

The Arizona Border Study

*An Extension of the
Arizona National Human Exposure Assessment Survey (NHEXAS) Study
Sponsored by the Environmental Health Workgroup of the Border XXI Program*

Quality Systems and Implementation Plan for Human Exposure Assessment

The University of Arizona
Tucson, Arizona 85721

Cooperative Agreement CR 824719

Standard Operating Procedure

RTI-Compendium

Title: Compendium of Method Summaries For Analysis of Trace Metals, Pesticides, and PAHs in Dietary Samples Using Total Diet Study Procedures

Source: The University of Arizona

U.S. Environmental Protection Agency
Office of Research and Development
Human Exposure & Atmospheric Sciences Division
Exposure & Dose Research Branch

Notice: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), partially funded and collaborated in the research described here. This protocol is part of the Quality Systems Implementation Plan (QSIP) that was reviewed by the EPA and approved for use in this demonstration/scoping study. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

RESEARCH TRIANGLE INSTITUTE



RTI/7196/08-03 QAPP

July 2, 1998

REVISED

QUALITY ASSURANCE PROJECT PLAN

ARIZONA BORDER STUDY - ANALYSIS OF FOOD SAMPLES

Analytical and Chemical Sciences
Research Triangle Institute
P.O. Box 12194
Research Triangle Park, NC 27709

Contract Number: 68-C5-0011
Work Assignment Number: 3-08

Prepared by:

J. M. Roberds
J. M. Roberds

K. W. Thomas
K. W. Thomas
Work Assignment Task Leader

R. Fernando
R. Fernando
Work Assignment Task Leader

Approved by:

E. D. Pellizzari
E. D. Pellizzari, Ph.D.
Program Manager and
Work Assignment Leader
Vice-President, Analytical
and Chemical Sciences

PREPARED FOR

Larry C. Butler, Work Assignment Manager
Angela Moore, Project Officer
Environmental Monitoring System Laboratory
U.S. Environmental Protection Agency
Cincinnati, OH 45268



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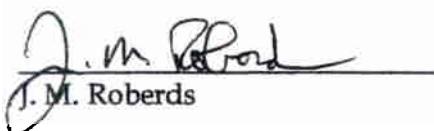
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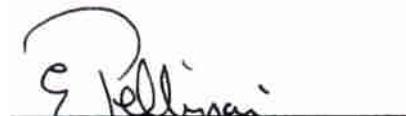

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Distribution List

E. Pellizzari
 K. Thomas
 R. Fernando
 M. Roberds
 J. Flanagan
 D. Smith
 T. Erexson

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SECTION 1.0

PROJECT DESCRIPTION

1.1 OVERVIEW

The Arizona Border Study is a pilot study to measure exposures to environmental contaminants for Arizona residents living near the U.S.- Mexican border. Exposure data from border residents will then be compared to exposure data for residents living in the remainder of the state. The Arizona Border Study will also act as a pilot study for other proposed exposure studies such as the Pesticides in Young Children study now in the planning stages. Part of the Arizona Border Study requires the analysis of food and beverage samples collected by study participants as duplicate diets. This work assignment applies to only the analysis of food and beverage samples collected during the study.

1.2 PURPOSE

The purpose of this work assignment is to analyze duplicate diet solid food samples collected for the Arizona Border Study for selected metals, pesticides, and polynuclear aromatic hydrocarbons (PAHs). A work plan describing this work has been previously prepared and submitted to EPA [1]. All samples will be processed and analyzed using procedures described in the National Environmental Research Laboratory (NERL) Manual of Analytical Methods for Determination of Selected Environmental Contaminants in Composite Food Samples [2] or recent modifications of those methods. Table 1 gives a complete listing of the target chemicals for testing.

1.3 STUDY DESIGN

1.3.1 Analytical Protocols

Sample processing and analysis are conducted using analytical protocols written for this work assignment. These protocols are versions of methods from the NERL food methods manual with recent modifications where applicable. A copy of each protocol is provided in Appendix A. The protocols and corresponding method are shown in Table 2.

TABLE 1. TARGET ANALYTES FOR FOOD SAMPLES

Metals			
Lead	Nickel	Manganese	Copper
Arsenic	Chromium	Selenium	Zinc
Cadmium	Barium	Vanadium	
Pesticides/Herbicides			
DDD, DDE, DDT	Lindane	Carbaryl	Acephate ^a
Chlordane	Chlorpyrifos	Propoxur	Methomyl
Heptachlor	Diazinon	cis-trans Permethrin	Pendimethalin ^a
Dieldrin	Malathion	Methyl parathion	Trifluralin
PAHs			
Naphthalene ^b	Anthracene	Benz[a]anthracene	Benzo[e]pyrene ^b
Acenaphthene	Retene ^b	Chrysene	Benzo[a]pyrene
Fluorene	Pyrene	Cyclopenta[c,d]pyrene ^b	Indeno[1,2,3-cd]pyrene
Phenanthrene	Fluoranthene	Benzofluoranthenes	Benzo[g,h,i]perylene

^a Pesticides that have not been evaluated using RTI/ACS-AP-211-132 (Method PAS-01).

^b PAHs that have not been evaluated using RTI/ACS-AP-211-136 (Method PAH-01).

TABLE 2. RTI/ACS ANALYTICAL PROTOCOLS FOR ARIZONA BORDER STUDY

Protocol	Title	Corresponding Method from NERL Food Methods Manual
RTI/ACS-AP-211-038	Sample Homogenization and Aliquot Preparation For Solid Food Samples	PH-00
RTI/ACS-AP-211-131	Determination of Base/Neutral Pesticides in Composite Food Samples	PAS-00
RTI/ACS-AP-211-132	Soxhlet Extraction of Base/Neutral Pesticides in Composite Food	PAS-01
RTI/ACS-AP-211-133	n-Hexane/Acetonitrile Partitioning of Base/Neutral Pesticides in Food Sample Extracts	PC-01
RTI/ACS-AP-211-134	Gel Permeation Chromatography of Pesticides and PAHs in Composite Food Samples	PC-02
RTI/ACS-AP-211-135	Analysis of Pesticides and PAHs From Extracts of Composited Food Samples by Gas Chromatography/Mass Spectrometry	PA-01
RTI/ACS-AP-211-136	Determination of Polynuclear Aromatic Hydrocarbons in Food Samples	PAH-01
RTI/ACS-AP-211-137	High Performance Liquid Chromatography (HPLC) Analysis of Carbamates	PA-02
RTI/ACS-AP-211-138	ICP/MS and GFAAS Analysis of Elements in Composite Food	M-00

1.3.2 Task 1: Metals

Homogenized food samples will be digested and analyzed using RTI/ACS-AP-211-138. Method M-00 originally used GFAA and ICP/AES for the analysis of sample digests. This method was modified to use low resolution ICP/MS for analysis instead of ICP-AES. Most of the metals listed in Table 1 should be amenable to this method. Selenium will be determined by GFAAS. Chloride may also cause interferences for vanadium analysis. The anticipated number of food samples to be digested and analyzed for metals is shown in Table 3.

1.3.3 Task 2: Pesticides

Homogenized food samples will be extracted, cleaned up, and analyzed for target pesticides using RTI/ACS-AP-211-131, 132, 133, 134, and 137 (Table 2). A schematic of the overall method is shown in Figure 1. This method has been modified to include an aqueous backwash step (pH 7) to remove residual fatty acids and water soluble coextractives from the sample extract.

The pesticide methods have not been evaluated for measuring acephate or pendimethalin. For these two pesticides, performance will be evaluated throughout the program using method controls and spiked matrix samples. During previous method evaluation work [3], a substantial HPLC interferent prevented the quantitation of methomyl in many samples. Since no work has been done or is proposed for identifying and removing this interferent, methomyl may not be determined depending upon the nature of the samples in this study.

The anticipated number of food, beverage and analysis QC samples to be processed and analyzed is shown in Table 3.

1.3.4 Task 3: PAHs

Homogenized food samples will be processed for PAHs using RTI/ACS-AP-211-133, and 136. GC/MS analysis of the PAHs will be performed using RTI/ACS-AP-211-135. The extraction procedure for PAHs is different than that used for pesticides so separate food samples aliquots will be used for PAH analysis. Although the GC/MS analysis procedure is the same for both pesticides and PAHs, the PAHs will not be analyzed in the extracts used for pesticide analysis. This method has not been evaluated for a subset of the target PAHs as designated on Table 1. It is anticipated that these additional target PAHs will be amenable to this method. Performance of the additional PAHs will be evaluated throughout the program.

TABLE 3. FOOD AND QC SAMPLES FOR ANALYSIS

Analytes	Field Samples	QC Samples				Total
		SRMs	Duplicate Aliquots	Spiked Matrix		
Metals	100	4	5	10	119	
Base/Neutral and Carbamate Pesticides	100	4	5	5	114	
PAHs	100	4	5	5	114	

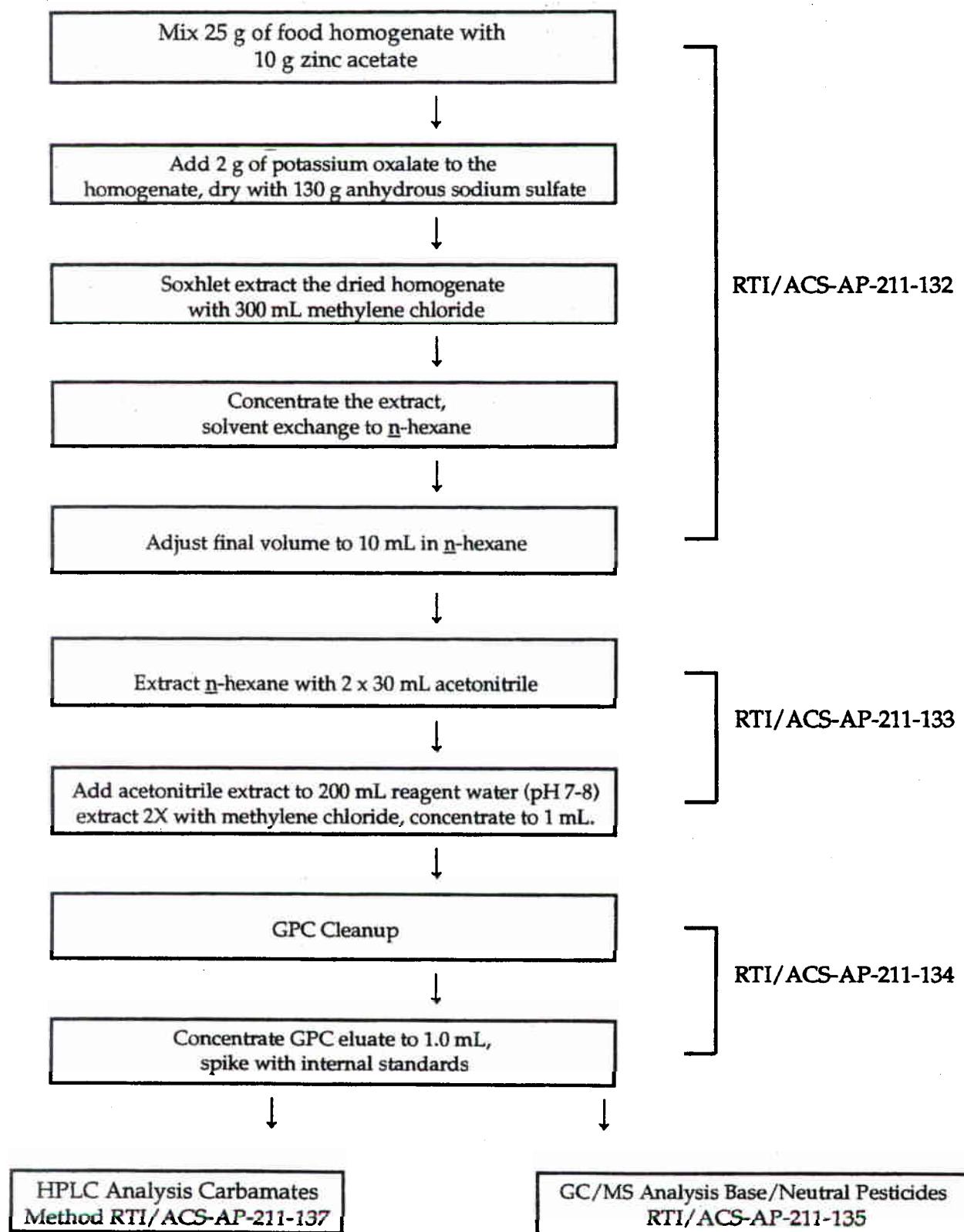


Figure 1. Flow Diagram of RTI/ACS-AP-211-131.

using method controls and spiked matrix samples. It is anticipated that naphthalene may show poor performance due to significant volatility losses. Similarly, cyclopenta[c,d]pyrene is a relatively unstable PAH which may show poor performance due to degradation during processing. Finally, it is often difficult to separate benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene during GC/MS analysis. We propose to analyze these three compounds together as benzofluoranthenes.

1.3.5 Task 4. Sample Homogenization and Archival

Samples will be one-day duplicate diet solid food samples collected as part of the Arizona Border Study provided by the University of Arizona. Once received at RTI, samples will be inspected and logged in, noting any breakage, spoilage, or other problems. A chain-of-custody record will be completed for each sample as it is logged in. Samples will be stored at -20°C until homogenization. Samples will be accumulated until a sufficient number have been received to begin the homogenization and analysis procedures.

Prior to testing, the solid food samples will be homogenized until uniform consistency is achieved. The samples will be homogenized according to RTI/ACS-AP-211-038. This method is a modified version of Method PH-00 in the NERL food methods manual [1] and will be used so that the homogenization procedure follows as closely as possible the FDA procedure in previous studies. In this modified procedure, a Robot Coupe 6-L scientific food processor will be used instead of the blender. No water will be added to the solid foods to aid homogenization when this food processor is used. In addition, Method PH-00 calls for adding methylene chloride to homogenized sample aliquots to inhibit enzymatic degradation of some analytes. However, this procedure was not used by FDA for NHEXAS samples and will not be used in this study. A copy of the protocol is provided in Appendix A. Homogenized samples will be stored protected from light at -20°C. Samples for metals analysis may be stored up to 6 months prior to digestion and analysis. Samples for pesticides and PAHs will be extracted within 3 months after homogenization.

Aliquots of each homogenized sample will be prepared immediately after homogenization is performed. Aliquots will be prepared for metals analysis, pesticide analysis, PAH analysis, and for archival. The proposed sample aliquot preparation scheme is presented in Table 4.

TABLE 4. SOLID FOOD HOMOGENATE ALIQUOT PREPARATION SCHEME

Preparation Order	Analytes ^a	Nominal Aliquot Weight (g)	Container Type	Tracking Code Suffix ^b	To Be Used For
1	Pesticides	50	120 mL Glass Jar	XXXXXX1	Analysis
2	PAHs	50	120 mL Glass Jar	XXXXXX2	Analysis
3	Metals	25	50 mL Plastic Tube	XXXXXX3	Analysis
4	Pesticides	50	120 mL Glass Jar	XXXXXX4	Backup
5	PAHs	50	120 mL Glass Jar	XXXXXX5	Backup
6	Pesticides/PAHs	50	120 mL Glass Jar	XXXXXX6	Archive ^a
7	Metals	25	50 mL Plastic Tube	XXXXXX7	Archive ^a
8	Pesticides/PAHs	50	120 mL Glass Jar	XXXXXX8	Archive ^a
9	Metals	25	50 mL Plastic Tube	XXXXXX9	Archive ^a

^aThere may not be sufficient mass in all samples to prepare all archival samples.

^bThese are proposed tracking codes where XXXXXX is the same as the original sample code.

SECTION 2.0

PROJECT ORGANIZATION

The project organization is outlined in Figure 2. Dr. Edo Pellizzari will serve as Work Assignment Leader and will directly supervise all efforts on this work assignment. He will have overall responsibility for all technical, fiscal, and scheduling aspects of the proposed work. He will also serve as the major point-of-contact with the EPA Work Assignment Manager. In addition, Dr. Pellizzari will ensure that high technical quality is obtained, that all tasks adhere to the schedule, and that work is performed within budget. He will be assisted by task leaders who will be responsible for specific technical activities. Each task leader will be responsible for the technical effort within his/her area. Specific duties include supervising all assigned work in preparation of data reports. Overall review of the activities on the Work Assignment will be the responsibility of the Project Manager, Dr. E. Pellizzari.

The QA Office will be staffed by the QA Officer, Dr. James Flanagan, and the QA Coordinator, Ms. Doris Smith. The QA Officer is administratively independent of the personnel proposed for this study. The QA Officer is responsible for all independent QA review of study data. The QA Coordinator is responsible for the following:

- Conducting periodic audits of data collection and measurement systems (Section 10.0),
- Monitoring situations requiring corrective action (Section 13.0),
- Submitting required QA reports (Section 14.0).

Any deficiencies in the execution of the Quality Assurance Project Plan identified by the QA Officer will be communicated to the Work Assignment Leader for immediate remedial action.

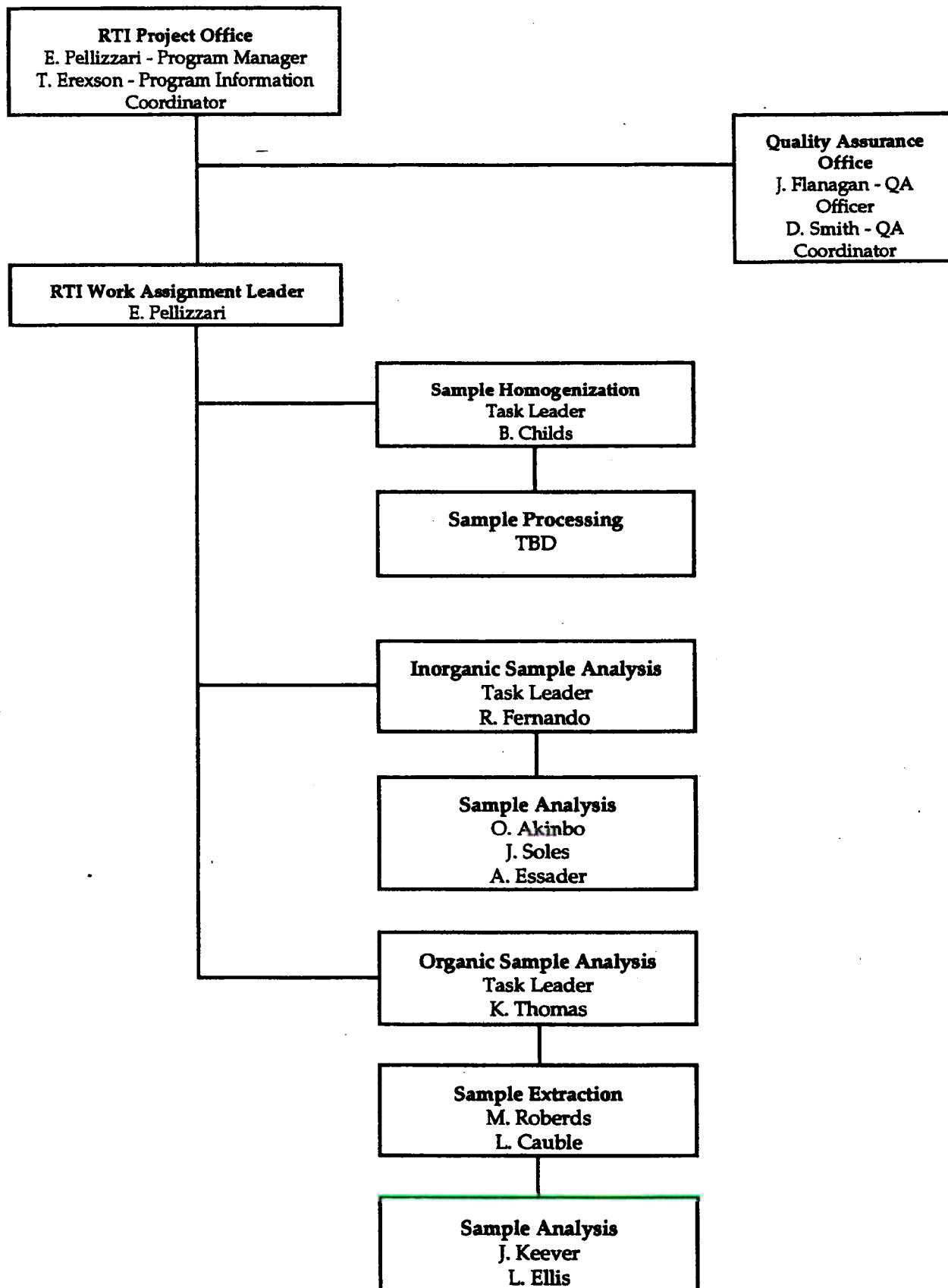


Figure 2. Project Organization.

SECTION 3.0

QUALITY ASSURANCE GOALS

3.1 ORGANICS

Method PA-00 and PAH-01 in the NERL food methods manual [1] defines performance criteria established for methods developed as part of EPA's dietary research program. These criteria were intended to account for the need to generate useful data for EPA's dietary exposure studies while considering technical feasibility and overall method cost. Methods for the determination of pesticides and PAHs in food samples must meet similar criteria as follows.

- The methods should be applicable to the determination of pesticides and PAHs in composite food samples.
- The methods should provide concentration data that are both accurate and precise. Acceptable recovery levels for laboratory fortified matrices and surrogate standards as defined values between 60 and 120% of the real value. Precision of 30% relative standard deviation is considered acceptable and achievable for the analysis of duplicate samples.
- The methods should minimize interferences and should provide sufficient selectivity to prevent misidentification of chemicals (i.e., reduce false positive or false negative errors). This requirement is especially important in a complex matrix such as food.
- Finally, the methods should be sufficiently sensitive to detect pesticides and PAHs in composite food samples collected from the general population. A detection limit (DL) of 2 to 5 µg/kg for pesticides and 0.2 to 1 µg/kg for PAHs is targeted as technically feasible.

The Quality Assurance Goals given in Table 5 are based on these performance criteria. During testing, experimental results will be compared against these criteria.

TABLE 5. QUALITY ASSURANCE GOALS

Parameter	Goal (Organics)	Goal (Inorganics)
Precision	< 30% RSD	<30% RSD
Accuracy	60 - 120% recovery	80-120%
Detection Limit	2-5 µg/kg (Pesticides) ^a 0.2-1.0 µg/kg (PAHs) ^a	See Table 6
% Completion	> 95%	> 95%

^aAnalytical detection limits equivalent to the shown concentrations in foods.

3.2 INORGANICS

The method for the determination of metals in food samples must meet the following criteria.

- The method should be applicable to the determination of metals in composite food samples.
- The method should provide concentration data for all analytes that are accurate and precise. Acceptable recovery levels (accuracy) for laboratory fortified matrices and surrogate standards are defined as between 80-120% of the true value. Precision, defined as % relative standard deviation, of 30% for the analysis of duplicate samples is considered acceptable.
- The method should minimize interferences and should provide sufficient selectivity to prevent misidentification of elements, false positive and false negative values. This requirement is especially important in a complex matrix such as food.
- The method should be sufficiently sensitive to determine metals in composite food samples collected from the general population. A method detection limit of 20-60 µg element/kg food is targeted.

The Quality Assurance Goals are summarized in Table 5 and Table 6. Detection limit goals in Table 6 are those for actual food samples and are based, in part, on the concentrations actually found in composite food samples. In RTI/ACS-AP-211-138 (Appendix A) the protocol Table 6 defines acceptance criteria for a quality control sample used to assess instrument

TABLE 6. GOALS FOR ELEMENTAL DETECTION LIMITS^a

Element	Detection Limit ($\mu\text{g/kg}$)
Al	62
As	6
Ba	2
Cd	2
Cr	250
Cu	19
Pb	19
Mn	6
Ni	19
Se	9 ^b
V	187
Zn	2875

^a Determined by RTI under EPA Cooperative Agreement CR 822070-01-D, "Evaluating Food Contamination in Scenarios in Dietary Exposure Studies - Child Dietary Lead Study."

^b Analyzed by GFAAS.

performance. The difference between concentrations for food detection limits and those in the quality control sample are the absence of any food matrix in the QC sample and the higher levels of some elements in real food samples.

3.3 ANALYSIS, ACCURACY AND PRECISION

Ambient precision will be expressed as the percent relative standard deviation (% RSD) between duplicate aliquots, since actual duplicate samples will not be collected from the study participants. The %RSD will be calculated using the following equation:

$$\% \text{ RSD} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100 \quad (1)$$

Accuracy of the method will be determined by using fortified reagent blanks, fortified sample matrices and analysis of Standard Reference Materials (SRM) or Certified Reference Material (CRM).

For spiked matrix samples the accuracy will be expressed as

$$\% \text{ Recovery} = \frac{C_m - C_u}{C_s} \times 100 \quad (2)$$

where C_m , C_u , and C_s are the concentration of each target analyte measured in the spiked sample, in the unspiked sample, and the spiked concentration, respectively.

For SRM or CRM samples, accuracy will be calculated as

$$\% \text{ Recovery} = \frac{C_m}{C_c} \times 100 \quad (3)$$

where C_m is the concentration of each target analyte measured in the Reference Material and C_c is the certified target analyte concentration.

RTI has three portions of NIST SRM 1548a (Typical Diet). These will be analyzed for elements; however, the certification status for these elements is unknown. NIST has also recommended that SRM 1548a be used for pesticides; however, a list of pesticides in this material has not been provided and may or may not include target analytes for this study. Since SRM 1548a is not currently available for purchase from NIST, EPA would need to obtain

from NIST a list of organic components and their certified values. If target analytes are present in the appropriate concentrations, RTI will analyze portions of SRM 1548a for target pesticides if at least three portions are provided by EPA or by NIST. Additionally, SRM 1491 and SRM 1492 will be realized for PAHs and pesticides, respectively.

3.4 COMPLETENESS

Completeness of study requirements with regard to sample analysis will be determined by comparison of analysis scheduled as specified in the study design to analyses acceptably completed. A high completion rate is expected ($\geq 95\%$) since laboratory-prepared homogenates will be utilized, with additional sample available for reanalysis, if needed.

3.5 REPRESENTATIVENESS

This study is being conducted to analyze duplicate diet samples for the Arizona Border Study for metals, pesticides, and PAHs. These results will be used to measure dietary exposure to environmental contaminants for a sample of Arizona residents along the U.S.-Mexican Border. Every effort is made to ensure that each analytical sample is representative of the dietary sample provided by the University of Arizona by using techniques such as thoroughly mixing to obtain homogeneity and the use of duplicate analysis of sample aliquots.

SECTION 4.0

SAMPLING PROCEDURES

4.1 SAMPLES

Sample collection is not included in this work assignment. The samples are shipped frozen overnight in insulated shippers. One-day duplicate diet food samples from Arizona participants will be received frozen in insulated shippers from the University of Arizona. The samples will be stored at -20°C immediately upon arrival. Once received, the samples will be inspected and logged in, noting any breakage, spoilage, or other problems. Any information on lost or compromised samples and documents will be communicated to the Work Assignment Leader. A chain-of-custody record will be completed for each sample as it is logged in and each sample identification label will be verified. An example of the chain-of-custody record is shown in Figure 3. Samples will be stored at -20°C until homogenization. The storage freezer(s) will be continuously monitored and temperatures will be electronically recorded at 3-h intervals. Freezers containing these samples will be connected to a backup generator system that will start automatically in the event of a power outage.

4.2 SAMPLE HOMOGENIZATION

Prior to extraction, the solid food samples will be homogenized until uniform consistency is achieved. The samples will be homogenized according to RTI/ACS-AP-209-038.

Aliquots of each homogenized sample will be prepared immediately after homogenization is performed. Aliquots will be prepared for metals analysis, pesticide analysis, PAHs analysis, and for archival. Unless the aliquots will be immediately processed for analysis, they will be stored at -20°C. The proposed sample aliquot preparation scheme is presented in Table 4.

4.3 SAMPLE MANAGEMENT

Food sample homogenates will be labeled with unique codes for sample identification. These aliquot codes will be provided by the University of Arizona. The aliquot codes will be placed on all sample aliquots and associated documents and will be used for data reporting. In addition to the aliquot codes, RTI will generate a tracking code for each sample homogenate.

Food Collection Data Sheet and Chain of Custody Record
Health & Environment Studies

HHID: 515245

/A

Participating Respondents First Name: NA

IRN: NA

Sample Collection Date: 5 / 21 / 98
 MO DAY YEAR

Solid Food Sample ID: 1T12142 OR Liquid Food Sample ID: NA

Sample Collected by Field Team on: 5 / 21 / 98 T. Leader: _____
 MO DAY YEAR

Sample Refrigerated by Respondent:

Yes or No

Sample Compared to diet recorded in 24 Hour Food Diary:

Yes or No

Sample Compared to diet recorded in 4 Day Diet Diary:

Yes or No

Comments: LAB BLANK

FOOD SAMPLE ALIQUOT IDENTIFICATION

Comments

Metals Aliquot ID:	<u>3623291</u>
Pesticide Aliquot ID:	<u>3723917</u>
PAH Aliquot ID:	<u>4313797</u>
Return Aliquot ID:	

Collection Comments

Laboratory Comments

Custody Record

Name

Mo\Day\Year

Operation Performed

Kira Joalin

5 121 1998

To Lab → Ship
Received at RTI

Banta Childs

5 122 1998

1

1

1

1

1

1

1

1

Figure 3. Example Chain-of-Custody Record.

The tracking code will be made up of the original sample code plus the last digit which represents the homogenate number. The homogenate tracking code scheme is shown in **Table 4.**

SECTION 5.0

SAMPLE CUSTODY

Sample custody procedures will be used to track samples and sub-samples generated during this work assignment. The overall responsibility for sample and document custody will be the sample homogenization task leader. Custody of sample aliquots taken for extraction and analysis will be tracked using a batch custody record form and chain-of-custody records. An example of a batch custody record form is shown in Figure 4. The batch custody form will assist in tracking samples and will include important processing information such as sample amount taken for extraction, spiking, extraction, cleanup, and analysis procedures and dates, final extract volume, and amounts of internal standards added. Detailed information regarding sample extraction, cleanup, and standards preparation will be recorded in RTI Laboratory Notebooks. During sample processing, the laboratory chemists is responsible for sample custody. When a batch of sample extracts are submitted to the analytical lab for analysis, the analyst is responsible for sample custody.

