexane, followed by 10 mL of 80-percent-benzene-in-hexane, and collect these fractions in a single tube.

17. Add 10 mL of benzene to the column, and collect eluate.

18. Concentrate fractions to 5 mL.

TUBES REQUIRED FOR THIS PROCEDURE*

- 12 15-mL screw-top centrifuge tubes
- 12 12- to 15-mL glass-stoppered centrifuge tubes for extracts
- 3 rows of 12 12- to 15-mL glass-stoppered centrifuge tubes for fractions
- 12 25- to 50-mL centrifuge tubes for the combined eluate from step 16.

19. The 5.0-mL fractions are analyzed on GC equipped with tritium foil electron-capture detector, a glass U-shaped column, 6 ft × ¼ in. packed with 35 SE 30/4.5 QFL on 80/100 mesh Gas chrom Q. The gas chromatographic column was operated under the following parameters: nigrogen carrier gas flow rate 70 mL/min; column temperature 168°; inlet, 225°, detector, 210°; transfer line, 235°. Standards: 3,5,6-Tc Pyr, 30 pg/µL; 2,4,5-TCP, 30 Pg/µL; PNP, 100 pg/µL; silvex 40 pg/µL; 2,4,5-T, 50 pg/µL; 2,4-D, 200 pg/µL; dicamba, 25 pg/µL; PCP, 7 pg/µL.

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*Required when analyzing 10 samples, 1 blank, and 1 spike.

"Multiresidue Procedure for Halo- and Nitrophenols. Measurement of Exposure to Biodegradable Pesticides Yielding these Compounds as Metabolites," J. Agric. and Food Chemistry. 21(2):295-298. 1973.

APPENDIX D

GLOSSARY OF SELECTED TERMS

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duplicate: either of two things (e.g., specimens) that exactly resemble or correspond to each other.

endogenous-compound: a compound found naturally in specimens as opposed to compounds added for quality assurance purposes.

endogenous-compounds data: measurements reported for endogenous compounds.

external reference duplicate analysis: chemical analysis performed by an external reference laboratory on one of the duplicate specimens; used to compare chemical analysis results of the corresponding duplicate specimen obtained by the primary laboratory.

field blank: one specimen of a large homogenous matrix pool that was shipped to the collection site and handled identically to specimens collected from sample persons (subjects).

field control: either a field blank or field spike.

field duplicate: either of two specimens that were collected in an identical manner from sample persons.

field spike: a field blank fortified (spiked) with selected target compounds.

field split: one of two duplicate specimens obtained from a large matrix pool.

lab-split duplicate: one of two aliquots obtained in the laboratory by splitting specimens from sample persons.

spiked-split duplicates: a pair of duplicate specimens, one of which was fortified with selected target compounds, and the other of which was not.

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Specimens of serum and urine were selected as part of the Hispanic HANES Pilot Study. Statistically designed quality assurance protocols were used to permit estimation of the field procedures and chemical analysis quality. The quality of field procedures was assessed by the use of field QA specimens, both spiked and unspiked to estimate the levels of contamination and degradation. The quality of the chemical analysis was assessed using duplicates and split samples (spiked and unspiked) to estimate chemical analysis precision and bias. The results of the quality assessment of the analysis of volatiles in serum and semivolatiles in serum and urine are summarized.

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