The batch custody records are kept in the analytical laboratory until the data has been electronically transferred from the analytical data system into a computer spreadsheet and final sample concentrations are calculated. Upon complete review of the data, the chain-of-custody and batch records will be returned to the Sample Homogenization Leader. Individual task leaders will be responsible for making sure that ~~sample tracking procedures are followed~~.

GC/MS Analysis

Matrix:

Batch No.:

Instrument

Operator

Size Equivalent (g)

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1

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Figure 4. Batch Custody Record

SECTION 6.0

ANALYTICAL PROCEDURES

6.1 ORGANIC ANALYSIS

6.1.1 Sample Extraction

Samples for pesticide analysis will be extracted according to RTI/ACS-AP-211-132, a schematic is shown in Figure 5. In summary, a 25 g aliquot of a composited, homogenized food sample is first mixed with zinc acetate to minimize fatty acid interferences then mixed with anhydrous sodium sulfate to remove water. The sample is Soxhlet extracted with methylene chloride overnight. The extract is solvent exchanged into n-hexane and concentrated to 10 mL for subsequent cleanup.

Samples for PAHs analysis will be extracted according to RTI/ACS-AP-211-136, a schematic of this method is shown in Figure 6. A 25 g homogenized food sample is digested with an ethanolic potassium hydroxide solution for 2 -3 hours, the PAHs are then partitioned from the alkaline digest by shaking into n-hexane. The resulting n-hexane extract is concentrated to 1 mL and cleaned up using solid phase extractions (SPE) silica gel columns.

6.1.2 Sample Extract Cleanup

6.1.2.1 Solvent Partitioning

After the initial extraction procedure, cleanup steps are necessary to remove interferences before further clean-up and analysis. Pesticide extracts are first cleaned up by solvent partitioning according to RTI/ACS-AP-211-133. This procedure separates fats from pesticides in the food extracts. The food extract in n-hexane is partitioned with acetonitrile, then further extracted with methylene chloride. The pesticides are partitioned into the methylene chloride while the fat remains in the n-hexane solvent. The extract is then concentrated for further cleanup. The final sample extract is in acetonitrile.

6.1.2.2 Gel-Permeation Chromatography

Prior to analysis by GC/MS the food extracts for pesticides and PAHs are cleanup by gel permeation chromatography according to RTI/ACS-AP-211-133. Food extracts containing

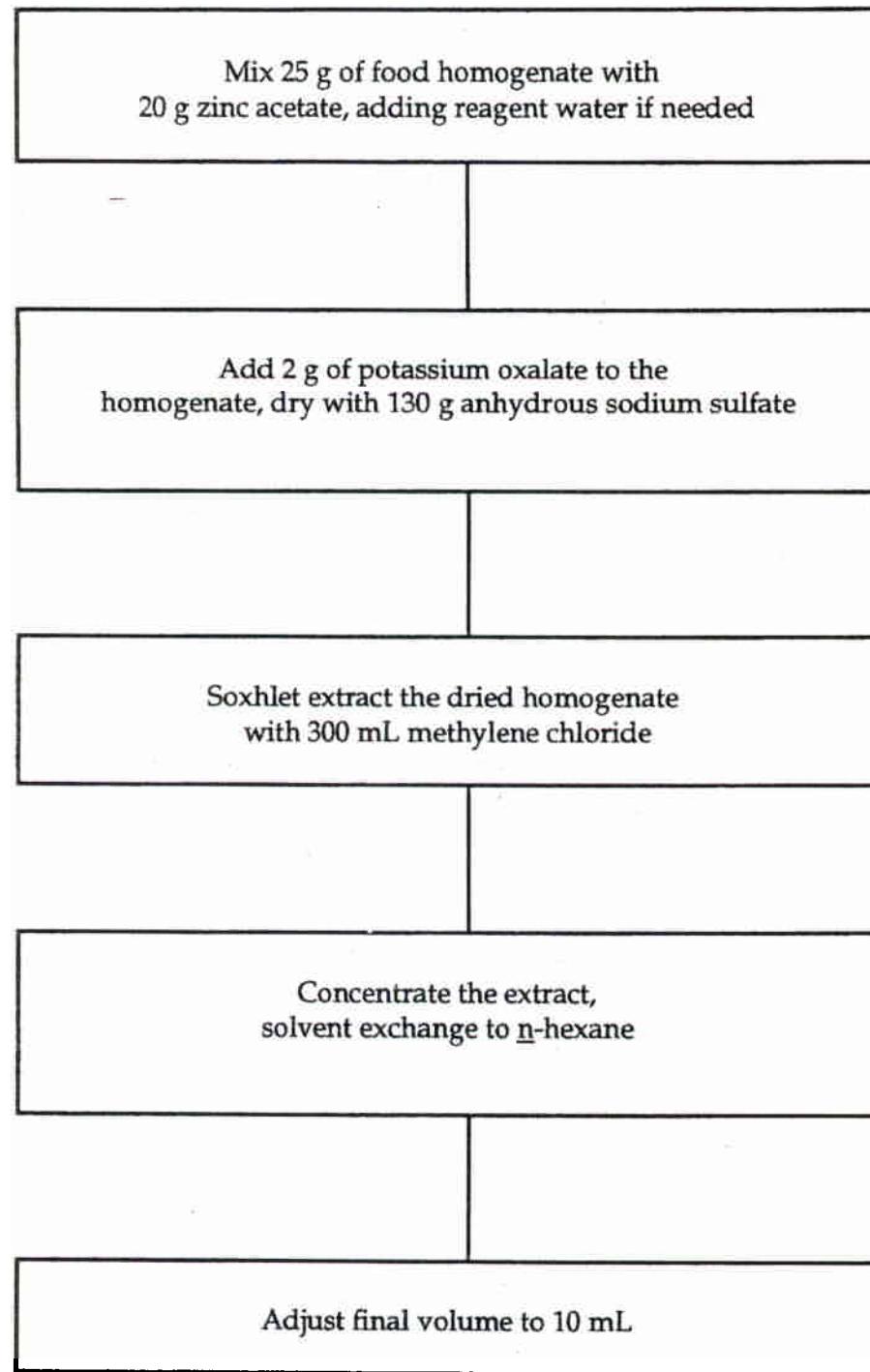


Figure 5. Flow Diagram of Pesticide Extraction by RTI/ACS-AP-211-132.

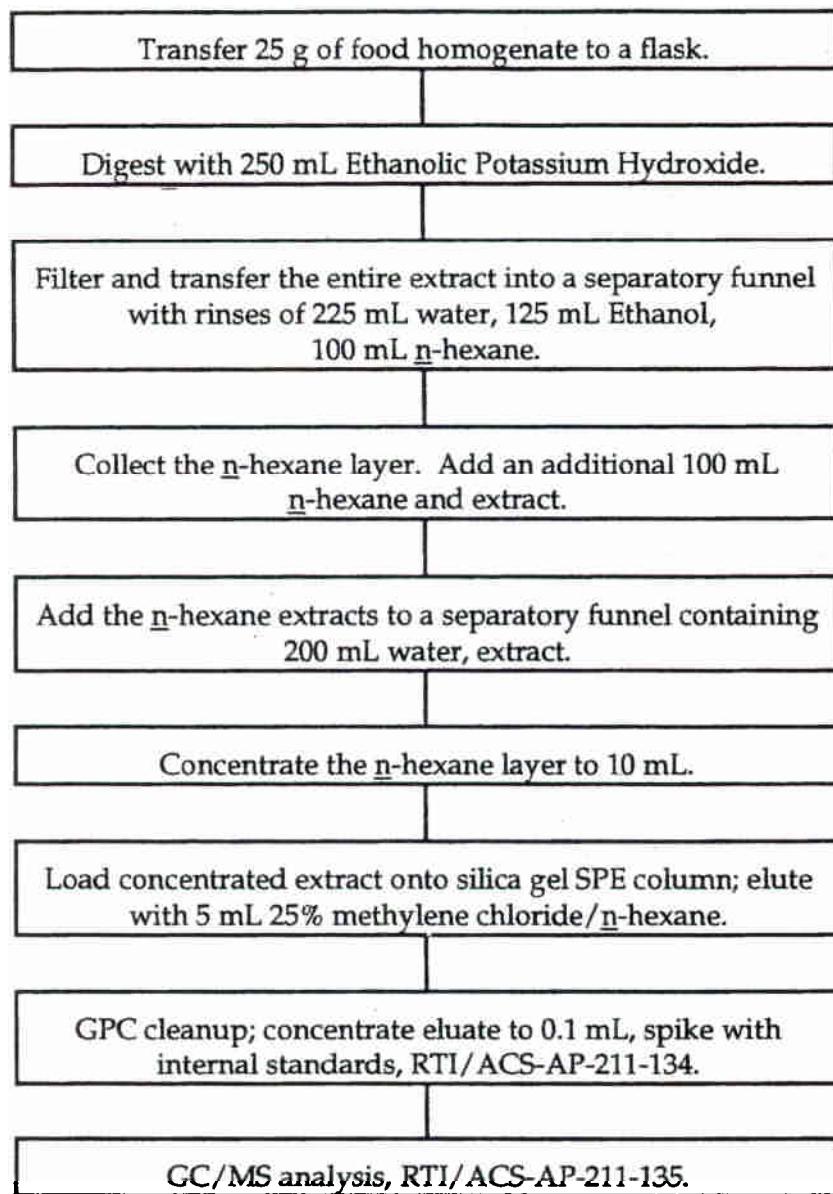


Figure 6. Flow Diagram of PAH Extraction by RTI/ACS-AP-211-136.

less than 0.5 g fat are injected onto a high performance liquid chromatographic column which separates the higher molecular weight coextractives from the lower molecular weight analytes using size exclusion chromatography.

6.1.3 Gas Chromatography/Mass Spectrometry Analysis

Base/neutral pesticides and PAHs will be measured in food extracts using the GC/MS procedures described in RTI/ACS-AP-211-135.

GC/MS analysis conditions for the GC/MS analysis are described in Table 7. Under current plans the analyses will be performed using a HP 5890 gas chromatograph and HP5988A mass spectrometer. However, a different laboratory or set of instruments may be selected. In any case, the GC/MS conditions must be suitable for analyzing the sample extracts.

6.1.4 High Performance Liquid Chromatography Analysis

Carbamate pesticides will be measured in food extracts using HPLC with post-column derivatization as described in RTI/ACS-AP-211-137. Proposed HPLC analysis conditions are described in Table 8. They may be modified as required to chromatographically separate and determine the target analytes in complex food matrices.

6.2 INORGANIC ANALYSIS

6.2.1 Sample Digestion

Samples for metals analysis will be digested according to RTI/ACS-AP-211-138. A schematic of this method is shown in Figure 7. A 4 g aliquot is decomposed with ultrahigh purity nitric acid using atmospheric pressure microwave digestion. Microwave heating is continued until a clear solution is obtained. Samples are allowed to cool, and the undigested fat is permitted to separate. Filtration is performed as needed. The sample solution is diluted to a final volume with internal standards added for analysis by ICP-MS or GFAAS (for selenium).

6.2.2 Inductively Coupled Plasma-Mass Spectrometry Analysis

All metal analytes except selenium will be determined by ICP-MS according to the procedures described in RTI/ACS-AP-211-138. The ICP-MS operating conditions are shown in Table 9.

TABLE 7. OPERATING PARAMETERS FOR GC/MS ANALYSIS

Parameter	Setting
GAS CHROMATOGRAPH	
Column	30M x 0.32 mm i.d. fused silica capillary column (0.5 µm film thickness)
Temperature Program	60°C to 130°C @ 15°C/min 130°C to 300°C at 4.5°C/5 min
Carrier Gas	helium
Capillary Injector	1 min splitless
Injection Volume	1.0 µL
Injector Temperature	240°C - 300°C
MASS SPECTROMETER	
Ionization Mode	Electron Ionization Selected Ion Monitoring
Emission Current	0.3 mA
Source Temperature	200°C
Electron Multiplier	1600 to 3000 volts

TABLE 8. HPLC OPERATING CONDITIONS FOR THE ANALYSIS OF CARBAMATES

Parameter	Setting
LIQUID CHROMATOGRAPH	
Column	Zorbax-C8, 250 mm x 4.6 mm i.d.
Mobile phases	A = 95% water/5% acetonitrile B = 30% water/70% acetonitrile
Gradient program	80% A:20% B (10 min) to 100% B in 25 min, then hold 16 min
Solvent flow rate	1.5 ml/min
Injection size	20 µL
Post column hydroxide reagent flow	0.5 ml/min
Post column OPA derivatization solution flow	0.5 ml/min
FLUORESCENCE DETECTOR	
Excitation wavelength	336 nm
Emission energy	455 nm

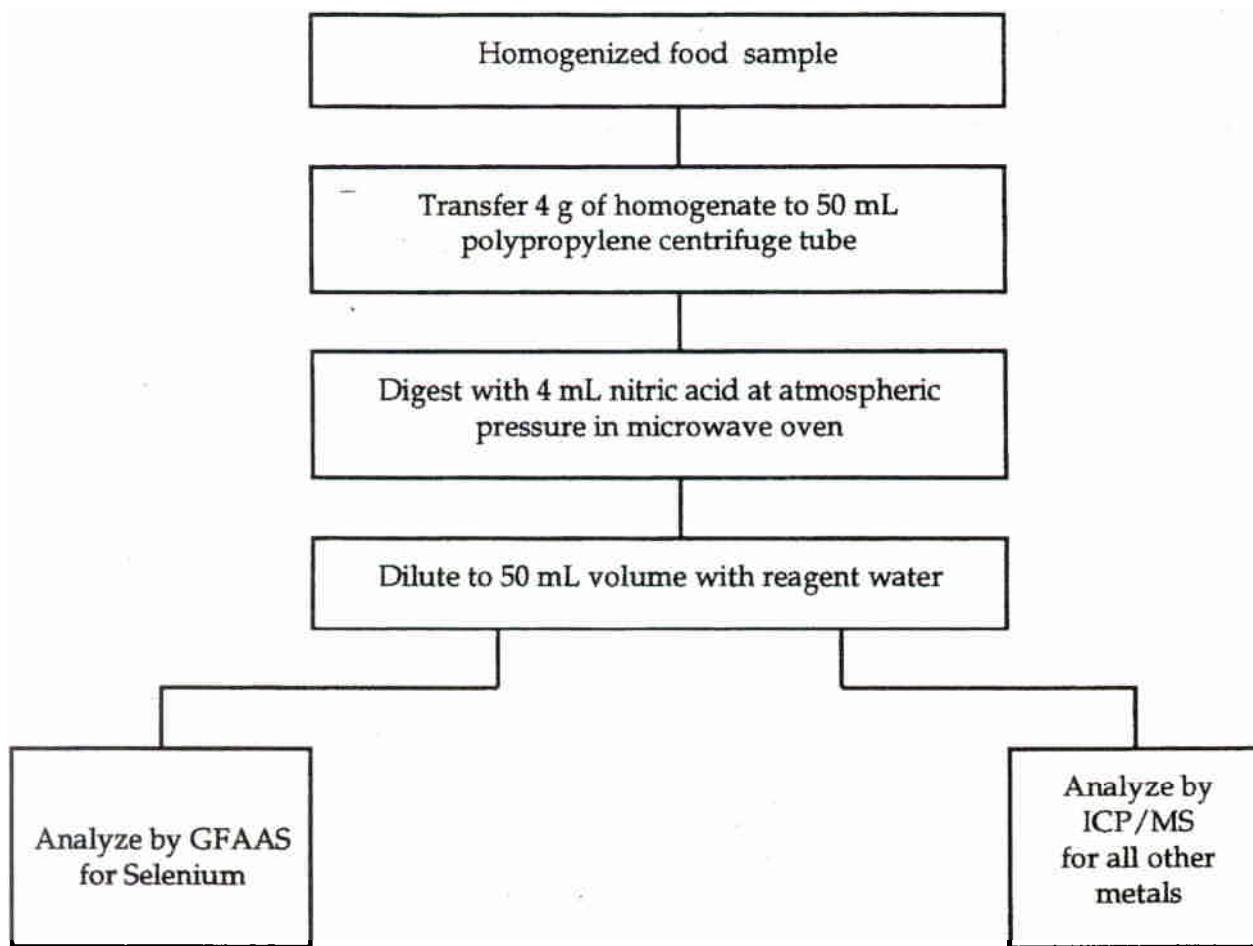


Figure 7. Flow Diagram for Analysis of Elements by Protocol (RTI/ACS-AP-211-138).

TABLE 9. ICP-MS INSTRUMENT OPERATING CONDITIONS

Instrument Parameter	Value
Instrument	Fisons PQXR ICP-MS
Forward RF power	1300-1350 watts
Reflected RF power	<= 2 watts
Argon flow rates:	
Nebulizer	0.80 L min ⁻¹
Coolant	13.75 L min ⁻¹
Auxiliary	0.9 L min ⁻¹
Sample uptake rate	1.0-2.0 mL min ⁻¹
Nebulizer type	Meinhard TR3-A30 glass nebulizer
Spray chamber	Water-cooled at 5°C
Vacuum system	
Expansion chamber P	2 mbar
Intermediate P	1.0 × 10 ⁻⁴ mbar
Analyzer P	2.0 × 10 ⁻⁶ mbar
Ion lens voltages	Optimized with ¹¹³ In
Detector	Electron multiplier
Mode	Peak jump
Dwell time	25 msec
Points/peak	3
DAC step	5
Acquisition time	60 sec
Resolution	0.7-0.8 amu

6.2.3 Graphite Furnace Atomic Absorption Spectrometry Analysis

Selenium will be determined by GFAAS using the procedures described in RTI/ACS-AP-211-138. The GFAAS operating conditions are shown in Table 10.

TABLE 10. GFAAS OPERATING CONDITIONS

Element	Selenium (Se)
Instrument	Perkin-Elmer ZL5100
Furnace Type	THGA with Zeeman
Wavelength	196.0 nm
Slit Width	2.00 nm, low
Source	Electrodeless discharge lamp (EDL)
Signal Measurement	Peak area
Read time	5.0 seconds
Matrix Modifiers	5 µg Pd + 3 µg Mg(NO ₃) ₂
Furnace Program	

Step	Temperature (°C)	Ramp (seconds)	Hold (seconds)
1	100	1	10
2	130	5	50
3	1100	10	20
4	2100	0	5
5	2500	1	4

SECTION 7.0

CALIBRATION PROCEDURES

7.1 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Quantitation of the target analytes for pesticides and PAHs is accomplished with an internal standard using peak areas from extracted ion current profiles. It is anticipated that the analysis for base/neutral pesticides and PAHs will be done with individual calibrations. For calibrations that will be applied across multiple run days, the calibration standards will be prepared at a minimum of six concentration levels (pesticides: 10-500 pg/ μ L; PAHs 5-500 pg/ μ L). For calibrations to be run and applied on a single analysis day (see Section 9.2) calibration standards will be prepared at a minimum of four concentration levels (pesticides 25-500 pg/ μ L; PAHs 10-500 pg/ μ L. One of the calibration standards should be at a concentration near, but above, the method detection limit; the others should bracket the expected range of concentrations (within the working range of the GC/MS system). Actual calibration levels may be adjusted based on the sensitivity of the instrument used for analysis. Each calibration standard will contain all target analytes, internal standards, and surrogates.

The method of internal standards will be used for quantitation. Relative response factors will be determined for each analyte relative to the closest chromatographically eluting internal standard.

Relative response factors (RF) are calculated for each analyte and surrogate relative to the **internal standard** using the following equation:

$$RF = \frac{(A_x)(Q_s)}{(A_{is})(Q_x)} \quad (4)$$

where:

A_x = integrated peak area of the quantitation ion of the analyte.

A_{is} = integrated peak area of the quantitation ion of the internal standard.

Q_x = concentration (pg/ μ L) of the analyte in the calibration standard.

Q_{is} = concentration (pg/ μ L) of internal standard in the calibration standard.

For each analyte and surrogate, the mean RF is calculated from the analysis of the calibration standards. If average RFs are used for quantitation, the relative standard deviation for average

RFs must be $\leq 25\%$. As an alternative to calculating RFs, a linear or second order regression calibration curve may be generated. Acceptable regression curves must have r^2 values greater than 0.98 and calculated concentrations for each calibration standard within $\pm 25\%$ of prepared concentrations.

The concentration of analytes and surrogates in food sample extracts or in solvent standards is calculated from the equation:

$$C_x = \frac{(A_x)(Q_{is})}{(A_{is})(RF)} \quad (5)$$

where:

- C_x = concentration of analyte or surrogate in pg/ μ L in the food extract.
 A_x = integrated peak area of the quantitation ion of target analyte.
 A_{is} = integrated peak area of the quantitation ion of the internal standard.
 Q_{is} = concentration of the internal standard in pg/ μ L.
RF = daily response factor of the analyte from the continuing calibration check standard or the mean response factor from the calibration curve.

The concentration of an analyte in food samples, C_f (ng/g) is calculated as:

$$C_f = \frac{C_x \cdot E_v}{1000 \cdot SW} \quad (6)$$

where:

- C_f = food concentration (ng/g)
 C_x = extraction concentration (pg/ μ L) as calculated in Equation 5
 E_v = extract volume (μ L)
SW = weight of sample aliquot extracted for analysis (g)

7.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Quantitation of the target analytes is accomplished with an internal standard using peak areas from sample analysis chromatograms. It is anticipated that the analysis for carbamates will be done with individual calibrations. Calibration standards will be prepared at a minimum of five concentration levels (10 to 2000 pg/ μ L) for each analyte. One of the

calibration standards should be at a concentration near, but above, the method detection limit; the others should bracket the expected range of concentrations (within the working range of the LC/MS system). Actual calibration levels may be adjusted based on the sensitivity of the instrument used for analysis. Each calibration standard will contain all target analytes and a internal standard.

The method of internal standards will be used for quantitation. Relative response factors will be determined for each analyte relative to the internal standard. Relative response factors (RF) are calculated for each analyte using the following equation.

$$RF = \frac{(A_x)(C_{is})}{(A_{is})(C_x)} \quad (7)$$

where:

- A_x = Area response for the analyte to be measured.
 A_{is} = Area response for the internal standard.
 C_{is} = Concentration of the internal standard (pg/ μ L).
 C_x = Concentration of the analyte to be measured (pg/ μ L).

If the RF value over the working range is constant (25% RSD or less), the average RF can be used for calculations. Alternatively, the individual analyte RF results can be used to plot a linear calibration curve.

The concentration of analytes and surrogates in food sample extracts or in solvent standards is calculated from the equation:

$$C_x = \frac{(A_x)(C_{is})}{(A_{is})(RF)} \quad (8)$$

where:

- C_x = Concentration of analyte or surrogate in pg/ μ L in the food extract.
 A_x = Integrated peak area of the quantitation ion of target analyte.
 A_{is} = Integrated peak area of the quantitation ion of the internal standard.
 C_{is} = Concentration of the internal standard in pg/ μ L.

RF = Daily response factor of the analyte from the continuing calibration check standard or the mean response factor from the calibration curve.

The concentration of analyte in food samples, C_f (ng/g) is calculated as:

$$C_f = \frac{C_x \cdot E_v}{1000 \cdot SW} \quad (9)$$

where:

C_f = food concentration (ng/g)

C_x = extraction concentration (pg/ μ L) as calculated in Equation 8

E_v = extract volume (μ L)

SW = weight of sample aliquot extracted for analysis

7.3 INDUCTIVELY COUPLED PLASMA/MASS SPECTROMETRY

Quantitation of all metals except selenium will be accomplished using calibration curves generated from the analysis of multiple point, multi-element calibration standard solutions. Calibration solutions and calibration check standards will be prepared from commercially available multi-element stock standards that are NIST traceable. Stock solutions for calibration and calibration check standards will be obtained from different vendors.

Calibration curves will be prepared from a minimum of five (5) standards and a calibration blank using an acid matrix similar to the sample digests. Internal standards will be added to all calibration standards, calibration check samples, blanks and sample digests. Internal standard elements to be added are Y, In, and Bi at a final concentration of 10 ng/mL each. Metal concentrations in the calibration will range from 0.05 to 100 ng/mL. The concentrations in the standards were selected to range from the instrumental quantitation limit to the highest expected digest concentrations.

Rinse times between samples/standards will be checked by analyzing a calibration or other blank solutions immediately following aspiration of the highest standard. The measured analyte concentration in the blank should not exceed a value three times the expected instrument detection limit (IDL). If the blank measurement exceeds this value the rinse time will be increased.

For each standard solution, the ratio of the peak intensity for the analyte to the peak intensity of the internal standard at a fixed concentration will be calculated. A calibration curve will then be generated for solution concentration versus intensity ratio. Calibration curves will be generated using linear least square regressions (equal weighting) with subtraction of the calibration blank from all points.

The equation used will be:

$$y = mx + b \quad (10)$$

where y is the ratio of the analyte raw counts to the internal standard raw counts (calculated by the ICP-MS software) and x is the concentration in ppb (ng/mL).

The calibration will be considered acceptable if $r \geq 0.999$ ($r^2 \geq 0.998$). The measured value of each standard must be within $\pm 10\%$ of the expected value ($\pm 20\%$ for the low standard). A calibration blank is then analyzed. A calibration check sample (1 ng/mL) prepared from an independent source will then be measured. The measured value must be within $\pm 10\%$ of the expected value. If not, corrective action will be taken and the calibration will be repeated until successful.

After initiation of sample analysis, a continuing calibration check standard (selected to cover the calibration range) will be analyzed every 10th sample. Measured values must be within $\pm 10\%$ of the expected value. If not, corrective action will be taken and all samples analyzed since the last successful check sample analysis will be reanalyzed. A calibration blank will also be analyzed after every 10th sample to verify the calibration intercept accuracy and efficiency. If the analyte value exceeds the initial calibration blank value by a factor of 10, corrective action will be taken and all samples analyzed since the last successful calibration blank check analysis will be reanalyzed.

The concentration of analyte in a food sample, C_f (ng/g), is calculated as:

$$C_f = \frac{C_s \cdot V_s}{SW} \quad (11)$$

where:

$$C_f = \text{analyte concentration in food (ng/g)}$$

C_s = analyte concentration in solution (ng/mL)
V_s = volume of solution (mL)
SW = food sample weight digested (g)

7.4 GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

Quantitation of selenium will be accomplished using a calibration curve generated from the analysis of multiple point, single element calibration standard solutions. Calibration solutions and calibration check standards will be prepared from commercially available stock standards that are NIST traceable. Stock solutions for calibration and calibration check standards will be obtained from different vendors.

Calibration curves will be prepared from a minimum of five (5) standards and a calibration blank using an acid matrix similar to the sample digests. Selenium concentrations in the calibration will range from approximately 0.05 to 100 ng/mL. The concentrations in the standards were selected to range from the instrumental quantitation limit to the highest expected digest concentrations.

Rinse times between samples/standards will be checked by analyzing a calibration or other blank solutions immediately following aspiration of the highest standard. The measured analyte concentration in the blank should not exceed a value three times the expected instrument detection limit (IDL). If the blank measurement exceeds this value the rinse time will be increased.

Each measured standard/sample value will be the result of a mean taken of three (3) integrations.

For each standard solution, the peak area will be integrated using the instrument software. A calibration curve will then be generated for solution concentration versus peak area. Calibration curves will be generated using linear least square regressions (equal weighting) with subtraction of the calibration blank from all points.

The calibration will be considered acceptable if $r \geq 0.999$ ($r^2 \geq 0.998$). The measured value of each standard must be within $\pm 10\%$ of the expected value ($\pm 20\%$ for the low standard). A calibration blank is then analyzed. A calibration check sample in the middle of the calibration range prepared from an independent source will then be measured. The measured

value must be within $\pm 10\%$ of the expected value. If not, corrective action will be taken and the calibration will be repeated until successful.

After initiation of sample analysis, a continuing calibration check standard (selected to cover the calibration range) will be analyzed every 10th sample. Measured values must be within $\pm 10\%$ of the expected value. If not, corrective action will be taken and all samples analyzed since the last successful check sample analysis will be reanalyzed. A calibration blank will also be analyzed after every 10th sample to verify the calibration intercept accuracy and efficiency. If the analyte value exceeds the initial calibration blank value by a factor of 10, corrective action will be taken and all samples analyzed since the last successful calibration blank check analysis will be reanalyzed.

The concentration of analyte in a food sample, C_f (ng/g), is calculated as:

$$C_f = \frac{C_s \cdot V_s}{SW} \quad (12)$$

where:

C_f = analyte concentration in food (ng/g)

C_s = analyte concentration in solution (ng/mL)

V_s = volume of solution (mL)

SW = food sample weight digested (g)

7.5 METTLER AT 261 ANALYTICAL BALANCE

The analytical balance used for weighing sample aliquots for metals determination will be calibrated in accordance with ACS-SOP-174-004 (Rev. 0).

7.6 MICROWAVE DIGESTION SYSTEM

The CEM MDS 2000 Laboratory Microwave Digestion System will be used to digest the samples for metals determination. The MDS 2000 will be calibrated in accordance with ACS-SOP-174-020 (Rev. 0) and operated in accordance with ACS-SOP-174-021.

7.7 MICROPIPETS

Variable volume micropipets from several manufacturers will be used in the metals determination procedure. These pipets will be calibrated by an external vendor on an annual basis.

SECTION 8.0

DATA REDUCTION, VALIDATION, AND REPORTING

8.1 CHEMICAL ANALYSIS DATA

The data flow is initiated by the chemist who either manually calculates the analyte concentration values or recovers the same from a computerized output. If the analysis did not meet acceptable performance standards, corrective action will be taken.

Sections 9.0 and 12.0 contain the discussion of routine procedures which will be used to assess accuracy and precision, the criteria for "in-control" conditions. When the analyses have been completed, the criteria for "in-control" conditions will be re-assessed. A summary will be made of data generated under analytical conditions judged to be "out-of-control".

Analytical values reported will contain two significant figures.

8.2 PERFORMANCE DATA

Method performance will be assessed on an ongoing basis during sample analysis. Analytical method performance evaluations will include an assessment of accuracy and precision and assessment of the detection limit (DL).

8.2.1 Accuracy and Precision

Accuracy of the methods will be assessed as analyte recovery from spiked samples and SRMs and calculated as shown in Equation (2). Precision will be assessed as %RSD of replicate sample aliquots and calculated as shown in Equation (1).

8.2.2 Detection Limit (DL)

The detection limit for each organic analyte will be that of the lowest calibration concentration from calibration standards which meets the method criteria of 5:1 signal-to-noise ratio and does not increase the average response factor RSD to >25%. (If the lowest calibration standard was in a non-linear portion of the GC/MS response range, its response factor would be substantially different than the response factors measured from higher concentration calibration standards).

The DL for each metal will be three times the standard deviation of the digested blank.

8.3 DATA VALIDATION

Data validation will be the responsibility of the individual task leaders. The following checks on data quality will be performed:

- The mechanisms used for all data transcriptions and transmissions will be reviewed, and a random subset (approximately 5%) of all transcriptions checked.
- The calculation of results of a random subset (approx 5%) of the raw data will be verified.
- The calculation of %RSD for replicate injections on analytical instruments will be verified by an individual other than the analyst.

8.4 DATA ANALYSIS

No statistical data analysis is included in the evaluation of results for these analysis.

8.5 DATA REPORTING

A Final Report will be prepared after the completion of all sample analysis. The final report will contain the results for samples analyzed as well as method performance data. In addition, final data will be provided in PC-compatible spreadsheet or SPSS statistical software files (hard copy and electronic). One hard copy of the instrumental output will be provided for each analyzed sample.

SECTION 9.0

INTERNAL QUALITY CONTROL

9.1 QUALITY CONTROL SAMPLES

Internal quality control procedures to monitor each measurement parameter will be implemented during the sample analysis in the laboratory. Results of samples will be compared against method performance requirements. An overview of the quality control samples is shown in Table 11.

Reagent blanks will be prepared and treated in the same manner as samples. These blank samples will be used to monitor background contamination during storage and analysis.

Fortified reagent blanks, fortified matrix blanks, or fortified replicate matrix samples will be prepared by adding known amounts of target compounds to reagent blanks, matrix blanks, and replicate sample. These control samples will be used to evaluate accuracy and precision of the test method.

9.2 INSTRUMENT PERFORMANCE

9.2.1 Gas Chromatography/Mass Spectrometry (GC/MS)

The mass spectrometer tune will be assessed using the tuning compound appropriate to the particular instrument before commencing target analyte calibration. The mass calibration will be checked each analysis day to maintain accurate mass assignment.

Each analysis day, prior to analysis of samples, the performance of the GC/MS system will be evaluated with respect to continuing calibration, peak resolution, and detector sensitivity. A mid-point calibration standard will be used for this purpose. The sequence of analyses on any given day of analysis will include, at a minimum:

- 250 pg/ μ L calibration standard
- up to four samples or QC samples
- 25 pg/ μ L calibration standard
- up to four samples or QC samples
- 50 pg/ μ L calibration standard
- up to four samples or QC samples
- 500 pg/ μ L calibration standard

TABLE 11. QUALITY CONTROL SAMPLES FOR ANALYSIS

Sample Type	Source	Number	Purpose
Metals			
Reagent blanks	Analysis reagents	5	Determine background contamination
Fortified reagent blanks	Analysis reagents spiked with target analytes	5	Determine analysis precision
Fortified matrix spikes	Matrix spiked with target analytes	5	Accuracy and precision
Duplicates	Duplicate aliquots	5	Determine analysis precision
SRM	SRM 1548A	4	Determine analysis Accuracy and precision
Organics			
Reagent blanks	Analysis reagents	5	Determine background contamination
Fortified reagent blanks	Analysis reagents spiked with target analytes	5	Determine analysis precision
Fortified matrix spikes	Matrix spiked with target analytes	5	Accuracy and precision
Duplicates	Duplicate aliquots	5	Accuracy and precision
SRM	SRM 1491, 1492	4	Accuracy and precision

The 250 pg/ μ L standard analysis will be used to determine if the GC/MS is operating in-control with respect to the original calibration. Calculated RFs from the 250 pg/ μ L standard must be within 30% of the mean values calculated in the initial calibration for at least 75% of the target analytes. In addition, the absolute area response for the internal standard(s) and analytes must not decrease by more than 50% from the response obtained during calibration. If these criteria are met, target analyte concentrations will be reported for all in-control analytes. If more than 2 out-of-control target analytes are present in a sample at concentrations above the detection limit, then the sample will be analyzed. If 1 or 2 out-of-control target analytes are present in a sample at concentrations above the detection limit, the concentrations will be calculated and reported along with a data flag indicating the calibration was not in-control. If the instrumental control criteria are not met, corrective action will be taken using one of the following two approaches.

Approach 1: The four calibration standards analyzed with the set of samples may be used for the purpose of measuring analyte concentrations on that day. In order for this approach to be used, the %RSD for the four calibration standard RFs must be within $\pm 25\%$ and concentrations for the 25 pg/ μ L calibration standard (equivalent to a concentration of 1 ng/g in food) must be measurable for 75% of the target analytes. If these criteria are met, target analyte concentrations will be reported for all in-control analytes. If more than 2 out-of-control target analytes are present in a sample at concentrations above the detection limit, then the sample will be reanalyzed. If 1 or 2 out-of-control target analytes are present in a sample at concentrations above the detection limit, the concentrations will be calculated and reported with a data flag indicating the calibration was not in-control.

Approach 2: Corrective action may be applied to the GC/MS instrument (cleaning, repair, etc.) or to the materials used to calibrate the instrument. The instrument will then be recalibrated, followed by reanalysis of all samples in the out-of-control sample set.

9.2.2 High Performance Liquid Chromatography System (HPLC)

Each analysis day, prior to the analysis of the sample the performance of the HPLC system will be evaluated with respect to gathering calculations, peak resolutions, chromatographic stability and detector sensitivity. A midpoint calibration standard will be used for this purpose. Calculated RFs for carbaryl and at least two other target analytes must be within 25% of the mean calculated in the initial calibration. In addition, the absolute area

response for the internal standard(s) and analytes must not be lower than 25% of the most recent calibration check standard analysis and no more than 50% lower than the response obtained during calibration. If these criteria are not met, corrective action will be taken and the samples from that day will be reanalyzed. Results for individual analytes that do not meet the check criteria of $\pm 25\%$ difference from the original calibration will not be accepted and will be reported as not calculated. In addition to the system performance checks at the beginning of each day, a retention check will be performed after the analysis of every nine samples.

9.2.3 Inductively Coupled Plasma/Mass Spectrometry

Prior to each analytical calibration, the instrumental performance checks summarized in Table 12 will be conducted according to the appropriate schedule. After the instrument has been allowed to warm up at least one hour (plasma, vacuum system, detector, etc.), various system parameters will be checked to ascertain that manufacturer specifications are met. These include the vacuum status (expansion chamber, intermediate and analyzer pressures), forward and reflected power of the ICP RF, and spray chamber temperature (if applicable), which will be checked daily. Instrument conditions will be optimized using the indium-113 mass. For In¹¹³, the acceptance criterion for sensitivity will be a count rate exceeding 20,000 cps/10 ppb.

On a weekly basis, a tuning solution containing elements representative of the desired mass regions will be aspirated. A solution containing Be, Co, In, Pb, and Bi at 10 ng/mL will be used. The mass calibration status will be checked for each tuning element and adjusted as necessary to ensure the difference between actual and calculated mass is less than 0.1 amu.

The detector calibration will be run monthly. If both pulse and analog detector modes are used, a tuning solution containing the above elements at a suggested concentration of 50 ng/mL will be aspirated. The pulse/analog count factor for each element should be between 100-400.

Mass resolution of the quadrupole will be checked by aspirating solutions that contain Mg and Pb. Mg peaks at masses 24, 25 and 26 and Pb peaks at masses 206, 207, and 208 will be examined. Resolutions will be deemed acceptable if the peak width at 5% peak height is approximately 0.75 amu or less. Mass resolution will be checked monthly.

TABLE 12. INSTRUMENT PERFORMANCE PARAMETERS

Parameters	Criteria
Vacuum	See manufacturer's recommendations
RF Power	± 5 W stability
Reflected Power	<5 W
Spray Chamber Temperature	5-10°C
Sensitivity	>20,000 cps/10 ng/mL In ¹¹³
Mass Calibration	Actual-calculated mass <0.1 amu
Detector Calibration	Pulse counts/analog counts = 100-400
Resolution	Peak width at 5% peak height <0.75 amu
Stability	10 minute test (5 integrations) RSD <2%

The instrumental stability will be checked after any portion of the torch, sample introduction system or cones have been cleaned or changed by aspirating a tuning solution that is at least 50 times the IDL for at least 5 integrations or 10 minutes. The RSD for each element should be less than 2%. The %RSD of the triplicate integrations for the samples must be equal to or less than 30% except for those samples falling below the method quantitation limit.

9.2.4 Graphite Furnace Atomic Absorption Spectrometry

Prior to analytical calibration, the instrument and lamp will be allowed to warm up for a minimum of 20 minutes, and a new furnace tube inserted. The tube will be conditioned according to the instrument manufacturer's procedure. The background and analyte peak areas from deionized water and a midrange Se standard will be checked to insure that the sensitivity and background are acceptable for analysis. A 50 ppb solution of Se should give an absorbance signal equivalent to approximately 0.16.

SECTION 10.0

PERFORMANCE AND SYSTEMS AUDITS

10.1 SYSTEMS AUDITS

All major study components will be audited once during the study by the QA Coordinator (or designee). These include:

- Preparation of materials,
- Laboratory systems,
- Analytical measurement systems,
- Data entry and processing.

A Quality Assurance Audit Checklist will be developed to aid in the evaluation of the different areas.

10.1.1 Preparation of Materials

The following system components will be assessed:

- Preparation of custody and tracking documents,
- Preparation of blanks and controls,
- Specific problem areas.

The results of the systems audit will be submitted in writing to the Work Assignment Leader and the Quality Assurance Officer.

10.1.2 Laboratory Systems

An objective assessment of the laboratory systems will be performed once by the Quality Assurance Coordinator. The audit will be conducted according to RTI/ACS-SOP-815-001. This audit will evaluate the following work areas:

- Adherence to analytical protocols,
- Sample preservation,
- Sample storage,
- Sample tracking and custody procedures,
- Work load,
- Specific problem areas.

The results of the systems audit will be submitted in writing to the Work Assignment Leader and Quality Assurance Officer within 5 working days of the audit.

10.1.3 Analytical Measurement Systems

Each analytical measurement system will be audited once during the study for adherence to established protocols and general work performance. These audits will assess the following system components:

- Instrument(s) used and performance criteria,
- Internal QC protocols,
- Preventative maintenance,
- Mechanism for detecting and resolving analytical out-of-control situations,
- Sample throughput, backlog,
- Problem areas.

The results of these systems audits will be submitted in writing to the Work Assignment Leader within 5 days of the audit.

10.1.4 Data Processing

Systems audits will be conducted by the QA Coordinator on the data and processing aspects for all data generated. Evaluation of transcription error rate, data tracking and storage and quality control will be performed.

10.1.5 Other

During the course of the study, a systems audit may be conducted by the EPA Quality Assurance Manager and/or the EPA Work Assignment Manager. The RTI project staff and QA Office staff will cooperate fully with an EPA audit team.

10.2 PERFORMANCE AUDITS

No performance audits are scheduled for this study.

SECTION 11.0

PREVENTIVE MAINTENANCE

Preventive maintenance will be performed on all instruments and equipment used according to the schedule defined prior in the appropriate protocol. A bound notebook will be maintained for each instrument used during this work assignment. When applicable, the following information will be recorded:

- Results of performance tests,
- Instrument calibration information, calibration checks,
- Dates on which routine maintenance is performed and a detailed account of what was done,
- Instrument failure,
- Record of all changes in location, instrument repairs, changes and modifications,
- Description of any problems encountered and steps taken to rectify them.

SECTION 12.0

SPECIFIC ROUTINE PROCEDURES USED TO ASSESS DATA PRECISION, ACCURACY, AND COMPLETENESS

12.1 PRECISION

The duplicate samples will not be provided by the University of Arizona since this is a duplicate diet collection. Analysis precision will be reported as percent relative standard deviation (%RSD) between replicate sample aliquots.

$$\% RSD = \frac{\text{Mean}}{SD} \times 100 \quad (13)$$

12.2 ACCURACY

Accuracy will be reported as recovery from spiked control samples.

$$\% Recovery = \frac{C_m - C_u}{C_s} \times 100 \quad (14)$$

where C_m , C_u , and C_s are the concentration of each target analyte measured in the spiked sample, in the unspiked sample, and the spiked concentration, respectively.

For SRM or CRM samples accuracy will be calculated as

$$\% Recovery = \frac{C_m}{C_c} \times 100 \quad (15)$$

where C_m is the concentration of each target blank measured in the Reference Material and C_c is the certified target analyte concentration.

12.3 COMPLETENESS

Completeness is defined as follows for all chemical and physical measurements:

$$\% \text{ Completeness} = \frac{V}{N} \quad (16)$$

where:

V = the number of measurements judged valid,

N = the number of measurements planned.

SECTION 13.0

CORRECTIVE ACTION

Every effort will be made in each phase of this task to anticipate and resolve potential problems before the quality of performance is compromised. One of the major objectives of this QA plan is to establish mechanisms necessary to ensure this end.

Internal quality control measures described in this QAPP, implemented by the technical staff and monitored by the Work Assignment Leader, will give information on data quality on a day-to-day basis. The responsibility for interpreting the results of these checks and resolving any potential problems resides with the Work Assignment Leader. Any problems that affect data quality or the ability to use the data in the overall method assessment will be reported to the RTI QA officer by the Work Assignment Leader.

Ideally, the quality control measures regulating the operation of each work area will be sufficient to maintain acceptable performance and data quality. However, in the event that a study component is not operating within the limits of acceptability, a formal account of the matter must be submitted by the RTI QA Officer to the Work Assignment Leader and the Project Leader. The report should contain the following information:

- Description and duration of problem,
- Probable cause and resolution of problem,
- Statement describing study data affected by problem,
- Feasibility of repeating work activity and/or generating new data.

No further work may be performed until the problem has been satisfactorily resolved, and the RTI QA Officer has acknowledged approval.

SECTION 14.0

QUALITY ASSURANCE REPORTS

A section will be included in the Final Report which discusses project data quality and describes the overall quality assurance program implemented during the study. Specifically, this section will contain the following information:

- The quality of data being generated or used in the project.
- A reliability summary for the measurement systems (internal QC).
- A delineation of problem areas and corrective action taken.

SECTION 15.0

REFERENCES

1. Sheldon, L.S. Arizona Border Study - Analysis of Food Samples. Work Plan. EPA Contract Number 68-C2-0103, Work Assignment 02-08. U.S. EPA, National Exposure Research Laboratory, Las Vegas, NV, July 15, 1997.
2. Sheldon, L.S. Manual of Analytical Methods for Determination of Selected Environmental Contaminants in Composite Food Samples. EPA-68-C2-0103, U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 1995.
3. Sheldon, L.S. Environmental Contaminants in Foods: Phase 3 - Validation of Analytical Methods. Final Report. EPA-68-C2-0103, U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 1994.

APPENDIX A
ARIZONA BORDER STUDY PROTOCOLS

TITLE: SAMPLE HOMOGENIZATION AND ALIQUOT PREPARATION FOR SOLID FOOD SAMPLES

SOURCE:
Research Triangle Institute
Post Office Box 12194
Analytical and Chemical Sciences
Research Triangle Park, NC 27709-2194

AUTHOR(s):

Mike R. Ford

Date: 5/26/98

Vincent W. Turner

Date: 5/26/98

APPROVED BY:

Principal Investigator:

E. Pelliopia

Date: 5/26/98

QA Officer:

Doris Smith

Date: 5/26/98

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**SAMPLE HOMOGENIZATION AND ALIQUOT PREPARATION
FOR SOLID FOOD SAMPLES**

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1.0 SCOPE AND APPLICATION

This protocol describes the procedure for homogenization and aliquot preparation for composite solid food duplicate diet samples. It is designed to produce samples that can be analyzed for both organic and inorganic analytes. It can also produce sample aliquots that can be archived for future analysis.

2.0 SUMMARY OF THE METHOD

The duplicate diet samples collected from each participant will be homogenized to produce aliquots for analysis. Samples are homogenized in a scientific grade food processor with stainless steel cutting surfaces and bowls. If there is sufficient sample, there will be ten aliquots prepared. The aliquoting scheme is shown in Table 1. Since sample size will vary for each participant the order of aliquots taken is described in Table 1. Sample aliquots will be stored in the freezer until analysis.

3.0 MATERIALS

3.1 Homogenization

- 3.1.1 Robot Coupe Vertical Batch Processor Model RSI 6V with a 6 liter bowl.
- 3.1.2 1 Liter bowl insert for Robot Coupe Model RSI 6V.
- 3.1.3 Liquid Nox phosphate-free liquid detergent.
- 3.1.4 Picotech water (for cleaning only).

3.2 Aliquot Preparation

- 3.2.1 120 mL Glass jars with Teflon capliners precleaned by the manufacturer according to EPA Protocol A.
- 3.2.2 50 mL Plastic conical tubes precleaned for trace elements by acid soaking at RTI.
- 3.2.3 Stainless steel spoons and spatulas, various sizes.
- 3.2.4 Balance capable of 1 g weight resolution.

3.2.5 Calculator.

3.2.6 Freezer capable of maintaining temperatures < -20°C.

4.0 IDENTIFICATION AND HANDLING SAMPLES FOR HOMOGENIZATION

- 4.1 Solid foods were collected by the participant then stored in separate ziplock bags at the field site and assigned a unique sample code.
- 4.2 Samples shipped to RTI are stored in a freezer at -20°C until implementation of the processing described in this protocol.
- 4.3 Prior to homogenization, samples are removed from the freezer, allowed to thaw, and inedible portions of food samples are removed if necessary.
- 4.4 Weigh the sample to be homogenized to the nearest gram. Record the weight in a laboratory notebook.

5.0 SAMPLE HOMOGENIZATION

- 5.1 Select a processor bowl of suitable size (6-L or 1-L) to hold the sample. This selection involves estimating the volume of the sample. If the minimum estimated volume is 1000 mL or greater, use the 6-L bowl. If the estimated sample volume is less than 1000 mL, use the 1-L bowl.
- 5.2 Assemble the bowl and blade on the processor base. Transfer the food sample to the clean bowl assembly, removing as much of the food from the storage bag as possible. Compress the food, if necessary, to insure that processor is able to adequately homogenize the sample during operation. Do not add any water to the food samples.
- 5.3 Place the top on the bowl and start the blades turning at a low speed. Slowly increase the speed of the blades to homogenize the sample. If large particles of food are thrown to the side of the bowl away from the blades during the homogenization process, stop the processor and then remove the bowl lid, using a spatula scrape the food from the bowl sides down towards the blades. Place the lid back on the bowl and start the blades to continue processing.

WARNING

The processor is equipped with an automatic shut-off switch when the lid is removed from the bowl, however, do not completely remove the lid until the blade has stopped turning.

5.4 Run the processor on the highest speed setting for 5 to 15 seconds. Examine the food homogenate. Repeat as necessary to achieve the final desired consistency. The desired consistency is a thick paste or batter with no solid inclusions. It may be impossible to avoid solid inclusions for some foods. These should be less than 2 mm, and preferably 1 mm in diameter.

NOTE: The processing may heat the sample. Take care not to overheat the homogenate.

5.5 The total time the processor motor is run should not exceed 2 to 4 minutes in order to avoid overheating of the sample. It may be necessary to allow the sample to cool before proceeding.

6.0 SAMPLE ALIQUOT CODING AND ALIQUOT PREPARATION AND ARCHIVAL

6.1 After homogenizing the composite foods, portions of the homogenized composite samples will be transferred to precleaned containers for storage until analysis or will be archived for potential future analyses.

6.2 The types, amounts, and codes for the sample aliquots are described in Table 1. Archival samples will be prepared in the order listed. Some food samples may not be large enough for preparation of some or all archival samples.

6.3 Affix a preprinted sample aliquot code label provided by the University of Arizona and a tracking code label to each container prior to homogenization. Wrap Scotch tape all of the way around the container to protect the label.

6.4 The procedure for determining the priority and order for preparing sample aliquots is presented in Table 1.

6.5 After preparing sample aliquots according to Table 1, all aliquots must be placed in a freezer for storage at -20°C, unless extraction is scheduled to begin immediately for a particular aliquot.

6.6 Archival samples will be stored at -20°C for a minimum of six months.

7.0 PROCESSOR CLEANING

7.1 After sample homogenization the processor bowl(s) is/are cleaned thoroughly using a diluted solution of phosphate-free detergent. The cleaning should remove all of the sample residue from the bowl and blades.

7.2 After washing, rinse the bowl(s) and blades thoroughly with Picotech (metals free) water twice. Make sure that the bowl(s) and blades are dry before processing another sample.

8.0 DOCUMENTATION

8.1 Maintain a log of all processed samples in the laboratory notebook. Include observations or comments about problems.

8.2 Sign and date the custody record for each sample. Include comments about the processing, as needed.

TABLE 1. SOLID FOOD HOMOGENATE CODING SCHEME

Preparation Order	Analytes	Nominal Aliquot Weight (g)	Tracking Code Suffix*	To Be Used For
1	Pesticides	50	XXXXXX1	Analysis
2	PAHs	50	XXXXXX2	Analysis
3	Metals	25	XXXXXX3	Analysis
4	Pesticides	50	XXXXXX4	Backup
5	PAHs	50	XXXXXX5	Backup
6	Pesticides/PAHs	50	XXXXXX6	Archival
7	Metals	25	XXXXXX7	Archival
8	Pesticides/PAHs	50	XXXXXX8	Archival
9	Metals	25	XXXXXX9	Archival

* These are proposed tracking codes where XXXXXXX is the same as the original sample code. The aliquot ID codes have been assigned by the University of Arizona and these labels will be attached to the appropriate aliquot container.

TITLE: DETERMINATION OF BASE/NEUTRAL PESTICIDES IN COMPOSITE FOOD SAMPLES

SOURCE:
Research Triangle Institute
Post Office Box 12194
Analytical and Chemical Sciences
Research Triangle Park, NC 27709-2194

AUTHOR(s):

Mike Rhead

Date: 3/24/98

Kurt W. Thumm

Date: 3/24/98

Wayne C. Koenig

Date: 3/24/98

APPROVED BY:

Principal Investigator: E. Tellingeri

Date: 3/26/98

QA Officer: Doris Smith

Date: 3/25/98

STATUS:

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DETERMINATION OF BASE/NEUTRAL PESTICIDES IN COMPOSITE FOOD SAMPLES

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1.0 SCOPE AND APPLICATION

This is a method for the determination of base/neutral pesticides in composited food samples. This method has been tested and is applicable to the pesticides in Table 1. For pesticides not listed in Table 1, method performance must be evaluated by the analysis laboratory.

2.0 SUMMARY OF THE METHOD

This is a linked analysis scheme that provides for the extraction, cleanup, and analysis of pesticides from a homogenized food sample. A schematic of the method is shown in Figure 1. The linked analysis scheme includes the following procedures.

- Extraction (RTI/ACS-AP-211-132) - A 25 g aliquot of the homogenized food sample is mixed with zinc acetate to minimize fatty acid interferences; it is then mixed with anhydrous sodium sulfate to remove water. The sample is Soxhlet extracted with methylene chloride. The extract is solvent exchanged into hexane and concentrated to 10 mL.
- Preliminary Cleanup (RTI/ACS-AP-211-133) - Pesticides in the hexane extract are partitioned into acetonitrile. The acetonitrile extract is concentrated to a small volume for further cleanup.
- GPC Cleanup (RTI/ACS-AP-211-134) - The concentrated acetonitrile extract is diluted in methylene chlorine then subjected to GPC cleanup to remove high molecular weight materials. The final sample extract is concentrated to 1.0 mL for analysis.
- Analysis (RTI/ACS-AP-211-135) - The base/neutral pesticides are measured by GC/MS in the selected ion monitoring mode.
- Analysis (RTI/ACS-AP-211-137) - The carbamate pesticides are measured by HPLC.

3.0 DEFINITIONS

3.1 Internal Standard

A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.2 Surrogate Analyte

A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 Laboratory Duplicates (LD1 and LD2)

An equivalent sample aliquot taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of laboratory duplicates give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 Laboratory Reagent blank (LRB)

Reagents treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.5 Laboratory Performance Check Solution (LPC)

A solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.6 Laboratory Fortified Blank (LFB)

A laboratory reagent blank to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to

determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.7 Laboratory Fortified Sample Matrix (LFM)

An aliquot of a food sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.8 Stock Standard Solution

An concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.9 Primary Dilution Standard Solution

A solution of several analytes prepared in the laboratory from stock standard solutions and used to prepare primary dilution standards.

3.10 Calibration Standard (CAL)

A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.11 Equivalent Sample Weight (ESW)

Weight of food homogenate used for extraction excluding the weight of any water added for homogenization.

4.0 INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.2.

- 4.1.1 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with hot tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 1 hour. Do not heat volumetric ware. Thermally stable materials might not be eliminated by this treatment. Thorough rinsing with acetone may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
- 4.1.2 Phthalate esters contaminate many types of products commonly found in the laboratory. Plastics, in particular, must be avoided because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Serious phthalate contamination may result at any time if consistent quality control is not practiced.
- 4.1.3 The use of high purity reagents and solvents help to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

WARNING

When a solvent is purified, stabilizers put into the solvent by the manufacturer are removed thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives put into the solvent by the manufacturer are removed thus potentially reducing the shelf-life.

- 4.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from sample to sample. Cleanup procedures are provided to remove interferences.
- 4.3 Variable amounts of pesticides may adhere to glass surfaces. It is recommended that sample transfers and glass surface contacts be minimized.
- 4.4 Glassware contamination may result in analyte degradation: Soap residue on glassware may cause degradation of certain analytes. Specifically, aldrin, heptachlor, and most organophosphorus pesticides will degrade in this situation. This problem is especially pronounced with glassware that may be difficult to rinse (e.g., 500-mL K-D flask). These items should be hand-rinsed very carefully to avoid this problem.

5.0 SAFETY

- 5.1 The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Additional references to laboratory safety are available for the information of the analyst.
- 5.2 Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens including aldrin, chlordane, dieldrin, and heptachlor. Pure standard materials and stock standard solutions of these compounds should be handled in a hood or glovebox with suitable protection to skin, eyes, etc.
- 5.3 Refer to specific SOPs for nonroutine safety procedures.

6.0 EQUIPMENT AND SUPPLIES

- 6.1 Extraction - Refer to RTI/ACS-AP-211-132.
- 6.2 Cleanup - Refer to RTI/ACS-AP-211-133 and RTI/ACS-AP-211-134.
- 6.3 Analysis - Refer to RTI/ACS-AP-211-135.

7.0 REAGENTS AND STANDARDS

- 7.1 Extraction - Refer to RTI/ACS-AP-211-132.
- 7.2 Cleanup - Refer to RTI/ACS-AP-211-133 and RTI/ACS-AP-211-134.
- 7.3 Analysis - Refer to RTI/ACS-AP-211-135.
- 7.4 Internal Standard Spiking Solutions

Base/neutral - A solution is prepared in toluene that contains 9,10-dichloroanthracene and triphenylphosphate at concentrations of 10 ng/ μ L. A 50 μ L aliquot of this solution will be used to spike the final sample extracts.

8.0 SAMPLE HOMOGENIZATION AND STORAGE

- 8.1 Samples will be homogenized according to RTI/ACS-AP-211-038. The homogenates will be stored at -20°C until extraction.

9.0 QUALITY CONTROL

- 9.1 Quality control (QC) requirements include initial demonstration of laboratory capability, assessment of background contamination using laboratory reagent blanks, assessment of pesticide recoveries using laboratory fortified blanks, laboratory fortified samples, and surrogate standards, assessment of method precision using laboratory duplicate samples, and evaluation of the analytical method.

9.2 Initial Demonstration of Capability

- 9.2.1 Prepare at least one laboratory reagent blank (LRB), one laboratory fortified reagent blanks (LFB), and four laboratory fortified sample matrices (LFM). All fortified samples should be prepared at the 10 ng/g level for each target pesticide. Extract and analyze each sample.
- 9.2.2 The laboratory reagent blank must meet the criteria given in Section 9.3.

- 9.2.3 The recovery value for each target pesticide and surrogate standard in the fortified laboratory blank must fall in the range of 60 to 120%.
 - 9.2.4 The recovery value for each of target pesticide and surrogate standard in at least three of the fortified sample matrices must fall in the range of 60 to 120%. The mean recovery value for each target pesticide and surrogate standard must also fall within this range. The %RSD of the recovery values for the four samples must be less than 30. For those compounds that meet the acceptance criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, performance must be repeated using four fresh samples until satisfactory performance has been demonstrated.
 - 9.2.5 A low level calibration standard must be analyzed to demonstrate the concentrations at which the analytes can be measured. Criteria for acceptance for each analyte include:
 - signal-to-noise ratio of 1-to-5.
 - measured concentration within 30% of prepared concentration.
 - 9.2.6 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of data will improve beyond those require here.
 - 9.2.7 The analyst is permitted to modify GC columns, GC conditions, GC detectors, concentration techniques (i.e., evaporation techniques), internal standards or surrogate compounds.
- 9.3 Assessing Background Contamination - Laboratory Reagent Blanks
- 9.3.1 Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a laboratory reagent blank (LRB) must be analyzed.

- 9.3.2 To be considered acceptable, the concentration of all target pesticides must be less than 1 ng/g assuming a 25 g sample is used.
- 9.3.3 If this criterion is not met, determine the source of contamination and eliminate the interference before processing samples.

9.4 Assessing Surrogate Recovery

- 9.4.1 When surrogate recovery is <60% or >120%, check (1) calculations to locate possible errors, (2) fortifying solutions for degradation, (3) contamination or other obvious abnormalities, and (4) instrument performance. If those steps do not reveal the cause of the problem, re-analyze the extract.
- 9.4.2 If a blank extract analysis fails the 60-120% recovery criterion, the problem must be identified and corrected before continuing.
- 9.4.3 If sample extract re-analysis meets the surrogate recovery criterion, report only data for the re-analyzed extract. If sample extract re-analysis continues to fail the surrogate recovery criterion, report all data for that sample as suspect.

9.5 Assessing Pesticide Recovery

- 9.5.1 Laboratory Fortified Blank—At least one LFB must be analyzed with every twenty samples or one per sample set (all samples extracted within a 24-h period) whichever is greater. The fortified concentration of each analyte in the LFB should be 10 ng/g. If the recovery of any target pesticide or surrogate standard falls outside the control limits (60 to 120%), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.
- 9.5.2 Laboratory Fortified Sample Matrix—At least one LFM must be analyzed with every twenty samples or one per sample set (all samples extracted within a 24-h period) whichever is greater. The fortified concentration of each target pesticides should be 10 ng/g. If the recovery of any such target pesticide or surrogate standard falls outside the designated range (60 to 120%), and the LFB is shown to be in control, the recovery problem encountered with the closed sample is judged to be matrix related, not system related. The result for that

analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.6 Assessing Method Precision

- 9.6.1 At least one duplicate sample aliquot must be extracted and analyzed with every twenty samples or one per sample set (all samples extracted within a 24-h period) whichever is greater.
- 9.6.2 The %RSD of any target pesticide measured above the detection limit should be less than 30. Pesticides in the replicate samples that do not meet this criteriom are labeled as suspect.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Cleanup - Refer to RTI/ACS-AP-211-134.

10.2 Analysis - Refer to RTI/ACS-AP-211-135.

11.0 PROCEDURE

11.1 Extraction

Refer to RTI/ACS-AP-211-132. The final extract is in 10 mL of *n*-hexane.

11.2 Preliminary Cleanup

Refer to RTI/ACS-AP-211-133. The final sample extract in acetonitrile is concentrated to <0.5 mL. The extract is then diluted to 1.0 mL in methylene chloride for GPC cleanup.

11.3 GPC Cleanup

Refer to RTI/ACS-AP-211-134. The GPC eluate in methylene chloride is concentrated to 0.5 mL using K-D evaporation with a 10 mL K-D receiver and a modified micro-Snyder column. The concentrated extract is spiked with internal standard. The extract sample is solvent exchanged into 1.0 mL of iso-octane. The sample extract is transferred then sealed in amber glass vials with either Teflon-lined septum-sealed screw caps or crimp seals. The

sample extracts may be stored at 4°C for short periods of time (< 48 hours). The sample extract should be stored at ≤10°C for longer periods of time.

11.4 Analysis

Refer to RTI/ACS-AP-211-135.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Concentrations of analytes in the food samples should be calculated as

$$C_f = \frac{C_e \cdot E_v}{ESW \cdot 1000}$$

where:

C_f = food concentration (ng/g)

C_e = extract concentration (pg/μL) as calculated in RTI/ACS-AP-211-135,
Section 12 for base/neutral pesticides

E_v = extract volume (μL)

ESW = equivalent sample weight (g) - weight of sample excluding water added
for homogenization

13.0 METHOD PERFORMANCE

13.1 In a single laboratory, pesticide recoveries from three sample matrices were determined at one concentration level. Data from these experiments are given in Tables 2 to 4.

13.2 This method was applied to the analysis of composite food samples collected as part of the EPA/NCI Agricultural Health Study. Results of QC analyses performed for this study are given in Table 5.

13.3 In a single laboratory, the stability of pesticides stored using the conditions specified in Reference 1 (Method PH-00) was determined over a 21 day period. Data from these experiments are given in Tables 6 to 7.

13.4 In a single laboratory, seven replicates of a single food sample were fortified at a low level, then processed and analyzed to determine EDLs. Data from this experiment are given in Table 8.

14.0 SAMPLE CUSTODY AND DATA MANAGEMENT

14.1 Sample Management

Food sample homogenates will be received with unique codes for sample identification. These sample codes will be placed on all samples and associated documents and will be used for all data reporting.

14.2 Sample Custody

Sample custody procedures will be used to track samples and sub-samples generated during this work assignment. Custody of sample aliquots taken for extraction and analysis will be tracked using the Batch Custody Record shown in Figure 2. This form will assist in tracking samples and will include important processing information such as sample amount taken for extraction, spiking, extraction, cleanup, and analysis procedures and dates, final extract volume, and amounts of internal standards added. Detailed information regarding sample extraction, cleanup, and standards preparation will be recorded in RTI Laboratory Notebooks. During sample processing, the laboratory chemists is responsible for sample custody. When a batch of sample extracts are submitted to the analytical lab for analysis, the MS analyst is responsible for sample custody.

The batch custody records are kept in the analytical laboratory until the data has been electronically transferred from the MS data system into a computer spreadsheet and final sample concentrations are calculated. Upon complete review of the data, the chain-of-custody and batch records will be returned to the Task Manager.

14.3 Datafile Management

Electronic datafiles for the MS system containing results for sample extracts as pg/ μ L will be created for each individual sample. These files will be transferred to an electronic spread sheet where sample concentrations will be calculated as described in Section 12.

The MS laboratory manager and Task Manager are responsible for reviewing the data prior to its submission to the Work Assignment Leader. This review will be for completeness and accuracy of the dataset to ensure that all samples, blanks, and QC samples have been included in the electronic datafile and the data in the file is the same as the data generated during analysis.

15.0 REFERENCES

1. Sheldon, L.S. Manual of Analytical Methods for Determination of Selected Environmental Contaminants in Composite Food Samples. EPA-68-C2-0103, U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 1995.
2. Sheldon, L. Environmental Contaminants in Foods: Phase 3 - Validation of Analytical Methods. Final Report, U.S. EPA EMSL-Cincinnati Contract Number 68-C2-0103, Final Report 2-01, 1994.
3. Sheldon, L. Support for AHS Human Exposure Monitoring Study. Final Report, U.S. EPA EMSL-Cincinnati Contract Number 68-C2-0103, Work Assignment 2-05, 1994.

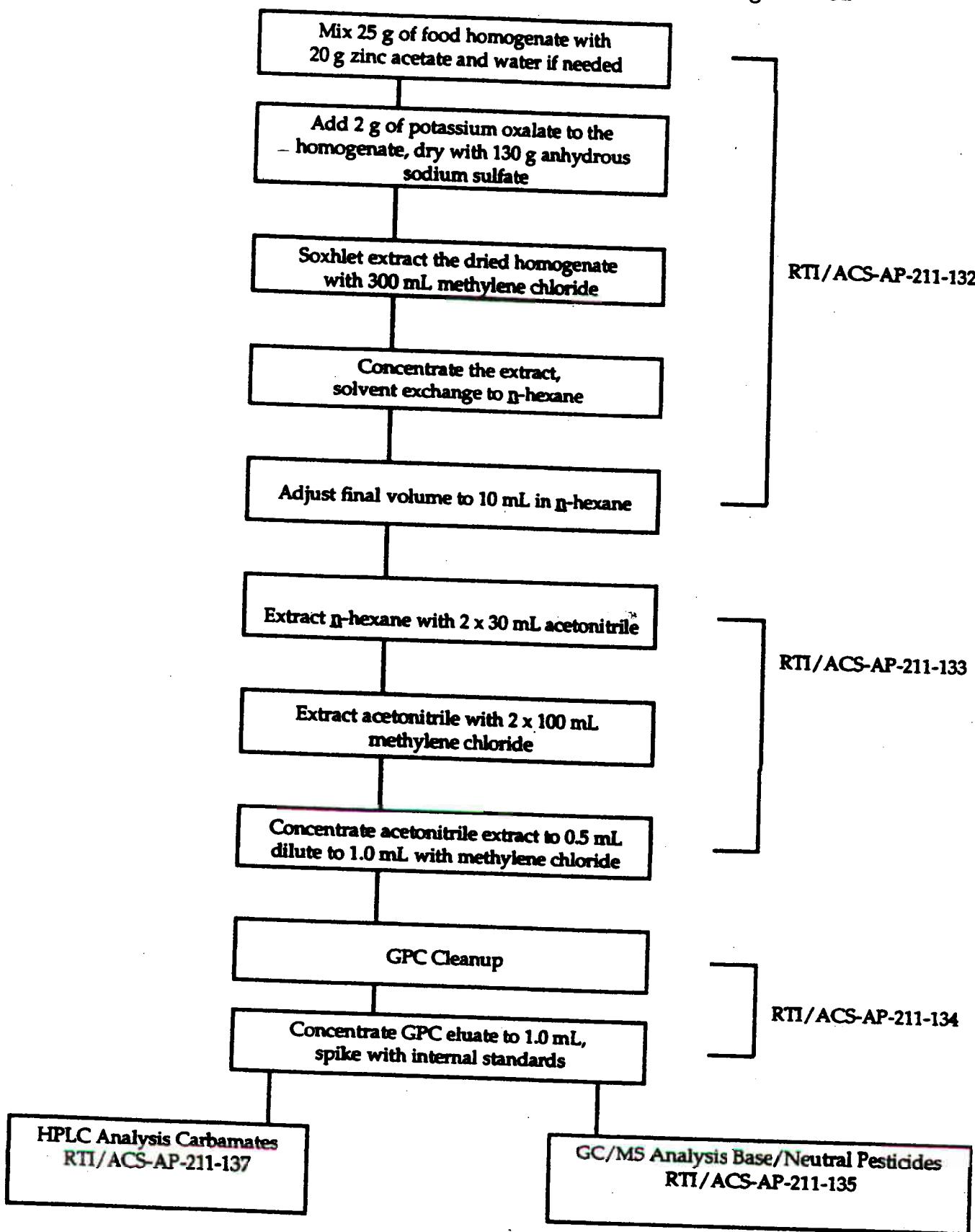


Figure 1. Flow Diagram of RTI/ACS-AP-211-131

GC/MS Analysis

Matrix:

Batch No.:

Instrument

Operator

Size Equivalent (g)

Figure 4. Batch Custody Record

TABLE 1. PESTICIDES DETERMINED BY RTI/ACS-AP-211-131

Compound	Comment
<u>Polar</u>	
Acetochlor	
Alachlor	
Atrazine	
Dacthal	chromatography can be affected by fatty acids in sample
Diazinon	
Dichloran	
Dichlorvos	
Fonofos	
Isofenphos	
Malathion	
Metolachor	
Parathion	
Phorate	
Simazine	chromatography can be affected by fatty acids in sample
Terbufos	
Trifluralin	
<u>Nonpolar</u>	
α -Chlordane	
γ -Chlordane	
Chlorpyrifos	
4,4'-DDD	
4,4'-DDE	
4,4'-DDT	
Dieldrin	
Endosulfan I	
Endrin	
Heptachlor	care must be taken to avoid contamination during processing
Lindane	
cis-Permethrin	
trans-Permethrin	
<u>Carbamates</u>	
Aldicarb	small interference during HPLC analysis
Carbaryl	
Carbofuran	
Methomyl	
Propoxur	substantial interference during HPLC analysis

TABLE 2. METHOD EVALUATION RESULTS - RTI/ACS-AP-211-131

Compound	Concentration (ppb)				% Recovery ^a		
	Laboratory		Mean Unspiked (n=3)	% RSD Unspiked	Fortified		Laboratory Fortified Sample Matrix (n=3)
	Reagent Blank (n=1)	Laboratory Blank (n=1)			Fortified Laboratory Blank (n=1)		
Polar							
Acetochlor	0.20	0.23	71		84	68	9
Alachlor	-	0.05	141		94	85	10
Atrazine	0.56	-	-		95	86	10
Captan	-	-	-		57	50	72
Dacthal	0.04	0.01	141		93	83	7
Diazinon	-	0.49	6		88	82	6
Dichloran	-	-	-		81	85	9
Dichlorvos	-	-	-		77	77	4
Folpet	-	-	-		56	59	16
Fonofos	-	-	-		90	82	6
Isofenphos	-	-	-		87	83	6
Malathion	-	INT ^b	-		81	112	29
Metolachor	-	-	-		98	92	7
Parathion	-	-	-		89	88	10
Phorate	-	-	-		79	81	7
Simazine	-	-	-		90	92	3
Terbufos	-	-	-		81	82	7
Trifluralin	-	-	-		79	86	7
Nonpolar							
Aldrin	-	-	-		66	59	7
γ-Chlordane	0.16	0.27	20		87	79	8
α-Chlordane	0.11	0.16	26		90	79	8
Chlorpyrifos	-	0.39	12		82	68	5
4,4'-DDD	-	0.08	72		93	83	9
4,4'-DDE	0.05	0.27	16		86	72	8
4,4'-DDT	0.05	0.08	20		95	82	7
Dieldrin	-	-	-		94	77	8
Endosulfan I	-	-	-		90	79	7
Endrin	-	-	-		100	82	4
Heptachlor	0.37	0.29	71		76	80	5
Hexachlorobenzene	-	-	-		56	51	9
Lindane	-	-	-		90	88	10
cis-Permethrin	-	-	-		94	110	27
trans-Permethrin	-	-	-		102	94	38

(continued)

TABLE 2. (continued)

Compound	Concentration (ppb)			% Recovery ^a		
	Laboratory Reagent Blank (n=1)	Mean Unspiked (n=3)	% RSD Unspiked	Fortified Laboratory Blank (n=1)	Laboratory Fortified Sample Matrix (n=3)	% RSD Spiked
<u>Surrogates</u>						
Atrazine-d ₅	NS ^b	NS	-	NS	NS	4
Chrysene-d ₁₂	93 ^d	91 ^d	1	85	82	32
Dimethylnitrobenzene	79 ^d	88 ^d	2	79	97	1
Fluorene-d ₁₀	90 ^d	92 ^d	0	81	86	4
Pentachloronitrobenzene	82 ^d	88 ^d	0	74	82	7
<u>Carbamates</u>						
Aldicarb	-	-	INT	55	80	10
Carbaryl	-	-	-	116	89	7
Carbofuran	-	-	-	93	76	8
Methomyl	-	-	-	93	73	12
Propoxur	-	-	-	90	71	7

^a Samples spiked at 10 ppb.

^b Not detected.

^c Not spiked.

^d % Recovery.

* Sample interference prevented quantitation.

TABLE 3. METHOD EVALUATION RESULTS - RTI/ACS-AP-211-131

Compound	Concentration (ppb)			% Recovery ^a		
	Laboratory		% RSD Unspiked	Fortified Laboratory Blank (n=1)	Laboratory	
	Reagent Blank (n=1)	Mean Unspiked (n=3)			Fortified Sample Matrix (n=3)	% RSD Spiked
<u>Polar</u>						
Acetochlor	- ^b	-	-	63	85	4
Alachlor	-	-	-	68	104	4
Atrazine	-	-	-	75	92	9
Captan	-	-	-	23	91	24
Dacthal	-	-	-	66	92	9
Diazinon	-	-	-	71	103	5
Dichloran	-	-	-	52	95	2
Dichlorvos	-	-	-	58	93	6
Folpet	-	-	-	28	72	21
Fonofos	-	-	-	64	91	5
Isofenphos	-	-	-	73	108	6
Malathion	-	-	-	77	112	29
Metolachor	-	-	-	67	92	3
Parathion	-	-	-	75	123	6
Phorate	-	-	-	56	85	4
Simazine	-	-	-	99	117	13
Terbufos	-	-	-	59	91	4
Trifluralin	-	0.1	35	51	88	4
<u>Nonpolar</u>						
Aldrin	-	-	-	58	84	10
α -Chlordane	-	0.3	34	70	89	3
γ -Chlordane	0.1	0.7	26	65	102	7
Chlorpyrifos	-	2.3	34	75	79	37
4,4'-DDD	-	-	-	72	139	2
4,4'-DDE	-	0.4	25	65	89	10
4,4'-DDT	-	-	-	71	93	5
Dieldrin	-	-	-	56	85	5
Endosulfan I	-	-	-	71	88	2
Endrin	-	-	-	66	76	6
Heptachlor	0.1	0.9	22	66	81	8
Hexachlorobenzene	-	-	-	51	65	8
Lindane	-	-	-	76	98	9
cis-Permethrin	-	-	-	66	60	16
trans-Permethrin	-	-	-	63	56	17

(continued)

TABLE 3. (continued)

Compound	Concentration (ppb)			% Recovery ^a		
	Laboratory Reagent Blank (n=1)	Mean		Fortified Laboratory Blank (n=1)	Fortified Sample Matrix (n=3)	% RSD Spiked
		Unspiked (n=3)	% RSD Unspiked			
<u>Surrogates</u>						
Atrazine-d ₅	NS ^b	NS	-	NS	NS	-
Chrysene-d ₁₂	65 ^c	61 ^d	2	61	63	17
Dimethylnitrobenzene	63 ^d	96 ^d	6	52	126	8
Fluorene-d ₁₀	69 ^d	81 ^d	3	61	93	2
Pentachloronitrobenzene	60 ^d	74 ^d	3	62	86	4
<u>Carbamates</u>						
Aldicarb	NT ^e	NT	NT	NT	NT	NT
Carbaryl	NT	NT	NT	NT	NT	NT
Carbofuran	NT	NT	NT	NT	NT	NT
Methomyl	NT	NT	NT	NT	NT	NT
Propoxur	NT	NT	NT	NT	NT	NT

^a Samples spiked at 10 ppb.

^b Not detected.

^c Not spiked.

^d % recovery.

^e Not tested.

TABLE 4. METHOD EVALUATION RESULTS - RTI/ACS-AP-211-131
 HOUSEHOLD COMPOSITE SAMPLE 3

Compound	Concentration (ppb)			% Recovery ^a		
	Laboratory Reagent Blank (n=1)	Mean Unspiked (n=3)	% RSD Unspiked ^b	Fortified Laboratory Blank (n=1)	Fortified Sample Matrix (n=3)	% RSD Spiked
Polar						
Acetochlor	- ^c	-	-	76	84	16
Alachlor	0.1	1.1	56	79	80	8
Atrazine	-	-	-	79	59	22
Captan	-	-	-	93	45	20
Dacthal	0.1	0.3	97	77	81	17
Diazinon	-	-	-	74	79	3
Dichloran	-	-	-	78	86	4
Dichlorvos	-	-	-	69	81	6
Folpet	-	-	-	80	83	1
Fonofos	-	-	-	76	81	3
Isofenphos	0.5	-	-	65	80	2
Malathion	0.1	5.0	26	74	80	15
Metolachor	0.1	0.2	141	83	90	7
Parathion	-	0.4	141	81	109	10
Phorate	-	0.2	141	69	71	3
Simazine	-	-	-	79	51	24
Terbufos	-	0.3	141	70	76	7
Trifluralin	0.1	0.4	78	70	88	4
Nonpolar						
Aldrin	-	-	-	60	70	15
α -Chlordane	0.1	0.3	87	72	67	4
γ -Chlordane	0.1	0.4	68	70	65	4
Chlorpyrifos	-	0.7	33	89	90	14
4,4'-DDD	0.1	0.3	141	76	77	4
4,4'-DDE	0.2	0.6	56	72	72	14
4,4'-DDT	0.1	1.5	24	85	77	7
Dieldrin	-	-	-	76	97	20
Endosulfan I	-	0.2	141	75	67	4
Endrin	-	-	-	76	70	5
Heptachlor	0.7	1.0	21	67	70	5
Hexachlorobenzene	-	0.2	110	51	50	8

(continued)

TABLE 4. (continued)

Compound	Concentration (ppb)			% Recovery ^a		
	Laboratory Reagent Blank (n=1)	Mean Unspiked (n=3)	% RSD Unspiked ^b	Fortified Laboratory Blank (n=1)	Laboratory Fortified Sample Matrix (n=3)	% RSD Spiked
Lindane	-	-	-	81	81	2
cis-Permethrin	-	-	-	81	122	0
trans-Permethrin	-	-	-	85	78	2
<u>Surrogates</u>						
Atrazine-d ₃	88 ^c	INT ^d	-	79	INT	INT
Chrysene-d ₁₂	75 ^d	64 ^d	0	67	61	6
Dimethylnitrobenzene	84 ^d	107 ^d	1	72	115	14
Fluorene-d ₁₀	92 ^d	85 ^d	0	77	80	7
Pentachloronitrobenzene	90 ^d	84 ^d	0	78	82	11
<u>Carbamates</u>						
Aldicarb	NT ^e	NT	NT	NT	NT	NT
Carbaryl	NT	NT	NT	NT	NT	NT
Carbofuran	NT	NT	NT	NT	NT	NT
Methomyl	NT	NT	NT	NT	NT	NT
Propoxur	NT	NT	NT	NT	NT	NT

^a Samples spiked at 10 ppb.

^b High %RSD values due to relatively high measured concentration in 3rd replicate.

^c Not detected.

^d % recovery.

^e Sample interference prevent quantitation.

Not tested.

TABLE 5. RESULTS OF QC ANALYSES FROM AHS STUDY FOR BASE/NEUTRAL PESTICIDES

Compound	Laboratory Reagent Blank (ng/g) (n=6)			% RSD Duplicate Samples (n=3)	Fortified Laboratory* Sample Matrix (n=7)	
	Mean	S.D.			Mean	% Recovery
Polar						
Acetochlor	- ^b	-		-	77	15
Alachlor	0.02	0.04		25	87	12
Atrazine	-	-		-	88	21
Captan	-	-		-	-	-
Dacthal	0.01	-		55	88	9
Diazinon	-	-		-	85	9
Dichloran	-	-		-	92	23
Dichlorvos	-	-		-	70	9
Folpet	-	-		-	32	69
Fonofos	-	-		-	83	8
Isofenphos	0.04	0.08		-	98	14
Malathion	-	-		25	63	41
Metolachor	0.02	-		-	88	12
Parathion	-	-		-	84	18
Phorate	-	-		-	83	6
Simazine	0.42	0.68		-	82	19
Terbufos	-	-		-	88	9
Trifluralin	0.02	-		19	87	17
Nonpolar						
Aldrin	-	-		-	70	11
γ -Chlordane	0.20	0.17		20	88	11
α -Chlordane	0.04	-		-	86	10
Chlorpyrifos	-	-		15	54	22
4,4'-DDD	0.01	-		-	97	16
4,4'-DDE	0.05	0.08		24	86	14

(continued)

TABLE 5. (continued)

Compound	Laboratory Reagent Blank (ng/g) (n=6)		% RSD Duplicate Samples (n=3)	Fortified Laboratory ^a Sample Matrix (n=7)	
	Mean	S.D.		Mean % Recovery	% RSD
4,4'-DDT	0.01	-	4	77	22
Dieldrin	-	-	-	88	14
Endosulfan I	-	-	-	86	17
Endrin	-	-	-	90	17
Heptachlor	0.50	0.28	19	81	9
Hexachlorobenzene	-	-	50	62	10
Lindane	-	-	-	91	18
cis-Permethrin	-	-	-	87	18
trans-Permethrin	-	-	-	81	26
<u>Surrogates</u>					
Atrazine-d ₅	NS ^c	-	21	NS	NS
Chrysene-d ₁₂	82 ^d	11	11	88	17
Dimethylnitrobenzene	74 ^d	14	11	90	30
Fluorene-d ₁₀	81 ^d	8.7	4	87	12
Pentachloronitrobenzene	76 ^d	9.1	4	83	11
<u>Carbamates</u>					
Aldicarb	-	-	14	138	24
Carbaryl	-	-	23	119	31
Carbofuran	-	-	22	145	18
Methomyl	NA ^b	NA	NA	NA	NA
Propoxur	-	-	6	138	12

^a Samples spiked at 10 ppb.

^b Not detected.

^c Not spiked.

^d % recovery.

^e Not analyzed; not a target pesticide of AHS study.

TABLE 6. STORAGE STABILITY STUDY FOR BASE/NEUTRAL PESTICIDES - COMPOSITE SAMPLE 4, T = 21 DAYS

Compound	Concentration (ppb)		% Recovery ^a		
	Laboratory Reagent Blank (n=1)	Mean Unspiked (n=3)	Fortified Laboratory Blank ^c (n=1)	Laboratory Fortified Sample Matrix (n=3)	% RSD Spiked
Polar					
Acetochlor	0.23	- ^b	102	80	9
Alachlor	-	-	91	82	13
Atrazine	-	0.40	98	87	10
Captan	-	-	145	-	-
Dacthal	0.15	0.15	97	80	11
Diazinon	0.18	-	93	78	9
Dichloran	0.17	-	101	94	11
Dichlorvos	0.10	-	99	71	11
Folpet	-	-	121	23	8
Fonofos	0.16	-	95	78	9
Isofenphos	-	-	111	126	60
Malathion	0.20	2.1	107	63	20
Metolachor	0.24	-	103	81	14
Parathion	-	-	97	82	12
Phorate	0.17	-	88	73	10
Simazine	0.37	-	110	92	14
Terbufos	0.41	-	91	77	10
Trifluralin	0.17	0.16	95	83	10
Nonpolar					
Aldrin	0.14	-	83	67	5
γ -Chlordane	0.31	0.49	94	78	11
α -Chlordane	0.29	0.22	91	80	11
Chlorpyrifos	0.20	0.41	96	61	15
4,4'-DDD	0.13	0.08	85	75	12
4,4'-DDE	0.15	0.24	90	76	8
4,4'-DDT	0.24	0.19	125	108	8
Dieldrin	-	-	100	87	10
Endosulfan I	-	-	97	82	14
Endrin	-	-	98	85	13

(continued)

TABLE 6. (continued)

Compound	Concentration (ppb)		% Recovery ^a		
	Laboratory Reagent Blank (n=1)	Mean Unspiked (n=3)	Fortified Laboratory Blank ^c (n=1)	Laboratory Fortified Sample Matrix (n=3)	% RSD Spiked
Heptachlor	0.39	1.0	96	76	8
Hexachlorobenzene	0.13	0.11	77	61	7
Lindane	0.49	0.18	96	88	10
cis-Permethrin	-	INT ^b	INT	INT	-
trans-Permethrin	0.29	-	66	65	4
<u>Surrogates</u>					
Atrazine-d ₅	NS ^d	NS	NS	NS	3
Chrysene-d ₁₂	82 ^d	83 ^d	78	83	3
Dimethylnitrobenzene	68 ^d	47 ^d	113	94	8
Fluorene-d ₁₀	80 ^d	94 ^d	102	99	4
Pentachloronitrobenzene	80 ^d	98 ^d	102	103	4
<u>Carbamates</u>					
Aldicarb	-	-	65	83	15
Carbaryl	-	1.3	97	89	6
Carbofuran	-	1.8	84	75	4
Methomyl	-	INT	INT	INT	-
Propoxur	-	-	96	87	6

^a Samples spiked at 10 ppb.

^b Not detected.

^c Not spiked.

^d % recovery.

^e Interferent in sample prevented quantitation.

TABLE 7. STORAGE STABILITY STUDY FOR BASE/NEUTRAL PESTICIDES - COMPOSITE SAMPLE 5, T = 21 DAYS

Compound	Concentration (ppb)		% Recovery ^a		
	Laboratory Reagent Blank (n=1)	Mean Unspiked (n=3)	Fortified Laboratory Blank ^c (n=1)	Laboratory Fortified Sample Matrix (n=3)	% RSD Spiked
Polar					
Acetochlor	- ^b	-	74	72	25
Alachlor	-	-	92	79	24
Atrazine	-	-	86	71	20
Captan	-	-	-	-	-
Dacthal	-	0.08	74	82	25
Diazinon	-	-	96	83	24
Dichloran	-	-	91	90	23
Dichlorvos	-	-	75	70	25
Folpet	-	-	26	3	55
Fonofos	-	-	97	85	23
Isofenphos	-	0.25	90	76	25
Malathion	-	2.09	97	78	39
Metolachor	-	-	88	67	39
Parathion	-	-	98	83	25
Phorate	-	-	86	69	25
Simazine	-	-	79	97	23
Terbufos	-	-	94	75	31
Trifluralin	-	0.06	93	82	25
Nonpolar					
Aldrin	-	-	66	62	33
γ -Chlordane	0.17	0.17	89	78	24
α -Chlordane	0.11	0.11	89	77	25
Chlorpyrifos	-	0.56	95	82	34
4,4'-DDD	-	0.12	76	74	26
4,4'-DDE	-	0.30	90	75	28
4,4'-DDT	-	0.63	96	78	28
Dieldrin	-	-	96	82	28

(continued)

TABLE 7. (continued)

Compound	Concentration (ppb)		% Recovery ^a		
	Laboratory Reagent Blank (n=1)	Mean Unspiked (n=3)	Fortified Laboratory Blank ^c (n=1)	Laboratory Fortified Sample Matrix (n=3)	% RSD Spiked
Endosulfan I	-	-	86	74	24
Endrin	-	-	91	77	24
Heptachlor	0.50	0.57	92	78	29
Hexachlorobenzene	-	0.04	63	57	32
Lindane	-	-	75	78	26
cis-Permethrin	-	-	65	31	25
trans-Permethrin	-	-	74	52	24
<u>Surrogates</u>					
Atrazine-d ₅	NS ^b	NS	NS	NS	NS
Chrysene-d ₁₂	90 ^d	27 ^d	88	74	10
Dimethylnitrobenzene	88 ^d	73 ^d	18	113	16
Fluorene-d ₁₀	94 ^d	67 ^d	96	93	17
Pentachloronitrobenzene	92 ^d	64 ^d	88	89	17
<u>Carbamates</u>					
Aldicarb	-	-	34	70	38
Carbaryl	-	-	95	95	23
Carbofuran	-	-	79	78	24
Methomyl	-	-	69	55	14
Propoxur	-	-	75	75	23

^a Samples spiked at 10 ppb.

^b Not detected.

^c Not spiked.

^d % recovery.

TABLE 8. ESTIMATED DETECTION LIMITS (EDL) - COMPOSITE SAMPLE FROM PARTICIPANT 5
 PREPILOT STUDY

Compound	Laboratory Reagent Blank (ng/g)	Laboratory Measured Concentration (ng/g)		EDL* (ng/g)	Spiking Level (ng/g)
		Mean (n=7)	S.D.		
<u>Polar</u>					
Acetochlor	- ^b	1.78	0.31	0.96	2.0
Alachlor	-	0.86	0.12	0.39	1.0
Atrazine	-	1.85	0.52	1.64	2.5
Captan	0.00	0.00	0.00	0.00	2.0
Dacthal	-	0.33	0.02	0.06	0.5
Diazinon	-	0.78	0.09	0.29	0.5
Dichloran	-	1.17	0.16	0.50	2.0
Dichlorvos	-	0.35	0.03	0.11	0.5
Folpet	0.00	2.48	0.45	1.41	5.0
Fonofos	-	0.98	0.10	0.32	1.0
Isofenphos	-	2.75	0.57	1.80	2.5
Malathion	-	3.60	0.65	2.03	3.0
Metolachor	-	1.86	0.26	0.80	2.0
Parathion	-	1.20	0.25	0.77	1.0
Phorate	-	0.39	0.10	0.31	0.5
Simazine	-	1.69	0.56	1.75	2.5
Terbufos	-	1.09	0.15	0.49	1.0
Trifluralin	-	0.30	0.06	0.19	0.5
<u>Nonpolar</u>					
Aldrin	-	0.57	0.07	0.23	1.0
γ -Chlordane	0.13	0.67	0.05	0.16	0.5
α -Chlordane	0.05	0.54	0.04	0.14	0.5
Chlorpyrifos	-	2.63	0.50	1.58	2.0
4,4'-DDD	-	0.38	0.02	0.06	0.5
4,4'-DDE	0.03	0.43	0.04	0.13	0.5
4,4'-DDT	-	0.13	0.04	0.13	0.1
Dieldrin	-	1.39	0.24	0.75	2.0
Endosulfan I	-	1.35	0.22	0.69	2.5
Endrin	-	1.47	0.26	0.81	2.0

(continued)

TABLE 8. (continued)

Compound	Laboratory	Laboratory Measured Concentration (ng/g)			EDL* (ng/g)	Spiking Level (ng/g)
	Reagent Blank (ng/g)	Mean (n=7)	S.D.			
Heptachlor	0.26	0.37	0.14	0.44	NS	
Hexachlorobenzene	-	0.15	0.01	0.05	0.25	
Lindane	-	1.92	0.15	0.46	2.5	
cis-Permethrin	-	3.5	1.68	5.28	5.0	
trans-Permethrin	0.03	6.4	0.96	3.03	5.0	
<u>Surrogates</u>						
Atrazine-d ₅	Ns ^c	NS	-	Na ^d	8.0	
Chrysene-d ₁₂	81 ^e	79	13	NA	10	
Dimethylnitrobenzene	78 ^e	102	10	NA	10	
Fluorene-d ₁₀	81 ^e	98	6	NA	10	
Pentachloronitrobenzene	81 ^e	103	11	NA	20	
<u>Carbamates</u>						
Aldicarb	-	5.3	0.08	0.27	5.0	
Carbaryl	-	6.3	0.19	0.64	5.0	
Carbofuran	-	5.2	0.29	0.97	5.0	
Methomyl	-	INT ^f	-	-	5.0	
Propoxur	-	5.2	0.36	1.2	5.0	

* Estimated detection limit based on a final sample volume of 0.5 mL. Current procedures have a final volume of 1.0 mL.

^b No instrumental signal.

^c Not spiked.

^d Not applicable.

^e % received.

^f Interferent in sample prevented quantitation.

LABORATORY OPERATIONS PROTOCOL	RESEARCH TRIANGLE INSTITUTE POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709-2194	RTVACS-AP-211-132																												
Page 1 of 11																														
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AUTHOR(s):	Mike R. Gord	Date: 3/24/98																												
	Kurt W. Warner	Date: 3/24/98																												
		Date: _____																												
APPROVED BY:																														
Principal Investigator:	E. Pelliyan	Date: 3/26/98																												
QA Officer:	Doris Smith	Date: 3/23/98																												
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**SOXHLET EXTRACTION OF BASE/NEUTRAL PESTICIDES
IN COMPOSITE FOOD SAMPLES**

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1.0 SCOPE AND APPLICATION

The procedures described in this protocol are designed to extract base/neutral pesticides from composited, homogenized food samples. The target analytes for this method are shown in Table 1. This protocol describes the extraction process.

2.0 SUMMARY OF THE METHOD

This method is for the extraction of a 25 g aliquot of a composited, homogenized food sample. A schematic of the method is shown in Figure 1. The homogenized food sample is first mixed with zinc acetate to minimize fatty acid interferences. The sample is then mixed with anhydrous sodium sulfate to remove water. The sample is Soxhlet extracted with methylene chloride. The extract is solvent exchanged into n -hexane and concentrated to 10 mL for subsequent cleanup.

3.0 DEFINITIONS

- 3.1 Refer to RTI/ACS-AP-211-131.
- 3.2 Equivalent sample weight (ESW)

When food samples are homogenized, water may be added to the sample to aid in the process. The equivalent sample weight is the weight of the food sample homogenate used for extraction excluding the addition of water.

4.0 INTERFERENCES

Refer to RTI/ACS-AP-211-131.

5.0 SAFETY

- 5.1 The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Additional references to laboratory safety are available for the information of the analyst.
- 5.2 Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens including aldrin, chlordane, dieldrin, and heptachlor. Pure standard materials and stock standard solutions of these compounds should be handled in a hood or glovebox with suitable protection to skin, eyes, etc.

5.3 Methylene Chloride

Avoid breathing vapors and contact with skin. TLV is 50 ppm (TWA over 8 h). May be carcinogenic to humans.

6.0 EQUIPMENT AND SUPPLIES

- 6.1 Micro Syringes - 10 and 100 μL .
- 6.2 Pipettes - 1.0 and 10.0 mL transfer.
- 6.3 Graduated Cylinders.
- 6.4 Standard Solution Storage Containers
Fifteen mL amber vials with Teflon-lined screw caps.
- 6.5 Stainless steel spoons.
- 6.6 Beakers - 250 mL and 500 mL.
- 6.7 Water bath for K-D concentrations.
- 6.8 Kuderna-Danish Concentrators
Five hundred mL capacity with Snyder columns and modified micro-Snyder columns.
- 6.9 Analytical Balance
Capable of weighing to 100 g.

6.10 Analytical Balance

Capable of weighing to nearest 0.001 g.

6.11 Aluminum weigh pans.

6.12 Boiling Chips

Solvent extracted, 10/40 mesh.

6.13 Soxhlet Extraction Equipment

6.13.1 Soxhlet Extractor - including round bottom flask, Soxhlet extractor, and condenser - 500 mL volume.

6.13.2 Heating Mantles.

6.13.3 Variable Transformers.

6.13.4 Extraction thimbles - 200 mL capacity.

7.0 REAGENTS AND STANDARDS

7.1 Reagents

7.1.1 Methylene chloride - demonstrated to be free of analytes.

7.1.2 n-Hexane - demonstrated to be free of analytes.

7.1.3 Zinc acetate - dihydrated, crystal, ACS Reagent Grade, demonstrated to be free of analytes.

7.1.4 Sodium sulfate, granular anhydrous, ACS grade, demonstrated to be free of analytes.

7.1.5 Potassium oxalate - demonstrated to be free of analytes.

7.2 Reagent Water

7.2.1 Reagent water is defined as water free of interference when employed in the procedure described herein.

7.2.2 A Millipore Super - Q Water System or its equivalent may be used to generate reagent water.

7.3 Surrogate Standards

A surrogate standard (i.e., a chemically inert compound not expected to occur in an environmental sample) should be added to each sample, blank, and matrix spike sample just prior to extraction or processing. The recovery of the surrogate standard is used to monitor for unusual matrix effects, gross sample processing errors, etc. Surrogate recovery is evaluated for acceptance by determining whether the measured concentration falls within the acceptance limits. Surrogate standard solutions are prepared in toluene such that a 50 µL aliquot added to the 25 g food homogenate will result in the desired spiking level (i.e., 10 ng/g). The following compounds have shown to be acceptable for surrogate use: atrazine-d₅, chrysene-d₁₂, and pentachloronitrobenzene.

7.4 Matrix Spike Standards

A spiking solution is prepared in toluene which contains all of the target pesticides such that a 25 µL aliquot added to the 25 g food homogenate or neutral reference material will result in the desired spiking level (10 ng/g). This solution will be used to prepare the laboratory fortified sample matrix and the laboratory fortified blank.

8.0 HOMOGENIZATION OF COMPOSITE SOLID FOOD SAMPLES

8.1 Samples will be homogenized according to RTI/ACS-AP-211-038. The homogenates will be stored at -20°C until extraction.

9.0 QUALITY CONTROL

Refer to RTI/ACS-AP-211-131.

10.0 CALIBRATION AND STANDARDIZATION

10.1 The calibration and standardization procedures are addressed in individual cleanup and analysis methods.

11.0 PROCEDURE

11.1 Sample Preparation

Remove samples from freezer and allow them to equilibrate to room temperature. Stir the sample well.

11.2 Soxhlet Extraction

11.2.1 Place an approximately 25 g aliquot of homogenized food sample in a 250 mL preweighed beaker. Weigh the sample plus beaker to the nearest 0.1 g. The homogenate sample weight (HSW) is the weight of the sample plus beaker minus the weight of the beaker. The equivalent sample weight (ESW) is the weight of the food sample prior to the addition of water during sample homogenization; it is calculated from the HSW as

$$ESW = HSW - (HWS \times P)$$

where P is the percentage of water added to the sample during homogenization. Record the HSW and the ESW to the nearest 0.1 g. Blend with 20 g zinc acetate and allow to react for 2 hours. If the zinc acetate does not sufficiently dissolve into the sample add reagent grade water until the zinc acetate is totally dissolved.

11.2.2 Add 2 g potassium oxalate to the food sample aliquot and blend. Add enough anhydrous sodium sulfate (~120 g) to completely dry the sample. Let the sample dry for 3 hours longer if needed. Stir the mixture occasionally to prevent clumping.

11.2.3 Place the mixture into a Soxhlet extraction thimble and spike the mixture by adding 25 μ L of the surrogate standard spiking solution. Spike laboratory fortified blanks and laboratory fortified sample matrices by adding 25 μ L of the matrix spike standard. Place the extraction thimble into a clean Soxhlet extractor body.

11.2.4 Extract the sample using 300 mL of methylene chloride for 12 to 20 hours.

11.3 Extract Concentration

- 11.3.1 Assemble a K-D concentrator by attaching a 10-mL concentrator tube to a 500 mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 9.0 of RTI/ACS-AP-209-131.
- 11.3.2 While the solvent is still warm add 5 g of sodium sulfate to the round bottom flask and swirl to remove any residual water that may be in the sample extract. Allow the solvent to cool to room temperature.
- 11.3.3 Decant the sample extract into the K-D concentrator. Rinse the round-bottom flask with two 25-mL portions of methylene chloride and decant the rinses into the K-D concentrator.
- 11.3.4 Add 1 to 2 clean boiling stones to the evaporative flask and attach a macro Snyder column. Place the K-D apparatus on a hot water bath, 65 to 70°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.
- 11.3.5 Add 50 mL of *n*-hexane to the K-D apparatus and repeat 11.3.3 adjusting the final volume to 10 mL with *n*-hexane if using RTI/ACS-AP-209-133.

12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 Concentrations of analytes should be based on the equivalent weight of the food sample (ESW); not the weight of homogenate used. The ESW is the weight of the food sample excluding the water added for homogenization.

13.0 METHOD PERFORMANCE

13.1 The method should be applicable to those pesticides and food types as shown in Table 1. Performance of the method in linked analysis schemes is given in RTI/ACS-AP-211-131.

14.0 POLLUTION PREVENTION

14.1 Always follow good laboratory practices when carrying out procedures. Consider air, soil and water pollution as the primary concerns. Order the least amount of solvent, materials, and reagents required. Do not discard solvents down the drain.

15.0 WASTE MANAGEMENT

15.1 Always dispose of spent or unused solvents by a properly licensed waste disposal company. Always properly label all waste. Store flammable waste in a storage container with a flash arrestor.

16.0 REFERENCES

1. Sheldon, L.S. Manual of Analytical Methods for Determination of Selected Environmental Contaminants in Composite Food Samples. EPA-68-C2-0103, U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 1995.
2. Test Methods for Evaluating Solid Waste (SW-846), Vol. IB. U.S. Environmental Protection Agency, Sept. 1986, Method 3600.
3. Sheldon, L., J. Keever, J. Beach, M. Roberds and L. Ellis. Environmental Contaminants in Foods: Phase 3 - Validation of Analytical Methods. Final Report, U.S. EPA EMSL-Cincinnati Contract Number 68-C2-0103, Work Assignment 2-01.

TABLE 1. APPLICABILITY OF RTI/ACS-AP-211-132

Compound	Applicable Food Type		
	Composite	Fatty	Nonfatty
POLAR BASE/NEUTRAL PESTICIDES			
Acetochlor	D	A	A
Alachlor	D	A	A
Atrazine	D	D	A
Dacthal	D	A	A
Diazinon	D	A	A
Dichloran	D	A	A
Dichlorvos	D	A	A
Fonofos	D	A	A
Isofenphos	D	A	A
Malathion	D	A	A
Metolachor	D	A	A
Parathion	D	A	A
Phorate	D	A	A
Simazine	D	A	A
Terbufos	D	A	A
Trifluralin	D	A	A
NONPOLAR BASE/NEUTRAL PESTICIDES			
Aldrin	D	A	A
α -Chlordane	D	A	A
γ -Chlordane	D	A	A
Chlorpyrifos	D	D	A
4,4'-DDD	D	A	A
4,4'-DDE	D	A	A
4,4'-DDT	D	D	A
Dieldrin	D	A	A
Endosulfan I	D	A	A
Endrin	D	A	A
Heptachlor	D	A	A
Hexachlorobenzene	D	A	A
Lindane	D	A	A
cis-Permethrin	D	A	A
trans-Permethrin	D	A	A
CARBAMATES			
Aldicarb	D	A	A
Carbaryl	D	A	A
Carbofuran	D	A	A
Methomyl	D	A	A
Propoxur	D	A	A

* 1° - Priority 1 pesticides; 2° - priority 2 pesticides.

D = Demonstrated through laboratory testing.

A = Should be applicable based on performance in other matrices.

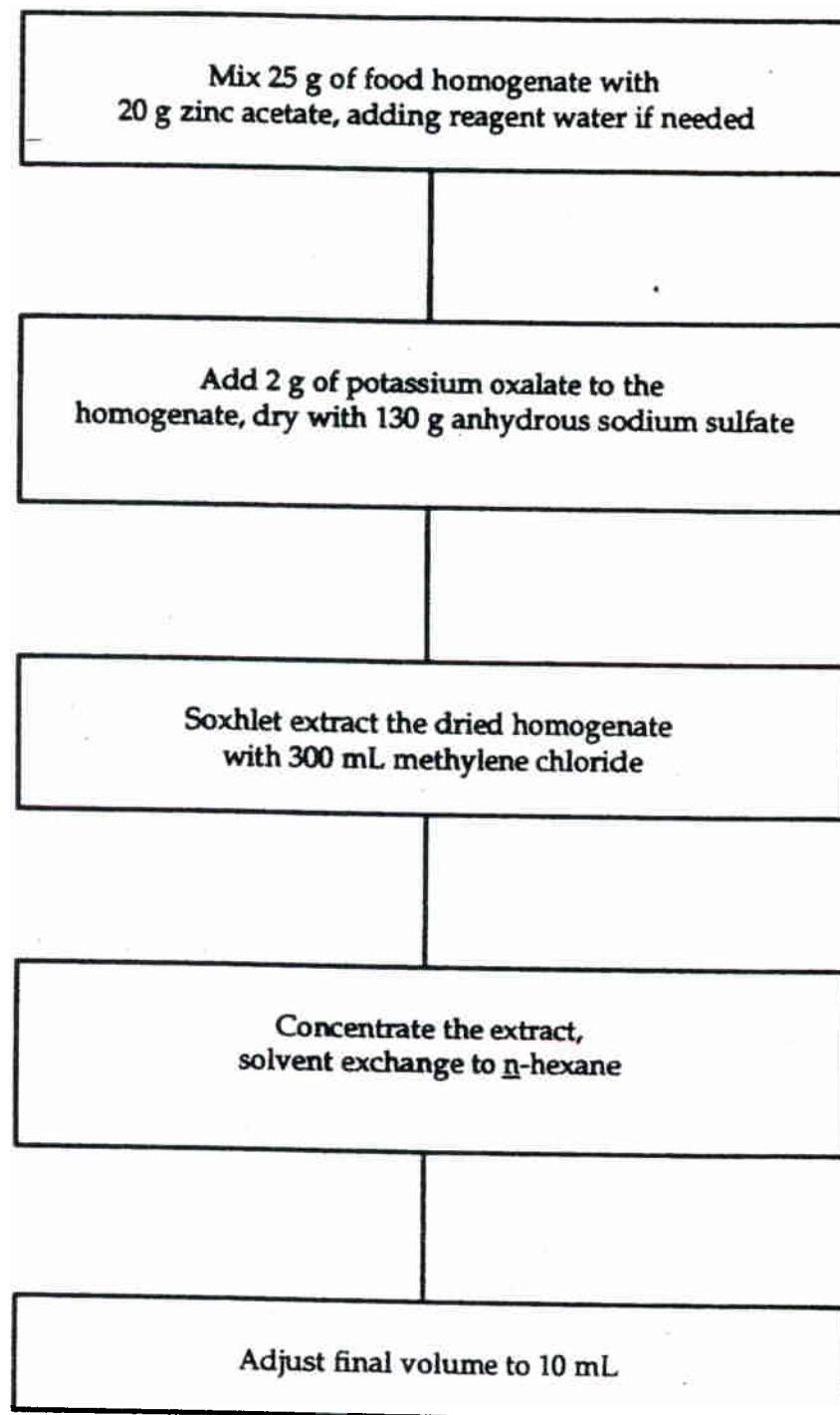


Figure 1. Flow diagram of RTI/ACS-211-132.

TITLE:

n-HEXANE/ACETONITRILE PARTITIONING OF BASE/NEUTRAL PESTICIDES IN FOOD SAMPLE EXTRACTS

SOURCE:

Research Triangle Institute
Post Office Box 12194
Analytical and Chemical Sciences
Research Triangle Park, NC 27709-2194

AUTHOR(s):

Mike Polcari

Date: 3/24/98

Kent W. Thurman

Date: 3/24/98

Date: _____

APPROVED BY:

Principal Investigator:

E. Pelliypin

Date: 3/26/98

QA Officer:

Doris Smith

Date: 3/25/98

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	DRAFT:	<input type="checkbox"/>
	FINAL VERSION:	<input checked="" type="checkbox"/>

REVISIONS:

No.	Date	No.	Date
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‡ Effective date of this version is the date of the last approval signature;
revision 0 is the original version.

**n-HEXANE/ACETONITRILE PARTITIONING OF BASE/NEUTRAL PESTICIDES
IN FOOD SAMPLE EXTRACTS**

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1.0 SCOPE AND APPLICATION

This method uses liquid-liquid partitioning to remove fats from extracts of composited, homogenized food samples. The target analytes for this method are shown in Table 1.

2.0 SUMMARY OF THE METHOD

This method is used to separate fats from pesticides in food extracts. This method is generally used as a preliminary cleanup step to remove large amounts of fats from sample extracts prior to gel permeation chromatography (RTI/ACS-AP-211-134). The food extract in η -hexane is partitioned with acetonitrile, then further extracted with methylene chloride. The pesticides are partitioned into the methylene chloride while the fat remains in the η -hexane solvent. The extract is then concentrated for further cleanup. The final sample extract is in acetonitrile.

3.0 DEFINITIONS - Refer to RTI/ACS-AP-211-131.

4.0 INTERFERENCES - Refer to RTI/ACS-AP-211-131.

5.0 SAFETY

5.1 The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Additional references to laboratory safety are available for the information of the analyst.

5.2 The following organohalides have been tentatively classified as known or suspected human or mammalian carcinogens: aldrin, chlordane, dieldrin, and heptachlor. Pure standard materials and stock standard solutions of these compounds should be handled in a hood or glovebox.

5.3 **Acetonitrile**

Avoid contact with skin. Threshold Limit Value (TLV) is 40 ppm (skin, time weighted average (TWA) over 8 h).

6.0 **EQUIPMENT AND SUPPLIES**

- 6.1 Separatory funnels, 60 or 125 mL capacity.
- 6.2 Graduated cylinders, 10 mL to 100 mL capacity.
- 6.3 Kuderna-Danish (K-D) concentrators, Snyder columns and modified micro-Snyder columns.
- 6.4 Water bath for K-D concentrations.
- 6.5 Boiling chips.

7.0 **REAGENTS AND STANDARDS**

7.1 **Reagents**

- 7.1.1 n-Hexane - demonstrated to be free of reagents.
- 7.1.2 Acetonitrile - demonstrated to be free of reagents.

8.0 **SAMPLE COLLECTION, PRESERVATION AND STORAGE**

8.1 It is anticipated that extracts will be processed shortly after extraction is complete. It is the analyst's responsibility to assure that any storage of extracts does not affect analytical results. All extracts should be protected from light. Extracts may be stored at 4°C for short periods of time (<48 hours). Extracts should be stored at $\leq -10^{\circ}\text{C}$ for longer periods of time.

9.0 QUALITY CONTROL - Refer to RTI/ACS-AP-209-131.**10.0 CALIBRATION AND STANDARDIZATION**

10.1 No calibration or standardization procedures are required for this cleanup method.

11.0 PROCEDURE

11.1 Take the sample extract in 10 mL of *n*-hexane and transfer to a 125 mL separatory funnel. Rinse the extract container with 2 x 2.5 mL of *n*-hexane.

NOTE: If the sample has a very high fat content (e.g., fat in the sample prevents extract concentration below 7.5 mL), the extract may be diluted to 15 mL with *n*-hexane.

11.2 Add 30 mL of acetonitrile saturated with *n*-hexane. Shake gently for 1 min, and let the layers separate. For samples that do not separate initially allow the layers to separate for up to an hour as needed. Drain the acetonitrile (lower) layer into a 500 mL separatory funnel containing 200 mL of reagent grade water (pH 7-8) and 6 g of sodium chloride taking care not to allow any emulsions to drain through with the sample.

11.3 Repeat Step 11.2.

11.4 Assemble a K-D concentrator by attaching a 10 mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if adequate performance can be demonstrated.

11.5 Add 100 mL of methylene chloride and shake vigorously for 1 min, and let the layers separate. Drain the methylene chloride (layer) into a K-D concentrator.

11.6 Repeat Step 11.5.

11.7 Extract Concentration

11.7.1 Add 1 to 2 clean boiling stones to the K-D concentrator and attach a macro Snyder column. Wet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 65 to 70°C, so that the concentrator tube is partially immersed in the hot water, and the entire

lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 5-10 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min. Rinse the K-D apparatus with methylene chloride. Remove the evaporative flask from the concentrator tube.

- 11.7.2 Add several boiling chips to the concentrator tube, then attach a modified micro-Snyder column. Place in a hot water bath at 45 to 50°C. Let solvent evaporate to <0.2 mL. Rinse modified micro-Snyder column. Adjust final volume to 1.0 mL with solvent to be used for additional cleanup or analysis.

12.0 DATA ANALYSIS AND CALCULATION

- 12.1 Since the entire sample extract is cleaned up, no adjustment in final sample concentration should be made for the cleanup procedure.

13.0 METHOD PERFORMANCE

- 13.1 Method performance data for this procedure are given in Table 1. Performance of linked analysis schemes incorporating this cleanup step are given in RTI/ACS-AP-209-131.

14.0 POLLUTION PREVENTION

- 14.1 Always follow good laboratory practices when carrying out procedures. Consider air, soil and water pollution as the primary concerns. Order the least amount of solvent, materials, and reagents required. Do not discard solvents down the drain.

15.0 WASTE MANAGEMENT

15.1 Always dispose of spent or unused solvents by a properly licensed waste disposal company. Always properly label all waste. Store flammable waste in a storage container with a flash arrestor.

16.0 REFERENCES

1. Sheldon, L.S. Manual of Analytical Methods for Determination of Selected Environmental Contaminants in Composite Food Samples. EPA-68-C2-0103, U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 1995.
2. U.S. FDA. Pesticide Analytical Manual, Vol. I., 3rd edition, U.S. FDA, Washington, DC, 1994.
3. DiMuccio, A., Cicero, A.M., Camoni, I., Pontecorvo, D., and Dommarco, R. On-Column Partition Cleanup of Fatty Extracts for Organophosphorus Pesticide Residue Determination. J. Assoc. Off. Anal. Chem., 70(1):106-108, 1987.
4. Sheldon, L., Keever, J., Beach, J., Roberds, M., and Ellis, L. Environmental Contaminants in Foods: Phase 3 - Validation of Analytical Methods. Final Report, U.S. EPA EMSL-Cincinnati Contract Number 68-C2-0103, Work Assignment 2-01, 1994.

TABLE 1. PERCENT RECOVERY OF BASE/NEUTRAL PESTICIDES
 DETERMINED USING RTI/ACS-AP-211-133

Compound	% Recovery ^{a,b}	
	Mean (n=2)	S.D.
Alachlor	97	1
Aldrin	56	6
Atrazine	102	6
Captan	0	0
Carbaryl	A ^c	-
α -Chlordane	95	2
γ -Chlordane	93	5
Chlorpyrifos	84	3
4,4'-DDD	94	0
4,4'-DDE	76	1
4,4'-DDT	95	1
Dacthal	96	1
Diazinon	87	1
Dichloran	67	0
Dieldrin	95	7
Folpet	0	0
Heptachlor	82	9
Malathion	105	0
Metolachor	99	0
cis-Permethrin	85	5
trans-Permethrin	95	4
Phorate	87	2
Propoxur	97	7
Trifluralin	77	0
Acetochlor	A	-
Aldicarb	A	-
Carbofuran	A	-
Dichlorvos	83	3
Endosulfan I	94	10
Endrin	100	0
Fonofos	87	2
Hexachlorobenzene	44	3
Isofenphos	93	2
Lindane	103	3
Methomyl	A	-
Parathion	77	7
Simazine	89	11
Terbufos	86	0

^a Analyzed by GC/MS as described in Method PA-01; recovery for cleanup method only.

^b All pesticides spiked at 250 ng, equivalent to 10 ng/g for a 25 g sample.

^c Not tested. Should be applicable based on results for linked analysis scheme using RTI/ACS-AP-211-133.

TITLE: GEL PERMEATION CHROMATOGRAPHY OF PESTICIDES AND PAHs
IN COMPOSITE FOOD SAMPLES

SOURCE: Research Triangle Institute
Post Office Box 12194
Analytical and Chemical Sciences
Research Triangle Park, NC 27709-2194

AUTHOR(s):

Mike Robard

Date: 3/24/98

Date: _____

Date: _____

APPROVED BY:

Principal Investigator:

E. Pellijsai

Date: 3/26/98

QA Officer:

Doris Smith

Date: 3/25/98

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**GEL PERMEATION CHROMATOGRAPHY OF PESTICIDES AND PAHs
IN COMPOSITE FOOD SAMPLES**

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1.0 SCOPE AND APPLICATION

1.1 This method is intended for removal of fats or other high molecular weight interferences from food extracts by gel permeation chromatography (GPC). The method should be applicable to extracts from composited, homogenized food samples. For food extracts to be analyzed for pesticides, a preliminary cleanup (RTI/ACS-AP-211-133) will be required to remove high amounts of fats. The target analytes for this method are shown in Table 1. This method describes the cleanup of samples using gel permeation chromatography.

2.0 SUMMARY OF THE METHOD

Food extracts containing less than 0.5 g fat are injected onto a high performance liquid chromatographic column which separates the higher molecular weight coextractives from the lower molecular weight analytes using size exclusion chromatography. High molecular weight compounds, including fats, elute quickly while smaller compounds (pesticides) take longer to elute. Once the column is calibrated, a fraction of the elute is taken which contains the analytes while removing the higher molecular weight interferences. This method is adapted from U.S. EPA Method 3640A (U.S. EPA, 1991).

3.0 DEFINITIONS - Refer to RTI/ACS-AP-211-131 and RTI/ACS-AP-211-136.

4.0 INTERFERENCES - Refer to RTI/ACS-AP-211-131 and RTI/ACS-AP-211-136.

5.0 SAFETY

5.1 The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining

awareness of OSHA regulations regarding safe handling of chemicals used in this method.

Additional references to laboratory safety are available for the information of the analyst.

5.2 The following organohalides have been tentatively classified as known or suspected human or mammalian carcinogens: aldrin, chlordane, dieldrin, and heptachlor. Pure standard materials and stock standard solutions of these compounds should be handled in a hood or glovebox.

5.3 Methylene Chloride

Avoid breathing vapors and contact with skin. Threshold Limit Value (TLV) is 50 ppm (skin, time weighted average (TWA) over 8 h). May be carcinogenic to humans.

6.0 EQUIPMENT AND SUPPLIES

6.1 Gel Permeation Chromatography System - may be automatic or manual.

- 6.1.1 A system capable of maintaining a flow rate of 4 to 6 mL/min and which meets the calibration requirements in Section 10.
- 6.1.2 An injector consisting of a 6-port valve with a 2 m x 3.2 mm i.d. stainless-steel loop (> 3.0 mL volume) and a short length of Teflon tubing to serve as a syringe port.

6.1.3 Chromatographic Column

- 6.1.3.1 Chromatographic column and packing - 250 mm x 22.5 mm stainless steel column with 5 μ m 100 angstrom porosity styrene - divinylbenzene copolymer packing with molecular weight exclusion limit of 1000 (e.g., Phenogel Prep-100, Phenomenex, Inc., Torrance, CA), or the equivalent.

- 6.1.3.2 Guard column (optional) - 75 mm x 21.2 - 22.5 mm column with the same packing as the main column (recommended).

- 6.1.4 UV Detector - Fixed wavelength (254 nm) with a semi-prep flow through cell.

- 6.1.5 Strip chart recorder or laboratory data system.

- 6.2 Syringe - 1 mL, with Luer-Lok fitting.

- 6.3 Syringe filter assembly, 25 mm Bio-Rad "Prep Disc" sample filter assembly #343-0005, and 0.45 µm filter discs, or equivalent. Rinsed with methylene chloride before use.
- 6.4 Analytical balance - capable of weighing to 0.0001 g.
- 6.5 Volumetric flasks - 10 mL to 100 mL, Class A.
- 6.6 Graduated cylinders.
- 6.7 Kuderna-Danish (K-D) concentrators, Snyder columns and modified micro-Snyder columns.
- 6.8 Water bath for K-D concentrations.
- 6.9 Boiling chips.

7.0 REAGENTS AND STANDARDS

7.1 Methylene Chloride

NOTE: Methylene chloride may have HCl present. Check by extracting HCl into equal volume of water and check pH of water. Correct if pH \leq 5 by filtering solvent through activated basic alumina or using another lot.

- 7.2 Corn oil solution – 5 mg/100 µL methylene chloride.
- 7.3 GPC calibration solution. Prepare a solution of the following in methylene chloride.

Compound	Conc., mg/L
corn oil	25,000
bis(2-ethylhexyl)phthalate	1,000
methoxychlor	200
perylene	20
sulfur*	80

*Soluble in warm corn oil.

7.4 Standards

Matrix spike standard - A spiking solution is prepared in toluene which contains all of the target pesticides such that a 50 μ L aliquot will result in the desired spiking level (250 ng/sample). This solution is used to prepare the laboratory fortified blank.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

It is anticipated that extracts will be processed shortly after extraction is complete. It is the analyst's responsibility to assure that any storage of extracts does not affect analytical results. All extracts should be protected from light. Extracts may be stored at 4°C for short periods of time (<48 hours). Extracts should be stored at \leq -10°C for longer periods of time.

9.0 QUALITY CONTROL

9.1 Before incorporating the GPC method into a linked analysis scheme, the analyst must process and analyze a minimum of one laboratory reagent blank to demonstrate that the method is free from background contamination. The analyst should also process and analyze a minimum of one laboratory fortified blank to demonstrate that the analytes are being recovered (75 to 125%) during the GPC step.

9.2 Refer to RTI/ACS-AP-211-131 and RTI/ACS-AP-211-136 for the quality control for the entire analysis scheme.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Load sample loop with GPC calibration solution (Section 7.3) using a 1 mL syringe. Follow this with 2.0 mL methylene chloride.

10.2 Inject the calibration solution and obtain a UV trace showing a discrete peak for each component. Adjust the detector and/or recorder sensitivity to produce a UV trace that meets the criteria in Section 10.3. ~~Differences between manufacturers' cell volumes and detector sensitivities~~ may require a dilution of the calibration solution to achieve similar results. An

analytical flow-through detector cell will require a much less concentrated solution than the semi-prep cell, and therefore the analytical cell is not acceptable for use.

10.3 Evaluate the UV Chromatogram for Column Condition using the Following Criteria

10.3.1 Peaks must be observed, and should be symmetrical, for all compounds in the calibration solution.

10.3.2 Corn oil and phthalate peaks must exhibit >85% baseline resolution.

10.3.3 Phthalate and methoxychlor peaks must exhibit >85% baseline resolution.

10.3.4 Methoxychlor and perylene peaks must exhibit >85% baseline resolution.

10.3.5 Perylene and sulfur peaks must not be saturated and must exhibit >90% baseline resolution.

10.3.6 It may be necessary to replace the chromatographic column or guard column if the column performance fails the criteria given in Section 10.3.1 to 10.3.5.

10.4 Calibration for Base/Neutral Pesticides

Determine the elution times for the phthalate, methoxychlor, perylene, and sulfur. Choose a dump time which removes all of the corn oil but collects 60% of the phthalate. Stop collection after the elution of perylene, but before the elution of sulfur.

10.5 Verify the flow rate by collecting the column eluate for 10 minutes in a graduated cylinder and measuring the volume. This should be 45-55 mL (4.5-5.5 mL/min). If the flow rate is outside of this range, corrective action must be taken. Once the flow rate is within the range of 4.5-5.5 mL/min, record the column pressure and temperature. Changes in pressure, solvent flow rate, and temperature conditions can affect analyte retention times, and must be monitored.

10.6 Reinject the calibration solution after appropriate collect and dump cycles have been set, and the solvent flow and column pressure have been established.

10.7 Measure and record the volume of collected GPC eluate in a graduated cylinder. The volume of GPC eluate collected for each sample extract processed may be used to indicate problems with the system during sample processing.

The retention times for bis(2-ethylhexyl)phthalate and perylene must not vary more than $\pm 5\%$ between calibrations. If the retention time shift is $>5\%$, take corrective action.

Excessive retention time shifts are caused by:

- Poor laboratory temperature control or system leaks.
- An unstabilized column that requires pumping solvent through it for several more hours or overnight.
- Excessive laboratory temperatures, causing outgassing of the solvent.

11.0 PROCEDURE

This technique is recommended for the elimination of lipids, polymers, proteins, steroids, and other dispersed high molecular weight compounds. This procedure is provided to describe the important parameters regardless of whether the system used is manual or automatic in operation.

11.1 Column preparation - follow the manufacturer's recommendations.

11.2 Extract Preparation

11.2.1 Adjust the extract volume to 1.0 mL with methylene chloride. The solvent extract must be primarily methylene chloride. All other solvents, e.g. 1:1 methylene chloride/acetonitrile, must be concentrated to 0.5 mL (or as low as possible if a precipitate forms) and diluted to 1.0 mL with methylene chloride. Thoroughly mix the sample before proceeding.

11.2.2 Filter the extract through a 0.45 μm filter by attaching a 1.0 mL syringe to the filter disk. Pipet the extract into the syringe barrel. Attach the plunger to the barrel and carefully but firmly push the extract through the filter. Draw the plunger out and add a 0.5 mL rinse of the extract container to the barrel, push the rinse through the filter. Repeat with another 0.5 mL rinse. If the filter breaks at any point, the extract must be reconcentrated and refiltered. Particles larger than 0.5 mm will damage the GPC column or clog the column frit.

NOTE: Viscosity of a sample extract should not exceed the viscosity of 1:1 water/glycerol. Dilute samples that exceed this viscosity.

11.3 GPC Cleanup

- 11.3.1 Calibrate the GPC at the start of each day following the procedure outlined in Section 10. Ensure that UV trace requirements, column flow rate, and column pressure criteria are acceptable. Also, the retention time shift must be <5% when compared to retention times in the last calibration UV trace.

NOTE: If these criteria are not met, try cleaning the column by flushing the column with mobile phase. Do not use any solvent on the column other than that used for the mobile phase. Check with the manufacturer before changing solvents, since this is a common cause of column failure. If a guard column is being used, replace it with a new one. This may correct the problem. If column maintenance does not restore acceptable performance, the column must be replaced.

- 11.3.2 Draw up a 1 mL portion of the extract into a 1 mL syringe and remove any air bubbles. Turn the injector valve to the load position. Attach the syringe to the syringe port on the injector valve. Pull up on the syringe plunger to drain out any air that may be left in the valve port. Push the extract onto the loop with the syringe pointed down so that air will remain in the syringe.
- 11.3.3 Repeat Step 11.3.2 until all of the sample extract is injected. Note the total volume of sample injected.
- 11.3.4 Add 1.0 mL of mobile phase to the sample extract container. Rinse the walls of the container and draw up the rinse with the syringe. Re-attach the syringe to the port, and again pull the plunger to withdraw air, then push the rinse onto the loop, keeping the air in the syringe.
- 11.3.5 Repeat 11.3.4 if necessary with sufficient methylene chloride rinse until the total volume of the rinse plus the sample volume are equal to 3.0 mL.
- 11.3.6 Inject the sample in the loop onto the column. Process each sample using the collect and dump cycle times established in Section 10.

11.3.7 Collect each sample in an Erlenmeyer flask or test tube, covered with aluminum foil to reduce solvent evaporation, or directly into a Kuderna-Danish evaporator or other concentrating device if desired. Monitor sample volumes collected. Changes in sample volumes collected may indicate air bubbles trapped in the pump or other pump failure.

11.4 Extract Concentration

- 11.4.1 Add 1 to 2 clean boiling stones to the evaporative flask and attach a macro Snyder column. Wet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 65 to 70°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches about 5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min. Rinse the K-D apparatus with methylene chloride. Remove the evaporative flask from the concentrator tube.
- 11.4.2 Add several boiling chips to the concentrator tube, then attach a modified micro-Snyder column. Place in a hot water bath at 45 to 50°C. Let solvent evaporate to <0.2 mL. Rinse modified micro-Snyder column. Adjust final volume to 1.0 mL with solvent to be used for additional cleanup or analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 Since the entire sample extract is cleaned up, no adjustment in final sample concentration should be made for the cleanup procedure.

13.0 METHOD PERFORMANCE

13.1 Method performance data for fortified reagent blanks are given in Table 1. Performance of linked analysis schemes incorporating this cleanup step are given in RTI/ACS-AP-209-131.

14.0 POLLUTION PREVENTION

14.1 Always follow good laboratory practices when carrying out procedures. Consider air, soil and water pollution as the primary concerns. Order the least amount of solvent, materials, and reagents required. Do not discard solvents down the drain.

15.0 WASTE MANAGEMENT

15.1 Always dispose of spent or unused solvents by a properly licensed waste disposal company. Always properly label all waste. Store flammable waste in a storage container with a flash arrestor.

16.0 REFERENCES

1. Sheldon, L.S. Manual of Analytical Methods for Determination of Selected Environmental Contaminants in Composite Food Samples. EPA-68-C2-0103, U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 1995.
2. U.S. EPA. Assessment and Control of Bioconcentratable Contaminants in Surface Waters, Draft Method. U.S. Environmental Protection Agency, Washington, DC, March, 1991.
3. Sheldon, L., Keever, J., Beach, J., Roberds, M., and Ellis, L. Environmental Contaminants in Foods: Phase 3 - Validation of Analytical Methods. Final Report, U.S. EPA EMSL-Cincinnati Contract Number 68-C2-0103, Work Assignment 2-01, 1994.

**TABLE 1. PERCENT RECOVERY OF BASE/NEUTRAL PESTICIDES FROM FORTIFIED
REAGENT BLANKS DETERMINED USING RTI/ACS-AP-211-134**

Compound	% Recovery ^{a,b}	
	Mean (n=3)	% RSD
Pesticides		
Alachlor	100	6
Aldrin	106	11
Atrazine	81	8
Captan	83	25
Carbofuran	80	20
α -Chlordane	107	8
γ -Chlordane	109	9
Chlorpyrifos	94	7
4,4'-DDD	103	6
4,4'-DDE	100	8
4,4'-DDT	85	6
Dacthal	108	9
Diazinon	96	7
Dichloran	86	16
Dichlorvos	101	7
Dieldrin	121	8
Folpet	76	30
Fonofos	95	8
Heptachlor	95	6
Lindane	101	10
Malathion	96	8
Metolachlor	108	8
Parathion	75	9
cis-Permethrin	104	0
trans-Permethrin	107	10
Phorate	132	4
Propoxur	97	15
Terbufos	78	8
Trifluralin	97	8
Acetochlor	A ^c	-
Aldicarb	A	-
Carbofuran	A	-
Dichlorvos	100	2
Endosulfan I	A	-

(continued)

TABLE 1. (continued)

Compound	% Recovery ^{a,b}	
	Mean (n=3)	% RSD
Endrin	A	-
Fonofos	A	-
Hexachlorobenzene	A	-
Isophenfos	A	-
Lindane	A	-
Methomyl	A	-
Parathion	A	-
Simazine	A	-
Terbufos	A	-
PAHs		
Benzo[a]anthracene	A	-
Benzo[a]pyrene	A	-
Acenaphthylene	A	-
Anthracene	A	-
Dibenzo[ghi]perylene	A	-
Fluoranthene	A	-
Indeno[1,2,3-cd]pyrene	A	-
Phenanthracene	A	-
Pyrene	A	-
Acenaphthene	A	-
Benzo[b]fluoranthene	A	-
Benzo[c]fluoranthene	A	-
Chrysene	A	-
Dibenzo[ah]anthracene	A	-
Fluorene	A	-
Benzo[e]pyrene-d ₁₂	A	-
Chrysene-d ₁₂	A	-
Acenaphthene-d ₁₀	A	-
Tetrachloronaphthalene	A	-
9,10-Dichloroanthracene	A	-
Perylene-d ₁₂	A	-
Fluorene-d ₁₀	A	-

^a Analyzed by GC/MS as described in RTI/ACS-AP-211-135; recovery for cleanup method only.

^b All pesticides spiked at 500 ng, equivalent to 10 ng/g for a 50 g sample.

Not tested. Should be applicable based on acceptable performance for linked analysis schemes using this method.

TITLE:ANALYSIS OF PESTICIDES AND PAHs FROM EXTRACTS OF
COMPOSITED FOOD SAMPLES BY GAS CHROMATOGRAPHY/MASS
SPECTROMETRYSOURCE:Research Triangle Institute
Post Office Box 12194
Analytical and Chemical Sciences
Research Triangle Park, NC 27709-2194AUTHOR(s):Mike RolerDate: 7/2/98Kurt W. ThunDate: 7/2/98John R. KennerDate: 7/2/98APPROVED BY:

Principal Investigator:

E. PellingaiDate: 7/2/98

QA Officer:

Doris SmithDate: 7/2/98STATUS:IN PROGRESS: DRAFT: FINAL VERSION: REVISIONS:

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**ANALYSIS OF PESTICIDES AND PAHs FROM EXTRACTS OF COMPOSITED
FOOD SAMPLES BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY**

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1.0 SCOPE AND APPLICATION

The procedures described in this protocol are designed to provide GC/MS analysis of pesticides or PAHs in extracts of composited food samples. This method has been tested and is applicable to the pesticides and PAHs in Table 1. For pesticides and PAHs not listed in Table 1, method performance must be evaluated by the analysis laboratory. Although the GC/MS analysis method is the same for pesticides and PAHs, are not analyzed at the same time since they must be extracted from food samples using different procedures.

2.0 SUMMARY OF THE METHOD

This method is for the analysis of base-neutral pesticides in extracts from composited food samples by GC/MS in the selected ion monitoring mode (SIM). The sample extract is injected into a GC/MS system, where analytes are separated with a fused silica capillary column. The compounds are identified based on chromatographic retention time of at least two representative mass fragment ions as compared to standard solutions analyzed under identical conditions. One ion, a primary ion, is used for the quantitation of a given compound. The secondary ion is utilized as a confirmation ion for a given compound. Quantitation is carried out by the method of internal standards by utilizing the areas of the quantitation ions for the analytes and internal standards to determine relative response factors for each specific analyte of interest. Surrogate standards are spiked into the sample matrix prior to extraction and cleanup, and are used to monitor the method performance for each sample.

3.0 DEFINITIONS

- 3.1 GC/MS - Gas Chromatography Mass Spectrometry
- 3.2 SIM - Selected Ion Monitoring

4.0 INTERFERENCES

See RTI/ACS-AP-211-131.

5.0 SAFETY

5.1 The toxicity and carcinogenicity of chemicals used in this method has not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Additional references of laboratory safety are available for the information of the analyst.

5.2 Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens including aldrin, chlordane, dieldrin, and heptachlor. Pure standard materials and stock standard solutions of these compounds should be handled with suitable protection to skin, eyes, etc.

6.0 EQUIPMENT

6.1 Laboratory Equipment

6.1.1 Volumetric flasks, various sizes.

6.1.2 Micro syringes, various sizes.

6.1.3 Vials. Various sizes of amber vials with Teflon-lined screw or crimpseal caps including autosampler vials.

6.1.4 Analytical balance. Capable of weighing 0.0001 g accurately.

6.2 Gas Chromatograph/Mass Spectrometer/Data System (GC/MS/DS)

6.2.1 The GC must be capable of temperature programming and be equipped for splitless/split injection. The injection tube liner should be quartz and about 3 mm in diameter. The injection system must not allow the analytes to contact hot stainless steel or other metal surfaces that promote decomposition.

- 6.2.2 The GC/MS interface should allow the capillary column or transfer line exit to be placed within a few mm of the ion source. Other interfaces, for example the open split interface, are acceptable as long as the system has adequate sensitivity.
- 6.2.3 The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 eV. The spectrometer must be capable of scanning from 45 to 450 amu or selected ion monitoring with a complete scan cycle time (including scan overhead) of 1.5 sec or less. (Scan cycle time = Total MS data acquisition time in sec divided by number of scans in the chromatogram). The spectrometer must produce a mass spectrum that meets all criteria for the tune of perfluorotributylamine (FC-43) specified by the manufacturer. This tuning criteria shall include the following parameters for resolution and mass accuracy. For the 69.0, 219.0 and 502.0 ions of FC-43 the mass accuracy shall be within ± 0.1 m/g. Acceptable mass resolution shall be determined by using the full width at half-max (FWHM) of the 502 and 503 ions of FC-43. The FWHM values shall be 0.50 ± 0.10 for both the 502 and 503 ions.
- 6.2.4 A data system is required to acquire, store, reduce, and output mass spectral data in both the full scan and SIM mode. The software must allow integration of the ion abundance of any specific ion between specified time or scan number limits, calculation of response factors as defined in Section 10.1.8 (or construction of a first or second order regression calibration curve), calculation of response factor statistics (mean and standard deviation), and calculation of concentrations of analytes using either the calibration curve or the equation in Section 12. Optionally, data may be transferred from the instrument to another computer to carry out calculations after identifications and integrations are complete.

7.0 REAGENTS AND STANDARDS

7.1 Helium Carrier Gas

7.2 Solvents

Methylene chloride, toluene, hexane, acetone, diethylether (pesticide grade or equivalent).

7.3 Stock Standard Solutions

Individual solutions of analytes, surrogates, and internal standards are prepared from certified solutions or from pure (neat) materials. The solutions are prepared in a suitable solvent (i.e., methylene chloride, toluene, iso-octane or hexane). The stock solutions are stored in vials with Teflon lined caps at -10°C or sealed in clean glass ampules for storage.

7.4 Primary Dilution Standard

The stock standards of the target pesticides and surrogates standards are used to prepare a primary dilution standard solution that contains multiple analytes. Aliquots of each of the stock standard solutions are combined to produce the primary dilution standard in which the concentration of the analytes is at least equal to the concentration of the highest calibration solution. Store the primary dilution standard solution in a vial sealed with a Teflon lined cap at 4°C or less.

7.5 Internal Standard Solution

9,10-Dichloroanthracene and Triphenylphosphate are recommended as internal standards although other compounds can be used. The stock internal standard solution(s) is used to prepare a primary dilution standard containing the internal standard(s). The solution is prepared at a level which facilitates the delivery of an appropriate amount of internal standard(s) to the final sample extracts with a small (i.e., 5-50 µL) spike. The solution is also used in the preparation of the calibration solutions.

7.6 Calibration Solutions

A series of calibration solutions are prepared to span the concentration range of approximately 10 to 750 pg/µL for each target pesticide and surrogate standard. The internal standard(s) should be at a constant mid-point level in all solutions. Table 2 lists the suggested

calibration levels, target analytes, internal standards and surrogates for the calibration curve standards. Calibration solutions should be prepared in the same solvent as the sample extracts. The solutions are stored in vials with Teflon lined caps at 4°C. Aliquots of the solutions are transferred to amber autosampler vials and sealed with Teflon lined septa for analysis by GC/MS.

8.0 EXTRACT STORAGE

- 8.1 Holding times for sample extracts prior to analysis have not been established. Sample extracts should be analyzed within thirty days of extraction.
- 8.2 Extracts are stored protected from light. For short periods of time, less than 48 hours, extracts may be stored at 4°C. For longer storage periods, the extracts should be stored at approximately -10°C or less.

9.0 QUALITY CONTROL

See RTI/ACS-AP-211-131 for quality control procedures.

10.0 INSTRUMENT CALIBRATION AND STANDARDIZATION

Demonstration and documentation of acceptable initial calibration of the GC/MS system are required before any samples are analyzed and are required intermittently throughout sample analysis as dictated by results of continuing calibration checks. After initial calibration is successful, a continuing calibration check is required at the beginning of each day during which analyses are performed. An additional three calibration standards of different concentrations are analyzed interspersed with the samples during each analysis day.

10.1 Initial Calibration

- 10.1.1 Calibrate the mass and abundance scales of the MS with calibration compounds and procedures prescribed by the manufacturer and as shown in Section 6.2.3.
- 10.1.2 Configure the GC/MS system as described in Table 3.
- 10.1.3 Inject a 1 μL aliquot of the highest concentration calibration solution, and acquire and store data from m/z 45-450 with a total cycle time (including scan overhead time) of 1.5 sec or less. Cycle time should be adjusted to measure at least five or more spectra during the elution of each GC peak. Identify target analytes and retention times for establishing SIM windows.
- 10.1.4 Follow manufacturer's procedures to configure the GC/MS software for acquisition of data in SIM mode. Inject a 1 μL aliquot of a medium concentration calibration solution and acquire and store data from the selected ions with a total cycle time (including scan overhead time) of 1.5 sec or less. If p,p' -DDT is a target analyte, inject a 1 μL aliquot of a solution that contains 250 pg/ μL of only p,p' -DDT under identical conditions.
- 10.1.5 Performance criteria for the medium calibration.
 - 10.1.5.1 MS sensitivity - The GC/MS/DS peak identification software should be able to recognize a GC peak in the appropriate retention time window for each of the compounds in the calibration solution, and make correct tentative identifications. If fewer than 99% of the compounds are recognized, system maintenance is required. See Section 10.2.6.
 - 10.1.5.2 Lack of degradation of p,p' -DDT - p,p' -DDT is easily degraded in the injection port if the injection port or front of the column is dirty. This is the result of buildup of high boiling residue from sample injection. Check for degradation problems by injecting a mid-level standard containing only p,p' -DDT. Look for the degradation products of p,p' -DDT (p,p' -DDE and p,p' -DDT). If degradation of DDT exceeds 20%, take corrective action before proceeding with calibration. Calculate percent breakdown as follows:

$$\% \text{ breakdown for } p,p'\text{-DDT} = \frac{\text{Amount of DDE + DDD}}{\text{Amount of DDT + DDE + DDD}} \times 100$$

- 10.1.6 If all performance criteria are met, inject a 1- μL aliquot of each of the other CAL solutions using the same GC/MS conditions.
- 10.1.7 Calculate a response factor (RF) for each analyte and surrogate for each CAL solution using the internal standard whose retention time is nearest the retention time of the analyte or surrogate. Table 1 contains suggested internal standards and quantitation ions for all selected compounds. RF is a unitless number, but units used to express quantities of analyte and internal standard must be equivalent. RF is calculated as:

$$RF = \frac{(A_a)(Q_s)}{(A_s)(Q_a)}$$

where:

- A_a = integrated abundance of the quantitation ion of the analyte.
 A_s = integrated abundance of the quantitation ion internal standard.
 Q_a = concentration of analyte in the calibration standard in pg/ μL .
 Q_s = concentration of internal standard in the calibration standard in pg/ μL .

For each analyte and surrogate, calculate the mean RF from the analysis of the six CAL solutions. Calculate the standard deviation (SD) and the percent relative standard deviation (%RSD) for each mean: %RSD = 100 (SD/M). If the RSD of any analyte or surrogate mean RF exceeds 25%, either analyze additional aliquots of appropriate CAL solutions to obtain an acceptable RSD of RFs over the entire concentration range, or take action to improve GC/MS performance.

NOTE: Some target analytes will not be detected in the least concentrated calibration standards. To include a RF from any standard in the calibration, the signal-to-noise ratio must be greater than 5- to 1- and the RF value must be within 35% of the mean RF. The quantitation range is then defined by the highest and lowest concentration calibration standards that meet this criteria. The RF from at least three calibration standards must be used to calculate the mean RRF for each analyte.

- 10.1.8 As an alternative to calculating mean response factors and applying the RSD test, use the GC/MS data system software or other available software to generate a linear or second order regression calibration curve. Acceptable regression curves must have r^2 values greater than 0.98, and calculated concentrations for each calibration standard within $\pm 25\%$ of prepared concentration. The lowest calibration standard used may be acceptable if within $\pm 30\%$ of prepared concentration.

10.2 Continuing calibration check - Verify the MS tune and initial calibration at the beginning of each day (daily RF) that analyses are performed using the following procedure, as well as after the analysis of every nine sample extracts.

- 10.2.1 Verify that the MS tune meets the criteria as described in Section 6.2.3.
- 10.2.2 Inject a 1- μL aliquot of a medium concentration calibration solution and analyze with the same conditions used during the initial calibration.
- 10.2.3 Demonstrate acceptable performance for the criteria shown in Section 10.1.6.
- 10.2.4 Each analysis day, prior to analysis of samples, the performance of the GC/MS system will be evaluated with respect to continuing calibration, peak resolution, and detector sensitivity. A mid-point calibration standard will be used for this purpose. The sequence of analyses on any given day of analysis will include, at a minimum:
- 250 pg/ μL calibration standard
 - up to four samples or QC samples
 - 25 pg/ μL calibration standard
 - up to four samples or QC samples
 - 50 pg/ μL calibration standard

- up to four samples or QC samples
- 500 pg/ μ L calibration standard

The 250 pg/ μ L standard analysis will be used to determine if the GC/MS is operating in-control with respect to the original calibration. Calculated RFs from the 250 pg/ μ L standard must be within 30% of the mean values calculated in the initial calibration for at least 75% of the target analytes. In addition, the absolute area response for the internal standard(s) and analytes must not decrease by more than 50% from the response obtained during calibration. If these criteria are met, target analyte concentrations will be reported for all in-control analytes. If more than 2 out-of-control target analytes are present in a sample at concentrations above the detection limit, then the sample will be analyzed. If 1 or 2 out-of-control target analytes are present in a sample at concentrations above the detection limit, the concentrations will be calculated and reported along with a data flag indicating the calibration was not in-control. If the instrumental control criteria are not met, corrective action will be taken using one of the following two approaches.

Approach 1: The four calibration standards analyzed with the set of samples may be used for the purpose of measuring analyte concentrations on that day. In order for this approach to be used, the %RSD for the four calibration standard RFs must be within $\pm 25\%$ and concentrations for the 25 pg/ μ L calibration standard (equivalent to a concentration of 1 ng/g in food) must be measurable for 75% of the target analytes. If these criteria are met, target analyte concentrations will be reported for all in-control analytes. If more than 2 out-of-control target analytes are present in a sample at concentrations above the detection limit, then the sample will be reanalyzed. If 1 or 2 out-of-control target analytes are present in a sample at concentrations above the detection limit, the concentrations will be calculated and reported with a data flag indicating the calibration was not in-control.

Approach 2: Corrective action may be applied to the GC/MS instrument (see Section 10.2.7) or to the materials used to calibrate the instrument. The instrument will then be recalibrated, followed by reanalysis of all samples in the out-of-control sample set.

- 10.2.7 Some possible remedial actions - Major maintenance such as cleaning an ion source, cleaning quadrupole rods, etc. require recalibration.
 - 10.2.7.1 Check and adjust GC and/or MS operating conditions; check the MS resolution, and calibrate the mass scale.
 - 10.2.7.2 Clean or replace the splitless injection liner; silanize a new injection liner.
 - 10.2.7.3 Flush the GC column with solvent according to manufacturer's instructions.
 - 10.2.7.4 Break off a short portion (about 1 meter) of the column from the end near the injector; or replace GC column. This action will cause a change in retention times.
 - 10.2.7.5 Prepare fresh CAL solutions, and repeat the initial calibration step.
 - 10.2.7.6 Clean the MS ion source and rods (if a quadrupole).
 - 10.2.7.7 Replace any components that allow analytes to come into contact with hot metal surfaces.
 - 10.2.7.8 Replace the MS electron multiplier, or any other faulty components.

11.0 ANALYSIS PROCEDURE

- 11.1 Analyze a 1-2 μL aliquot of each sample with the GC/MS system under the same conditions used for the initial and continuing calibrations (Section 10.1.3).
- 11.2 At the conclusion of data acquisition, use the same software that was used in the calibration procedure to tentatively identify peaks in retention time windows of interest.
- 11.3 Identification of analytes - identify a sample component by its retention time and extracted ion profiles. The GC retention time of the sample components should be within 12 sec of the time observed for that same compound when a calibration solution was analyzed. Manually check the peak integration to verify that the analyte was currently identified, the

extracted ion profile was properly integrated and the most accurate peak area was obtained.
Regenerate peak where necessary.

12.0 DATA CALCULATIONS

Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations if *unique* ions with adequate intensities are available for quantitation.

12.1 Calculate analyte and surrogate concentrations using the following equation:

$$C_x = \frac{(A_x)(Q_s)}{(A_{is})(RF)}$$

where:

C_x = concentration of analyte or surrogate in either pg or pg/ μ L in the sample extract.

A_x = integrated abundance of the quantitation ion of the analyte in the sample.

A_{is} = integrated abundance of the quantitation ion of the internal standard in the sample.

Q_s = total quantity in pg or pg/ μ L of internal standard added to the sample.

RF = mean response factor of analyte from continuing calibration checks.

12.2 Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty).

13.0 METHOD PERFORMANCE

13.1 Method quantitation limits (MQLs) are based upon the lowest calibration concentration used for the sample analysis.

13.2 Control charts will be utilized to monitor overall performance as determined by the daily RF and continuing calibration checks.

14.0 SAMPLE CUSTODY AND SAMPLE MANAGEMENT

See RTI/ACS-AP-211-131, Section 14.0.

15.0 REFERENCES

1. Sheldon, L. Environmental Contaminants in Foods: Phase 3 - Validation of Analytical Methods. Final Report, U.S. EPA EMSL-Cincinnati Contract Number 68-C2-0103, Final Report 2-01, 1994.
2. Sheldon, L. Support for AHS Human Exposure Monitoring Study. Final Report, U.S. EPA EMSL-Cincinnati Contract Number 68-C2-0103, Work Assignment 2-05, 1994.

TABLE 1. APPLICABLE PESTICIDES AND PAHs FOR RTI/ACS-AP-211-135

Compound	Suggested Quantitation Ions (m/z)		IS ^a	RT ^b (minutes)
	1°	2°		
BASE/NEUTRAL PESTICIDES				
Alachlor	188.0	166.0	DCA	19.62
Aldrin	262.8	66.0	DCA	21.38
Atrazine	200.0	215.0	DCA	16.43
α-Chlordane	272.8	274.8	DCA	24.11
γ-Chlordane	272.8	274.8	DCA	24.68
Chlorpyrifos	314.0	196.9	DCA	21.38
Dacthal	300.9	298.9	DCA	21.55
p,p'-DDD	235.0	237.0	DCA	27.54
p,p'-DDE	317.8	246.0	DCA	25.72
p,p'-DDT	235.0	237.0	DCA	29.12
Diazinon	304.0	141.0	DCA	17.31
Dichloran	206.0	176.0	DCA	15.79
Dieldrin	344.8	202.8	DCA	25.83
Heptachlor	273.8	271.8	DCA	19.82
Malathion	173.0	158.0	DCA	21.15
Metolachlor	162.0	238.0	DCA	21.26
cis-Permethrin	183.0	184.0	TPP	35.33
trans-Permethrin	183.0	184.0	TPP	35.33
Phorate	260.0	75.0	DCA	15.09
Trifluralin	306.0	264.1	DCA	14.51
Acetochlor	146.0	162.0	DCA	19.24
Dichlorvos	185.0	187.0	DCA	6.32
Endosulfan I	338.8	338.6	DCA	24.67
Endrin	278.9	244.9	DCA	26.71

(continued)

TABLE 1. (continued)

Compound	Suggested Quantitation Ions (m/z)			RT ^b (minutes)
	1°	2°	IS ^a	
Fonofos	246.0	109.0	DCA	17.07
Hexachlorobenzene	283.8	285.8	DCA	15.33
Isophenfos	213.0	58.0	DCA	23.25
Lindane	216.9	182.9	DCA	16.68
Parathion	291.0	137.0	DCA	21.71
Simazine	201.0	186.0	DCA	16.21
Terbufos	231.0	288.0	DCA	16.95
<u>Surrogates</u>				
Pentachloronitrobenzene	134.0	—	DCA	5.66
Fluorene-d ₁₀	174.1	176.1	DCA	12.75
Atrazine-d ₆	205.0	220.2	DCA	16.33
Pentachloronitrobenzene	236.8	—	DCA	16.43
Chrysene-d ₁₂	240.1	241.1	TPP	31.18
<u>Internal Standards</u>				
9,10-Dichloroanthracene	248.0	246.0	NA	26.20
Triphenylphosphate	325.0	65.0	NA	29.92
<u>PAHs</u>				
Benzo[a]anthracene	228.1	229.1	DCA	29.94
Benzo[a]pyrene	252.1	253.1	DCA, Per	36.83
Acenaphthylene	152.0	151.0	Ace	9.28
Anthracene	178.1	179.1	TCN	16.53
Dibenzo[ghi]perylene	276.1	277.1	DCA, Per	42.57
Fluoranthene	202.1	203.1	TCN	22.30
Indeno[1,2,3-cd]pyrene	276.1	277.1	DCA, Per	41.61
Phenanthracene	178.1	179.1	TCN	16.27

(continued)

TABLE 1. (continued)

Compound	Suggested Quantitation Ions (m/z)			RT ^b (minutes)
	1°	2°	IS ^a	
Pyrene	202.1	203.1	DCA	23.41
Acenaphthene	154.1	152.1	Ace	9.90
Benzo[b]fluoranthene	252.1	253.1	DCA	35.38
Benzo[c]fluoranthene	252.1	253.1	DCA,Per	35.31
Chrysene	228.1	229.1	DCA	30.12
Dibenzo[ah]anthracene	278.1	279.1	DCA	41.79
Fluorene	166.1	165.1	Ace	11.94
<u>Surrogates</u>				
Benzo[e]pyrene-d ₁₂	264.2	265.2	DCA, Per	36.47
Chrysene - d ₁₂	240.1	244.1	DCA	29.98
<u>Internal Standards</u>				
Acenaphthene-d ₁₀	162.1	164.1	NA	9.79
Tetrachloronaphthalene	265.9	263.9	NA	19.95
9,10-Dichloroanthracene	246.0	248.0	NA	25.00
Perylene-d ₁₂	264.2	265.2	NA	32.00
Flourene-d ₁₀	174.1	176.1	NA	26.20

^a Recommended internal standard for quantitation DCA - 9,10-dichloroanthracene, TPP-triphenylphosphate, Ace - acenaphthene-d₁₀, Per - perylene-d₁₂.

^b Typical retention time.

^c Not applicable.

TABLE 2. NOMINAL CALIBRATION SOLUTIONS

Compound	Concentration of Analytes in (pg/ μ L) Levels							
	10X	25X	50X	100X	250X	400X	500X	750X
BASE/NEUTRAL PESTICIDES								
Alachlor	10	25	50	100	250	400	500	750
Aldrin	10	25	50	100	250	400	500	750
Atrazine	10	25	50	100	250	400	500	750
Captan	10	25	50	100	250	400	500	750
Carbaryl	10	25	50	100	250	400	500	750
α -Chlordane	10	25	50	100	250	400	500	750
γ -Chlordane	10	25	50	100	250	400	500	750
Chlorpyrifos	10	25	50	100	250	400	500	750
Dacthal	10	25	50	100	250	400	500	750
p,p'-DDD	10	25	50	100	250	400	500	750
p,p'-DDE	10	25	50	100	250	400	500	750
p,p'-DDT	10	25	50	100	250	400	500	750
Diazinon	10	25	50	100	250	400	500	750
Dichloran	10	25	50	100	250	400	500	750
Dieldrin	10	25	50	100	250	400	500	750
Folpet	10	25	50	100	250	400	500	750
Heptachlor	10	25	50	100	250	400	500	750
Malathion	10	25	50	100	250	400	500	750
Metolachlor	10	25	50	100	250	400	500	750
cis-Permethrin	10	25	50	100	250	400	500	750
trans-Permethrin	10	25	50	100	250	400	500	750
Phorate	10	25	50	100	250	400	500	750
Propoxur	10	25	50	100	250	400	500	750

(continued)

TABLE 2 . (continued)

Compound	Concentration of Analytes in (pg/ μ L) Levels							
	10X	25X	50X	100X	250X	400X	500X	750X
Trifluralin	10	25	50	100	250	400	500	750
Acetochlor	10	25	50	100	250	400	500	750
Aldicarb	10	25	50	100	250	400	500	750
Benomyl	10	25	50	100	250	400	500	750
Carbofuran	10	25	50	100	250	400	500	750
Dichlorvos	10	25	50	100	250	400	500	750
Endosulfan I	10	25	50	100	250	400	500	750
Endrin	10	25	50	100	250	400	500	750
Fonofos	10	25	50	100	250	400	500	750
Hexachlorobenzene	10	25	50	100	250	400	500	750
Isophenfos	10	25	50	100	250	400	500	750
Lindane	10	25	50	100	250	400	500	750
Methomyl	10	25	50	100	250	400	500	750
Parathion	10	25	50	100	250	400	500	750
Simazine	10	25	50	100	250	400	500	750
Terbufos	10	25	50	100	250	400	500	750
<u>Surrogates</u>								
Pentachloronitrobenzene	10	25	50	100	250	400	500	750
Fluorene-d ₁₀	10	25	50	100	250	400	500	750
Atrazine-d ₆	10	25	50	100	250	400	500	750
Pentachloronitrobenzene	10	25	50	100	250	400	500	750
Chrysene-d ₁₂	10	25	50	100	250	400	500	750

(continued)

TABLE 2 . (continued)

Compound	Concentration of Analytes in (pg/ μ L) Levels							
	10X	25X	50X	100X	250X	400X	500X	750X
<u>Internal Standards</u>								
9,10-Dichloroanthracene	400	400	400	400	400	400	400	400
Triphenylphosphate	500	500	500	500	500	500	500	500
PAH's								
Benzo[a]anthracene	10	25	50	100	250	400	500	750
Benzo[a]pyrene	10	25	50	100	250	400	500	750
Acenaphthylene	10	25	50	100	250	400	500	750
Anthracene	10	25	50	100	250	400	500	750
Dibenzo[ghi]perylene	10	25	50	100	250	400	500	750
Fluoranthene	10	25	50	100	250	400	500	750
Indeno[1,2,3-cd]pyrene	10	25	50	100	250	400	500	750
Phenanthracene	10	25	50	100	250	400	500	750
Pyrene	10	25	50	100	250	400	500	750
Acenaphthene	10	25	50	100	250	400	500	750
Benzo[b]fluoranthene	10	25	50	100	250	400	500	750
Benzo[c]fluoranthene	10	25	50	100	250	400	500	750
Chrysene	10	25	50	100	250	400	500	750
Dibenzo[ah]anthracene	10	25	50	100	250	400	500	750
Fluorene	10	25	50	100	250	400	500	750
<u>Surrogates</u>	10	25	50	100	250	400	500	750
Benzo[e]pyrene-d ₁₂	10	25	50	100	250	400	500	750
Chrysene-d ₁₂	10	25	50	100	250	400	500	750

(continued)

TABLE 2 . (continued)

TABLE 3. RECOMMENDED CONDITIONS FOR GC/MS ANALYSIS

Parameter	Setting
<u>Gas Chromatograph</u>	
Column	30 m x 0.32 mm I.D. fused silica capillary column (0.5 μ M film thickness)
Temperature Program	60°C to 130°C at 15°C/min 130 to 300°C at 4.5°C/min
Carrier Gas	Helium
Capillary Injector	1 min splitless
Injection Volume	1.0 μ L
Injector Temperature	240°C - 300°C
<u>Mass Spectrometer</u>	
Ionization Mode	Electron ionization Selected ion monitoring
Emission Current	0.3 mA
Source Temperature	200°C
Electron Multiplier	1600 to 3000 volts

TITLE: DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS IN FOOD SAMPLES

SOURCE: Research Triangle Institute
Post Office Box 12194
Analytical and Chemical Sciences
Research Triangle Park, NC 27709-2194

AUTHOR(s):

Mike Rosen

Date: 3/24/98

Jeffrey T. Keener

Date: 3/25/98

Date:

APPROVED BY:

Principal Investigator:

E. Bellizzi

Date: 3/26/98

OA Officer:

Doris Smith

Date: 3/28/98

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**DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS
IN FOOD SAMPLES**

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1.0 SCOPE AND APPLICATION

1.1 This method is for the determination of polynuclear aromatic hydrocarbons (PAHs) in individual fatty and nonfatty foods as well as composite food samples. Gas chromatography/mass spectrometry (GC/MS) is used for the determination of the target PAHs. This method has been tested and is applicable to the PAHs in Table 1. For PAHs not listed in Table 1, method performance must be evaluated by the analysis laboratory.

1.2 This method has been validated in a single laboratory and estimated detection limits (EDLs) have been determined (Section 13). Observed detection limits may vary between food samples, depending upon the nature of interferences in the sample matrix and the specific instrumentation used.

1.3 This method is restricted to use by or under the supervision of analysts experienced in low level residue analysis and the use of GC/MS for the measurement of PAHs at trace levels. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 9.

2.0 SUMMARY OF THE METHOD

2.1 This is a method for the extraction, cleanup, and analysis of PAHs from a homogenized food sample. A schematic of the method is shown in Figure 1. The homogenized food sample is digested with an ethanolic potassium hydroxide solution. PAHs are then partitioned from the alkaline digest by shaking into η -hexane. The resulting η -hexane extract is concentrated to 1 mL and cleaned up using solid phase extractions (SPE) silica gel columns. The extract is further cleaned up by gel permeation chromatography (RTI/ACS-AP-211-134) to remove high molecular weight materials. The final sample extract is concentrated to 0.1 mL for analysis using GC/MS in the selected ion monitoring mode (RTI/ACS-AP-211-135).

3.0 DEFINITIONS

3.1 Internal Standard

A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.2 Surrogate Analyte

A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 Laboratory Duplicates (LD1 and LD2)

An equivalent sample aliquot taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of laboratory duplicates give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 Laboratory Reagent Blank (LRB)

An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.5 Laboratory Performance Check Solution (LPC)

A solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.6 Laboratory Fortified Blank (LFB)

An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LC is analyzed exactly like a sample, and its purpose is to

determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required estimated detection limit.

3.7 Laboratory Fortified Sample Matrix (LFM)

An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.8 Stock Standard Solution

An concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.9 Primary Dilution Standard Solution

A solution of several analytes prepared in the laboratory from stock standard solutions are used to prepare primary dilution standards.

3.10 Calibration Standard (CAL)

A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

4.0 INTERFERENCES

4.1 During analysis, major contaminant sources are reagents and liquid-solid extraction columns. Analysis of field and laboratory reagent blanks provide information about the presence of contaminants.

4.2 Interfering contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentration of compounds. Syringes and splitless injection port liners must be cleaned carefully or replaced as needed. After analysis of a sample containing high concentrations of compound, a

laboratory reagent blank should be analyzed to ensure that accurate values are obtained for the next sample. To minimize interferences for food samples, a solvent rinse may be injected after every sample.

4.3 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.2.

- 4.3.1 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with hot tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 1 hour. Do not heat volumetric ware. Thermally stable materials might not be eliminated by this treatment. Thorough rinsing with acetone may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
- 4.3.2 The use of high purity reagents and solvents help to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

WARNING

When a solvent is purified, stabilizers put into the solvent by the manufacturer are removed thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives put into the solvent by the manufacturer are removed thus potentially reducing the shelf-life.

4.4 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from sample to sample. Cleanup procedures are provided for removal of any interferences.

4.5 Variable amounts of PAHs from aqueous solutions may adhere to glass surfaces. It is recommended that sample transfers and glass surface contacts be minimized.

5.0 SAFETY

5.1 The toxicity and carcinogenicity of all chemicals used in this method has not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Additional references to laboratory safety are available for the information of the analyst.

5.2 The following method analytes have been tentatively classified as known or suspected human or mammalian carcinogens: benzo[a]pyrene, and benzo[a]anthracene. Pure standard materials and stock standard solutions of the compounds should be handled in a hood or glovebox.

5.3 Methylene Chloride

Avoid breathing vapors and contact with skin. TLV is 50 ppm (TWA over 8 h). May be carcinogenic to humans.

6.0 EQUIPMENT AND SUPPLIES

6.1 Alkaline Digestion/Extraction

- 6.1.1 Micro Syringes - 10 and 100 μL .
- 6.1.2 Pipettes - 1.0 and 10.0 mL transfer.
- 6.1.3 Graduated Cylinders.
- 6.1.4 Standard Solution Storage Containers - 15-L amber vials with Teflon-lined screw caps.
- 6.1.5 Analytical Balance - capable of weighing to 100 g.
- 6.1.6 Analytical Balance - capable of weighing to nearest 0.00001 g.

- 6.1.7 Digestion Apparatus - including 500 mL volume round bottom flask, and reflux condenser.
- 6.1.8 Heating Mantles.
- 6.1.9 Powder Funnels.
- 6.1.10 Glasswool.
- 6.1.11 Separatory Funnels, 1 L capacity.
- 6.1.12 Beakers - 250 mL.
- 6.1.13 Kuderna-Danish concentrators, Snyder columns, and modified micro-Snyder columns.
- 6.1.14 Water bath or equivalent.
- 6.1.15 Boiling Chips - solvent extracted, 10/40 mesh.
- 6.1.16 Aluminum Weigh Boats.

6.2 Solid Phase Extraction

- 6.2.1 Solid phase extraction columns - Silica Gel (SiOH) - 6 mL high capacity, Bakerbond SPC or equivalent.

6.3 GPC Cleanup

Refer to RTI/ACS-AP-211-134.

6.4 GC/MS Analysis

Refer to RTI/ACS-AP-211-135.

7.0 REAGENTS AND STANDARDS

7.1 Alkaline Digestion/Extraction

- 7.1.1 Ethanol- 100%-demonstrated to be free of analytes.
- 7.1.2 n-Hexane - demonstrated to be free of analytes.
- 7.1.3 Potassium hydroxide - pellet, ACS Reagent Grade.
- 7.1.4 Ethanolic KOH solution - dissolve 40 g KOH in 1 L of ethanol.

7.2 Solid Phase Extraction

- 7.2.1 n-Hexane.

7.2.2 Methylene Chloride - demonstrated to be free of analytes.

7.3 Reagent Water

- 7.3.1 Reagent water is defined as water free of interference when employed in the procedure described herein.
- 7.3.2 A Millipore Super - Q Water System or its equivalent may be used to generate deionized reagent water.

7.4 Stock Standard Solutions

Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure.

- 7.4.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in toluene and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.
- 7.4.2 Transfer the stock standard solutions into Teflon-lined screw cap amber vials. Store at 4°C and protect from light.
- 7.4.3 Stock standard solutions should be replaced after two months or sooner if comparison with laboratory fortified blanks, or QCS samples indicate a problem.

7.5 Matrix Spike Standards

The spiking levels for PAHs in food samples may range from 0.1 to 10 ng/g. Once the spiking level is established, a spiking solution is prepared in a suitable solvent which contains all of the target PAHs such that a small aliquot (25 µL to 50 µL) added to a homogenized food sample (25 g to 50 g), reagent water, or neutral reference material will result in the desired spiking level. This solution will be used to prepare the laboratory fortified sample matrix and the laboratory fortified blank.

7.6 Surrogate Standards

A surrogate standard (i.e., a chemically inert compound not expected to occur in an environmental sample) should be added to each sample, blank, and matrix spike sample just prior to extraction or processing. The recovery of the surrogate standard is used to monitor for unusual matrix effects, gross sample processing errors, etc. Surrogate recovery is evaluated for acceptance by determining whether the measured concentration falls within the acceptance limits. Surrogate standard solutions in toluene are prepared such that a 25 µL aliquot added to the 25 g food homogenate will result in the desired spiking level. Compounds shown to be acceptable for surrogate use are benzo[e]pyrene-d₁₂ and chrysene-d₁₂.

7.7 Internal Standard Spiking Solution

A solution is prepared in toluene that contains acenaphthalene-d₁₀, tetrachloronaphthalene, 9,10-dichloroanthracene, and perylene-d₁₂ at concentrations of 0.5 to 1.0 ng /µL, although other internal standard compounds may be used. A 50 µL aliquot of this solution will be used to spike the final sample extracts.

8.0 SAMPLE HOMOGENIZATION AND STORAGE

8.1 Sample Homogenization

Refer to RTI/ACS-AP-211-038.

8.2 Sample Storage

8.2.1 Store all homogenized samples in a freezer at -20°C.

8.2.2 Holding times for the homogenates have been established for up to 21 days. Analyte stability may be affected by the matrix, therefore, the analyst should verify that storage times are acceptable.

9.0 QUALITY CONTROL

9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries in each sample and blank,

monitoring internal standard peak area or height in each sample and blank (when internal standard calibration procedures are being employed), analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples.

9.2 Laboratory Reagent Blanks

Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a laboratory reagent blank (LRB) must be analyzed. If within the retention time window of any analyte of interest the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

9.3 Initial Demonstration of Capability

- 9.3.1 Select a representative concentration for each analyte about 10 times EDL. Methods in this manual were tested at 0.2 to 10 ng/g for polynuclear aromatic hydrocarbons. Prepare at least four laboratory fortified reagent blanks (LFB) or laboratory fortified sample matrices (LFM) at the selected spiking level. Extract and analyze each LFB or LFM with the selected methods. If LFMs are used for testing, a single unspiked aliquot of the food sample should also be extracted and analyzed.
- 9.3.2 For each analyte, the recovery (R) value for at least three of the samples must fall in the range of 60 to 140%. For those compounds that meet the acceptance criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, performance must be repeated using four fresh samples until satisfactory performance has been demonstrated.
- 9.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of data will improve beyond that required here.

9.4 The analyst is permitted to modify GC columns, GC conditions, GC detectors, continuous extraction techniques, concentration techniques (i.e., evaporation techniques), internal standards or surrogate compounds.

9.5 Assessing Surrogate Recovery

- 9.5.1 When surrogate recovery from a sample or method blank is <60% or >140%, check (1) calculations to locate possible errors, (2) fortifying solutions for degradation, (3) contamination or other obvious abnormalities, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.
- 9.5.2 If a blank extract reanalysis fails the 60-140% recovery criterion, the problem must be identified and corrected before continuing.
- 9.5.3 If sample extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract. If sample extract reanalysis continues to fail the surrogate recovery criterion, report all data for that sample as suspect.

9.6 Assessing the Internal Standard

- 9.6.1 When using the internal standard (IS) calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the daily calibration check standards IS response by more than 30%.
- 9.6.2 If a deviation greater than 30% occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract.
 - 9.6.2.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.
 - 9.6.2.2 If a deviation of greater than 30% is obtained for the re-injected extract, analysis of the sample should be repeated beginning with Section 11, provided the sample is still available. Otherwise, report results obtained from the re-injected extract, but annotate as suspect.

- 9.6.3 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.
 - 9.6.3.1 If the check standard provides a response factor (RF) within 25% of the value of the previous check standard or calibration, then follow procedures in Section 9.6.2 for each sample failing the IS response criterion.
 - 9.6.3.2 If the check standard provides a response factor which deviates more than 25% of the predicted value, then the analyst must recalibrate, as specified in Section 10.

9.7 Assessing Laboratory Performance - Laboratory Fortified Blank

The laboratory must analyze at least one laboratory fortified blank (LFB) sample with every twenty samples or one per sample set (all samples extracted within a 24-h period) whichever is greater. The fortified concentration of each analyte in the LFB should be 1 ng/g. If the recovery of any analyte falls outside the control limits (60 to 120%), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.8 Assessing Method Performance - Laboratory Fortified Sample Matrix

At least one LFM must be analyzed with every 20 samples or one per sample set (all samples extracted in a 24-h period) whichever is greater. The fortified concentration of each target PAH should be 1 ng/g. If the recovery of any target PAH or surrogate standard falls outside the designated range (60 to 120%), and the LFB is shown to be in control, the recovery problem is judged to be matrix related. The result for that analyte in the unfortified sample is labeled as suspect.

10.0 CALIBRATION AND STANDARDIZATION

10.1 GPC Cleanup

Refer to RTI/ACS-AP-211-134.

10.2 GC/MS Analysis

Refer to RTI/ACS-AP-211-135.

11.0 PROCEDURE

11.1 Sample Preparation

Remove samples from freezer and allow them to equilibrate to room temperature.

11.2 Alkaline Digestion

- 11.2.1 Place 25 g equivalent weight of homogenized food sample in a 500 mL round bottom flask. The equivalent weight is the weight of the food sample prior to the addition of water during sample homogenization. Record the food homogenate and equivalent weight to the nearest 0.01 g.
- 11.2.2 Spike the sample by adding 25 μ L of the surrogate standard spiking solution. Spike fortified matrix samples and fortified reagent blanks with 25 μ L of the matrix spike standard. Add 250 mL of ethanolic KOH to the flask.
- 11.2.3 Digest the sample by refluxing for 2 hours. At the completion of digestion, remove the sample from the heat and allow it to cool to room temperature.
- 11.2.4 Filter the sample digest through a funnel lightly packed with glasswool into a 1 L separatory funnel. Rinse the round-bottom flask sequentially with 225 mL reagent water, 125 mL ethanol and 100 mL n -hexane. Filter each rinse through the glasswool into the separatory funnel.
- 11.2.5 Shake the separatory funnel for 1 minute and allow layers to separate.
- 11.2.6 Prepare a second 1 L separatory funnel containing 200 mL of reagent water.
- 11.2.7 Remove the lower aqueous layer into a large beaker. Transfer the upper n -hexane layer into the separatory funnel containing reagent water.
- 11.2.8 Return the lower aqueous layer to the first separatory funnel with a 100 mL n -hexane rinse of the beaker. Shake the separatory funnel and allow to settle for 1 min.
- 11.2.9 Discard the lower aqueous layer. Transfer the n -hexane layer to the separatory funnel containing the first n -hexane extract along with a 50 mL n -hexane rinse. Shake the separatory funnel and allow layers to separate.

- 11.2.10 Assemble a K-D concentrator by attaching a 10-mL concentrator tube to a 500 mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 9.0 are met.
- 11.2.11 Discard the lower water layer and transfer the n -hexane layer to the K-D concentrator. Rinse the separatory funnel with 50 mL of n -hexane and add to the K-D concentrator.
- 11.2.12 Add 1 to 2 clean boiling stones to the evaporative flask and attach a Snyder column. Place the K-D apparatus on a hot water bath, 85 to 90°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches \leq 5 L, remove the K-D apparatus and allow it to drain and cool for at least 10 min.
- 11.2.13 Add 50 mL of methylene chloride (to remove residual water) to the K-D apparatus and repeat 11.3.12 adjusting the final volume to 10 mL with n -hexane. The extract should be entirely in n -hexane.

11.3 Solid Phase Extraction (SPE) Cleanup

- 11.3.1 Rinse the silica gel SPE column with 20 mL of methylene chloride followed by 3 mL of n -hexane.
- 11.3.2 Transfer the entire sample extract to the column.
- 11.3.3 Rinse the column with 1 mL of n -hexane twice. Discard the eluant.
- 11.3.4 Elute the column with 5 mL of 25% methylene chloride/ n -hexane, discarding the first 1 mL of eluant, then collecting the remaining eluant in a 10 mL Kuderna-Danish receiver.
- 11.3.5 Add several boiling chips to the concentrator tube, then attach a modified micro-Snyder column. Place in a hot water bath at 60°C. Let solvent evaporate to

<0.5 mL. Rinse modified micro-Snyder column. Adjust final volume to 1.0 mL with methylene chloride.

11.4 GPC Cleanup - Refer to RTI/ACS-AP-211-134

The GPC eluate in methylene chloride is concentrated to 0.5 L then solvent exchanged into 0.1 mL of toluene in a conical vial under a gentle stream of purified nitrogen. The concentrated extract is spiked with 25 µL of the GC/MS internal standard spiking solution. The sample extract is transferred then sealed in an amber glass vial with either Teflon-lined septa-sealed screw caps or crimp seals. The sample extracts may be stored at 4°C for short periods of time (<48 hours). The sample extract should be stored at -10°C for longer periods of time.

11.5 GC/MS Analysis

Refer to RTI/ACS-AP-211-135.

12.0 DATA ANALYSIS AND CALCULATIONS

$$C_f = \frac{C_e \cdot E_v}{ESW \cdot 1000} \quad (9)$$

where

C_f = food concentration (ng/g)

C_e = extract concentration (pg/µL) as calculated in RTI/ACS-AP-211-135,
Section 12

E_v = extract volume (µL)

ESW = equivalent sample weight - weight of sample excluding water added for
homogenization (g)

13.0 METHOD PERFORMANCE

13.1 In a single laboratory, pesticide recoveries from two sample matrices were determined at one concentration level. Data from these experiments are given in Tables 2 and 3.

13.2 In a single laboratory, seven replicates of a single food sample were fortified at a low level, then processed and analyzed to determine EDLs. Data from this experiment are given in Table 4.

14.0 POLLUTION PREVENTION

14.1 Always follow good laboratory practices when carrying out procedures. Consider air, soil and water pollution as the primary concerns. Order the least amount of solvent, materials, and reagents required. Do not discard solvents down the drain.

15.0 WASTE MANAGEMENT

15.1 Always dispose of spent or unused solvents by a properly licensed waste disposal company. Always properly label all waste. Store flammable waste in a storage container with a flash arrestor.

16.0 REFERENCES

1. Sheldon, L.S. Manual of Analytical Methods for Determination of Selected Environmental Contaminants in Composite Food Samples. EPA-68-C2-0103, U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 1995.
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3. Perfetti, G., Nyman, P., Fisher, S., Joe, F., Diachenko, G. Determination of Polynuclear Aromatic Hydrocarbons in Seafood by Liquid Chromatography with Fluorescence Detection, Journal of AOAC International Vol 75, No.5, 1992.
4. Methods for the Determination of Organic Compounds in Drinking Water, U.S. EPA, Cincinnati, Ohio, PB89-220461, Dec. 1988, Method 525 (rev. 2.1).

TABLE 1. APPLICABLE PAHs FOR RTI/ACS-AP-211-136

Compound	Analysis Method*	Comment
Benzo[a]anthracene	GC/MS	Applicable
Benzo[a]pyrene	GC/MS	Applicable - SPE cleanup must be performed to achieve low detection limits
Acenaphthylene	GC/MS	Applicable
Anthracene	GC/MS	Applicable
Dibenzo[ghi]perylene	GC/MS	Applicable - SPE cleanup must be performed to achieve low detection limits
Fluoranthene	GC/MS	Applicable
Indeno[1,2,3-cd]pyrene	GC/MS	Applicable - SPE cleanup must be performed to achieve low detection limits
Phenanthracene	GC/MS	Applicable
Pyrene	GC/MS	Applicable
Acenaphthene	GC/MS	Applicable - Low recoveries through SPE cleanup step
Benzo[b]fluoranthene	GC/MS	Applicable - SPE cleanup must be performed to achieve low detection limits
Benzo[k]fluoranthene	GC/MS	Applicable - SPE cleanup must be performed to achieve low detection limits
Chrysene	GC/MS	Applicable
Dibenzo[ah]anthracene	GC/MS	Applicable - SPE cleanup must be performed to achieve low detection limits
Fluorene	GC/MS	Applicable

* RTI/ACS-AP-211-135.

TABLE 2. METHOD EVALUATION RESULTS - RTI/ACS-AP-211-136
 COMPOSITE SAMPLE FROM PARTICIPANT 5, PREPILOT STUDY

Compound	Concentration (ng/g)			% Recovery				Spiked Concentration (ng/g)
	Laboratory Reagent Blank (n=1)	Mean Unspiked (n=3)	% RSD Unspiked	Laboratory Fortified Blank (n=1)	Laboratory Fortified Sample Matrix (n=3)	% RSD Spiked		
Benzo[a]anthracene	0.08	0.19	3	87	81	8		1
Benzo[a]pyrene	0.07	0.18	3	81	81	12		1
Acenaphthylene	0.14	0.24	9	47	59	7		2
Anthracene	0.14	0.18	3	66	77	8		2
Dibenz[ghi]perylene	0.07	0.17	3	82	88	11		1
Fluoranthene	0.13	0.47	7	83	80	5		5
Indeno[1,2,3-cd]pyrene	0.06	0.17	5	89	86	12		1
Phenanthracene	0.87	1.74	6	69	76	4		10
Pyrene	0.18	0.54	11	77	73	10		3
Acenaphthene	0.14	0.28	11	58	73	11		1
Benzo[b]fluoranthene	0.08	0.22	2	87	81	12		1
Benzo[k]fluoranthene	0.07	0.11	4	82	80	9		1
Chrysene	0.11	0.27	8	84	77	12		1
Dibenzo[ah]anthracene	0.06	INT	-	107	102	3		1
Fluorene	0.46	0.90	11	64	74	5		5
<u>Surrogates</u>								
Benzo[e]pyrene-d ₁₂	68*	77*	4	80	81	13		1
Chrysene-d ₁₂	71*	72*	2	73	71	9		1

* % recovery.

TABLE 3. METHOD EVALUATION RESULTS - RTI/ACS-AP-211-136 - WITHOUT CLEAN, COMPOSITE SAMPLE

Compound	Concentration (ng/g)			% Recovery ^a			
	Laboratory Reagent Blank (n=1)	Mean Unspiked (n=3)	% RSD Unspiked	Laboratory Fortified Blank (n=1)	Laboratory Fortified Sample Matrix (n=3)	% RSD Spiked	Spiked Concentration (ng/g)
Benzo[a]anthracene	0.00	0.11 ^b	-	89	99	12	1
Benzo[a]pyrene	0.00	0.05	82	92	121	8	1
Acenaphthylene	0.46	1.67	1	66	229	7	2
Anthracene	0.02	0.07	12	61	54	3	2
Dibenz[ghi]perylene	0.00	INT ^c	INT	79	INT	-	1
Fluoranthene	0.06	0.73	6	90	87	14	5
Indeno[1,2,3-cd]pyrene	0.00	INT	INT	78	INT	-	1
Phenanthracene	0.48	1.83	5	75	71	6	10
Pyrene	0.11	0.56	27	92	79	14	3
Acenaphthene	0.00	0.83	5	108	0	-	1
Benzo[b]fluoranthene	0.00	0.01 ^b	-	99	115	13	1
Benzo[k]fluoranthene	0.03	0.01 ^b	-	105	118	12	1
Chrysene	0.06	0.15 ^b	--	89	108	17	1
Dibenzo[ah]anthracene	0.00	INT	INT	88	INT	-	1
Fluorane	INT	0.88	13	INT	103	4	5
<u>Surrogates</u>							
Benzo[e]pyrene-d ₁₂	78 ^d	115 ^d	14	95	139	22	1
Chrysene-d ₁₂	87 ^d	63 ^d	4	80	87	13	1

^a Method performed without solid phase extraction cleanup.

^b Only quantitated in one sample, interference prevented quantitation in other two samples.

^c Interference in sample extract prevented quantitation.

^d % recovery.

**TABLE 4. METHOD EVALUATION RESULTS - ESTIMATED DETECTION LIMIT - RTI/ACS-AP-211-136
COMPOSITE SAMPLE FROM PARTICIPANT 5, PREPILOT STUDY**

Compound	Laboratory Reagent Blank (ng/g) (n=1)	% Recovery ^a Laboratory Fortified Blank (n=1)	Measured Concentration (ng/g)			Spiking ^d Level (ng/g)
	Mean	S.D. ^b	EDL ^c (ng/g)			
Benzo[a]pyrene	0.08	68	0.38	0.03	0.11	0.20
Benzo[a]anthracene	0.11	77	0.38	0.01	0.03	0.20
Acenaphthylene	0.17	49	1.61	0.14	0.45	0.40
Anthracene	0.23	75	0.91	0.05	0.15	0.40
Dibenz[ghi]perylene	0.08	59	0.34	0.01	0.03	0.20
Fluoranthene	0.18	81	1.72	0.07	0.23	1.0
Indeno[1,2,3-cd]pyrene	0.08	67	0.33	0.03	0.10	0.20
Phenanthracene	1.5	72	5.90	0.39	1.23	2.0
Pyrene	0.22	71	1.50	0.06	0.19	0.60
Acenaphthene	0.26	59	0.59	0.05	0.16	0.20
Benzo[b]fluoranthene	0.09	76	0.39	0.01	0.03	0.20
Benzo[k]fluoranthene	0.08	75	0.29	0.02	0.05	0.20
Chrysene	0.13	72	0.42	0.01	0.04	0.20
Dibenz[ah]anthracene	0.08	69	0.23	0.01	0.04	0.20
Fluorane	0.63	62	2.47	0.17	0.54	1.0
Surrogates						
Benzo[e]pyrene-d ₁₂	65 ^e	69 ^e	76 ^e	3.2	NA ^f	1.0
Chrysene-d ₁₂	63 ^e	65 ^e	69 ^e	1.1	NA	1.0

^a Spiked at concentrations shown in Table 2.

^b Standard deviation.

^c Estimated detection limit; EDL = S.D. x 3.67 for n = 6.

^d Spike level for EDL samples.

^e % recovery.

^f Not applicable.

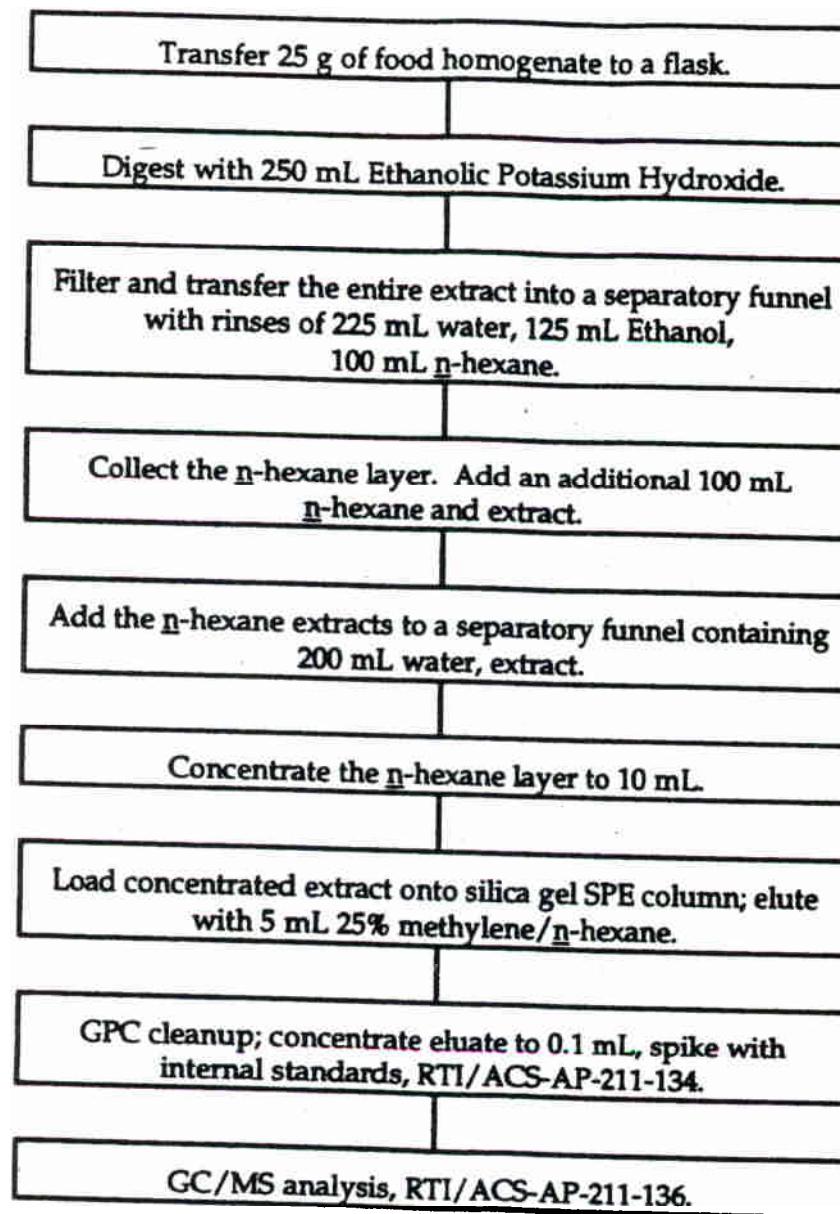


Figure 1. Flow diagram of RTI/ACS-AP-211-136

TITLE: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)
ANALYSIS OF CARBAMATES

SOURCE: Research Triangle Institute
Post Office Box 12194
Analytical and Chemical Sciences
Research Triangle Park, NC 27709-2194

AUTHOR(s):

Mike R. Ford

Date: 3/24/98

Jeffrey T. Kuehn

Date: 3/24/98

Date: _____

APPROVED BY:

Principal Investigator:

E. Pellegrini

Date: 3/26/98

QA Officer:

Doris Smith

Date: 3/25/98

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1		7	
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revision 0 is the original version.

**HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS
OF CARBAMATES**

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1.0 SCOPE AND APPLICATION

- 1.1 This is a high performance liquid chromatographic method for the determination of N-methylcarbamates in extracts from food samples. The extract is assumed to be relatively free of coextractive (lipids, proteins, etc.) prior to the application of this method. The method has been tested and is applicable to the carbamates in Table 1.
- 1.2 For compounds other than those listed in Table 1 or for other sample types, the analyst must demonstrate the applicability of the method by collecting precision and accuracy data on fortified samples (i.e., food composites) and provide qualitative confirmation of results by an acceptable analytical method.
- 1.3 Estimated detection limits (EDL) for the above pesticides must be experimentally determined for each linked analysis scheme.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 9 of RTI/ACS-AP-211-131.
- 1.5 When this method is used to analyze unfamiliar samples for any or all of the analytes above, analyte identifications must be confirmed by at least one additional qualitative technique.

2.0 SUMMARY OF THE METHOD

This method is for the analysis of carbamates in extracts from food samples by HPLC with post-column derivatization and fluorescence detection. The extract is filtered and a 20 µL aliquot is injected into a reverse phase HPLC column. Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, the analytes are hydrolyzed with 0.05 N sodium hydroxide (NaOH) at 95°C. The methyl amine formed during

hydrolysis is reacted with α -phthalaldehyde (OPA) and 2-mercaptoethanol (or N,N-dimethyl-2-mercaptoethylamine hydrochloride) to form a highly fluorescent derivative which is detected by a fluorescence detector.

3.0 DEFINITIONS

- 3.1 Refer to RTI/ACS-AP-211-131.
- 3.2 High Performance Liquid Chromatography (HPLC)

4.0 INTERFERENCES

- 4.1 Carryover contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of compounds. Syringes and injector must be cleaned carefully or replaced as needed.
- 4.2 Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference will vary considerably from sample to sample. Positive identifications must be confirmed.

5.0 SAFETY

- 5.1 Refer to RTI/ACS-AP-211-131.

6.0 EQUIPMENT AND SUPPLIES

- 6.1 Balance

Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2 Filtration Apparatus

- 6.2.1 Macrofiltration – to filter derivatization solutions and mobile phases used in HPLC. Recommend using 47 mm filters (Millipore Type HA, 0.45 µm for water and Millipore Type FH, 0.5 µm for organics or equivalent).
- 6.2.2 Microfiltration – to filter samples prior to HPLC analysis. Use 13 mm filter holder (Millipore stainless steel XX300/200 or equivalent), and 13 mm diameter 0.2 µm polyester filters (Nuclepore 180406 or equivalent).

6.3 Syringes and Syringe Valves

- 6.3.1 Hypodermic syringe – 50 mL glass, with Luer-Lok tip.
- 6.3.2 Micro syringes – various sizes.

6.4 Miscellaneous

- 6.4.1 Solution storage bottles – Amber glass, 500 to 4000 mL with Teflon-lined screw caps.
- 6.4.2 Helium, for degassing solutions and solvents.
- 6.4.3 Volumetric flasks.

6.5 High Performance Liquid Chromatography (HPLC)

- 6.5.1 HPLC system capable of injecting 20 µL aliquots, and performing binary gradients at a constant flow rate. A data system is recommended for measuring peak areas. Table 1 lists retention times observed for method analytes using the column and analytical conditions described below.
- 6.5.2 Column – 250 mm x 4.6 mm i.d. Zorbax-C8 (Dupont 880952.706) maintained at a constant 35°C temperature using an HPLC column heater.
- 6.5.3 Mobile Phases: A = 95:5 water:acetonitrile; B = 30:70 water:acetonitrile. Gradient program at 1.5 mL/min as follows: 80:20 A:B for 10 min then to 0:100 A:B in 25 min with a 16 min final hold.

- 6.5.4 Post column hydrolysis chamber – Capable of mixing reagents into the mobile phase. Reactor should be constructed using stainless steel tubing (3 m x 0.4 mm i.d.) and equipped with a solvent delivery system capable of delivering 0.1 to 2.0 mL/min of the NaOH reagent while maintaining a constant uniform 100°C temperature.
- 6.5.5 Fluorescence detector – Capable of excitation at 230 nm and detection of emission energies greater than 418 nm. A McPherson Instrument Model FL7508 fluorescence detector was used to generate the data presented in Methods PAS-01 to PAS-07.
- 6.5.6 Post column reaction chamber – Capable of mixing the derivatization solution into the mobile phase plus NaOH reagent flow; constructed of stainless steel tubing (3 m x 0.4 mm i.d.) and equipped with a solvent delivery system capable of delivering 0.1 to 3 mL/min of the derivatizing solution to the column plus NaOH flow.

7.0 REAGENTS AND STANDARDS

WARNING

When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed, thus potentially reducing the shelf-life.

7.1 Reagent Water

Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. Reagent water used to generate the validation data for this method was Hydro-Systems Picotech 18 M Ohm water.

7.2 Methanol – HPLC grade.

7.3 Acetonitrile – demonstrated to be free of analytes.

7.4 HPLC Mobile Phase

- 7.4.1 Reagent water. Filter and degas with helium before use.
- 7.4.2 Acetonitrile – HPLC grade. Filter and degas with helium before use.

7.5 Post Column Derivatization Solutions

- 7.5.1 Sodium hydroxide, 0.05 N – Dissolve 2.0 g of sodium hydroxide (NaOH) in reagent water. Dilute to 1.0 L with reagent water which has been filtered and degassed with helium just before use.
- 7.5.2 N,N-dimethyl-2-mercaptoproethylamine hydrochloride. Thiofluor crystals (Pickering Laboratories 3700-2000). Two grams of Thiofluor is equivalent to 1 mL of 2-mercaptoproethanol.
- 7.5.3 α -Phthalaldehyde (OPA). Dissolve 100 mg of OPA (Pickering Laboratories 0120) into 10 mL of UV grade methanol.
- 7.5.4 α -Phthalaldehyde diluent. Add one 950 mL bottle of α -phthalaldehyde diluent (Pickering Laboratories B910) into a reservoir and thoroughly degas.
- 7.5.5 Reaction solution. (To the degassed α -phthalaldehyde diluent). Add the dissolved α -phthalaldehyde mixture. Mix well. Add 2 grams of Thiofluor directly to the solution and gently mix.

7.6 4-Bromo-3,5-diethylphenyl N-methylcarbamate (BDMC)

Ninety-eight percent purity, for use as internal standard (available from Aldrich Chemical Co.).

7.7 Stock Standard Solutions (1.00 μ g/ μ L)

Stock standard solutions may be purchased as certified solutions or prepared from pure standard material using the following procedure.

- 7.7.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in HPLC grade acetonitrile and dilute to volume in a 10 mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any

concentration if they are certified by the manufacturer or by an independent source.

7.7.2 Transfer the stock standard solutions into Teflon-lined screw cap vials. Store in refrigerator and protect from light.

7.8 Internal Standard Solution

Prepare an internal standard solution by accurately weighing approximately 0.0010 g of pure BDMC. Dissolve the BDMC in acetonitrile and dilute to volume in a 10 mL volumetric flask. Transfer the internal standard fortification solution to a Teflon-lined screw cap bottle and store at room temperature. The internal standard fortification results in a final internal standard concentration of 500 µg/L.

8.0 SAMPLE HOMOGENIZATION AND STORAGE

8.1 Holding Time

Holding times for sample extracts prior to analysis have not been established. It is recommended that sample extracts be analyzed within 30 days of extraction.

8.2 It is anticipated that extracts will be analyzed shortly after extraction is complete. It is the analyst's responsibility to assure that any storage of extracts does not affect analytical results. All extracts should be protected from light. Extracts may be stored at 4 °C for short periods of time <48 hours. Extracts should be stored at ≥ 10 °C for longer periods of time.

9.0 QUALITY CONTROL

9.1 Refer to RTI/ACS-AP-211-131.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Establish HPLC operating parameters equivalent to those indicated in Section 6.5. The HPLC system should be calibrated using the internal standard technique.

10.2 Internal Standard Calibration Procedure

The analyst must select one or more internal standards similar in analytical behavior to the analytes of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. BDMC has been identified as a suitable internal standard.

10.2.1 Prepare calibration standards at a minimum of five concentration levels (10 to 2000 pg/ μ L) for each analyte of interest by adding volumes of one or more of the stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetonitrile. The lowest standard should represent analyte concentrations near, but above, their respective EDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

10.2.2 Analyze each calibration standard according to the procedure (Section 6.5). Tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factors (RF) for each analyte using Equation 1.

$$\text{where: } RF = \frac{(A_x)(C_s)}{(A_i)(C_s)} \quad (1)$$

A_x = Area response for the analyte to be measured.

A_i = Area response for the internal standard.

C_s = Concentration of the internal standard (μ g/L).

C_x = Concentration of the analyte to be measured (μ g/L).

10.2.3 If the RF value over the working range is constant (25% RSD or less), the average RF can be used for calculations. Alternatively, the individual analytes RF results can be used to plot a linear calibration curve.

10.2.4 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than 25%, the test must be repeated using a fresh calibration standard. If the repetition also fails, a new calibration curve must be generated for that analyte using freshly prepared standards.

10.3 Continuing Calibration Check

Verify the instrument calibration at the beginning of each day that analyses are performed using the following procedure, as well as after the analysis of every nine sample extracts.

- 10.3.1 Inject a 1- μ L aliquot of a mid level concentration calibration solution and analyze with the same conditions used during the initial calibration.
- 10.3.2 Determine that the absolute areas of the internal standards has decreased by more than 25% from the areas measured in the most recent continuing calibration check, or by more than 50% from the areas measured during initial calibration. If these areas have decreased by more than these amounts, adjustments must be made to restore system sensitivity or other maintenance as indicated in Section 10.3.5 and recalibration.
- 10.3.3 Calculate the RF for each analyte and surrogate from the data measured in the continuing calibration check. The RF for each analyte and surrogate is in control if its RF is within \pm 25% from the RF in the calibration curve. Acceptable performance for the analytical system is met if all analytes are in control. If the instrument is in control, the daily RF values are used for sample quantitation. If these conditions are not met, remedial action (Section 10.3.7) must be taken which may require recalibration.
- 10.3.4 After the analysis of every nine samples, the mid level calibration standard is reanalyzed. If the RF values for this analysis have not varied by more than 25% from the daily RF values, then the instrument is in control and samples may be quantitated using the daily RFs. If these conditions are not met, then the

quantitative data for the sample analyzed prior to the check standard are considered suspect for the analytes that are out-of-control. Sample extracts must be reanalyzed if any of the target pesticides that are out-of-control are detected or if the RF values for any of the pesticides in the check standard are less than 50% of the calibration value. Check standards may be analyzed more often to minimize the need to repeatedly reanalyze samples.

- 10.3.5 Some possible remedial actions - Major maintenance such as cleaning the HPLC, injector syringe and detector.
 - 10.3.5.1 Check and adjust HPLC operating conditions.
 - 10.3.5.2 Check and adjust the fluorescence detector.
 - 10.3.5.3 Flush the HPLC column with solvent according to manufacturer's instructions.
 - 10.3.5.4 Replace HPLC guard column if utilized.
 - 10.3.5.5 Prepare fresh CAL solutions, and repeat the initial calibration step.
 - 10.3.5.6 Replace the HPLC syringe.
 - 10.3.5.7 Replace any components that allow analytes to come into contact with hot metal surfaces.

11.0 PROCEDURE

- 11.1 Analyze 20 µL aliquot of each sample with the HPLC system under the same conditions used for the initial and continuing calibrations (Section 10).
 - 11.1.1 Section 6.5 summarizes the recommended operating conditions for HPLC. Table 1 lists retention times observed using this method.
 - 11.1.2 Verify system calibration daily as described in Section 10. The standards and samples must be in acetonitrile.
 - 11.1.3 Inject 20 µL of the sample extract. Record the volume injected and the resulting peak size in area units.
 - 11.1.4 If the response for the peak exceeds the working range of the system, dilute the sample extract with acetonitrile and reanalyze.

11.2 Identification of Analytes

- 11.2.1 Identify a sample component by comparison of its retention time to the retention time of a standard compound. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.
- 11.2.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 11.2.3 Identification requires expert judgement when sample components are not resolved chromatographically. When peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak on a chromatogram, appropriate alternate techniques, to help confirm peak identification, need to be employed. For example, more positive identification may be made by the use of an alternate detector which operates on a chemical/physical principle different from that originally used; e.g., mass spectrometry, or the use of a second chromatography column.

12.0 CALCULATIONS

12.1 Calculate Analyte Concentrations

where: $C_x = \frac{(A_x)(Q)}{(A_s)(RF)}$ (2)

C_x = concentration of analyte pg/ μ L in the food extract.
 A_x = area response of the analyte in the sample.

A_{is} = area response of the internal standard in the sample.
 Q_{is} = concentration of internal standard in $\text{pg}/\mu\text{L}$ in the food extract.
RF = daily response factor of analyte from the continuing calibration check standard.

12.2 Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty). Experience indicates that three significant figures may be used for extract concentrations above $99 \mu\text{g}/\text{L}$, two significant figures for concentrations between $1-99 \mu\text{g}/\text{L}$, and one significant figure for lower concentrations.

13.0 METHOD PERFORMANCE

13.1 Table 1 shows the target carbamates that can be analyzed by this method. Additional carbamates may be applicable with the method performance evaluated by the analysis laboratory. The table also indicates the lowest concentration standard that has been demonstrated to be in the linear range of the instrument.

13.2 The overall method performance will be dictated by the performance of the sample extraction in combination with the cleanup and analytical determinative method. Method performance for linked analysis schemes is provided in RTI/ACS-AP-211-131.

13.3 Substantial interferences were found in many food extracts for methomyl. Small interferences were found for the analysis of aldicarb. Additional cleanup or modifications to the chromatographic conditions might be used to minimize the effect of these interferences.

14.0 POLLUTION PREVENTION

14.1 Refer to RTI/ACS-AP-211-131.

15.0 WASTE MANAGEMENT

15.1 Refer to RTI/ACS-AP-211-131.

16.0 REFERENCES

1. Sheldon, L.S. Manual of Analytical Methods for Determination of Selected Environmental Contaminants in Composite Food Samples. EPA-68-C2-0103, U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 1995.
2. Methods for the Determination of Organic Compounds in Drinking Water, U.S. EPA, Cincinnati, Ohio, PB89-220461, Dec. 1988, Method 525 (rev. 2.1).
3. U.S. FDA, Pesticide Analytical Manual, Volume 1, 3rd Edition, Section 401, Washington, DC, 1994.
4. Sheldon, L., J. Keever, J. Beach, M. Roberds and L. Ellis. Environmental Contaminants in Foods: Phase 3 - Validation of Analytical Methods. Final Report, U.S. EPA EMSL-Cincinnati Contract Number 68-C2-0103, Work Assignment 2-01.

TABLE 1. APPLICABLE CARBAMATES FOR RTI/ACS-AP-211-137

Carbamate	RT ^a (min)	Lowest Calibration Standard (pg/ μ L)
<u>Pesticide</u>		
Methomyl	15.15	10
Aldicarb	28.31	10
Propoxur	32.32	10
Carbofuran	32.79	10
Carboxyl	33.95	10
<u>Internal Standard</u>		
4-Bromo-3,5-Dimethylphenyl-N-Methylcarbamate	37.60	NA ^b

^a Retention time.

^b Not applicable.

**LABORATORY
OPERATIONS
PROTOCOL**

**RESEARCH TRIANGLE INSTITUTE
POST OFFICE BOX 12194
RESEARCH TRIANGLE PARK, NC 27709-2194**

RTI/ACS-AP-211-138

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TITLE:

SAMPLE PREPARATION AND DETERMINATION OF TOTAL
RECOVERABLE ELEMENTS IN FOOD BY MICROWAVE DIGESTION,
INDUCTIVELY-COUPLED PLASMA MASS SPECTROMETRY AND
GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

SOURCE:

Research Triangle Institute
Post Office Box 12194
Analytical and Chemical Sciences
Research Triangle Park, NC 27709-2194

AUTHOR(s):

Eileen Kelly Frame

Date: 6/2/98

Date: _____

Date: _____

APPROVED BY:

Principal Investigator: *Ed Thompson*

Date: 6/3/98

QA Officer: *Doris Smith*

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SAMPLE PREPARATION AND DETERMINATION OF
TOTAL RECOVERABLE ELEMENTS IN FOOD BY MICROWAVE DIGESTION,
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1.0 SCOPE AND APPLICATIONS

1.1 This method provides sample preparation procedures and procedures for the determination of total recoverable elements in food and beverage samples. This method is based on EPA Methods 200.2 Rev. 2.8, 200.8 Rev. 5.4 and 200.9 Rev 2.2, all dated 1994. This method is applicable to the following elements:

Analyte	Symbol	Chemical Abstract Services Registry Number (CASRN)
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3
Cadmium	(Cd)	7440-43-9
Chromium	(Cr)	7440-47-3
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Manganese	(Mn)	7439-96-5
Nickel	(Ni)	7440-02-0
Selenium	(Se)	7782-49-2
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

Estimated solution method detection limits (MDLs) for these elements are listed in Tables 1 and 2. These are intended to be representative of what has been obtained in similar matrices after sample digestion using the VG PQXR inductively-coupled plasma-mass spectrometer (ICP-MS) optimized for multielement determinations and employing pneumatic sample introduction. Actual method detection limits and linear working ranges will depend on the sample matrix and selected operating conditions. The MDL presented for selenium is for determination by graphite furnace atomic absorption spectrometry, not ICP-MS. The

interference from the argon dimer in ICP-MS is too great to permit accurate determination of Se by this method.

1.2 Samples prepared by this method can be analyzed by ICP-MS, stabilized temperature graphite furnace atomic absorption spectrometry (STPF GFAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and atomic fluorescence spectrometry(AFS). ICP-AES and AFS analytical methods would need to be optimized for use.

1.3 Food and beverage samples are both treated as samples containing solid/suspended particulate matter and both require acid digestion prior to analysis. The elements determined in the digestate represent the total recoverable analyte present in the sample.

1.4 The total recoverable sample digestion procedure given in this method will solubilize and hold in solution only minimal concentrations of barium in the presence of free sulfate. For the analysis of barium in samples having varying and unknown concentrations of sulfate, analysis should be completed as soon as possible after sample preparation.

1.5 This method is not suitable for the determination of mercury, organomercury compounds, silicon, silver, tin, antimony and other volatile elements or those forming insoluble hydrated oxides or other insoluble compounds.

1.6 This method should be used by analysts experienced in the use of microwave digestion equipment, ICP-MS and GFAAS instrumentation and experienced in the interpretation of spectral interferences, matrix interferences and procedures for their correction. A minimum of six months experience with commercial instrumentation is recommended. Training will be documented in each analyst's training file.

1.7 Users of the method must document and have on file the required initial demonstration performance data described in Section 9.2 prior to using the method for analysis.

2.0 SUMMARY OF THE METHOD

2.1 Homogeneous, well-mixed samples of food or beverage are dispensed into acid-cleaned polypropylene digestion tubes and accurately weighed. Concentrated nitric acid is added and the samples are allowed to stand for a minimum of one hour to oxidize easily-oxidizable

material and expel the gases generated. The samples are then digested in an atmospheric pressure microwave digestion system using a multistep heating program. After digestion, the solubilized analytes are diluted to specific volumes with ASTM Type I water, mixed, refrigerated and allowed to settle. Diluted samples are to be analyzed by the appropriate technique as soon as possible after preparation.

2.2 The method describes the multielement determination of trace elements by ICP-MS, and the determination of traces of selenium by GFAAS. For the ICP-MS determination, sample material in solution is introduced by pneumatic nebulization into a radiofrequency argon plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a quadrupole mass spectrometer having a minimum resolution of 1 amu peak width at 5% peak height. The ions transmitted through the quadrupole are detected by an electron multiplier or a Faraday detector and the ion information processed by a data handling system. Interferences related to the technique must be recognized and corrected for (Section 4.0). Such corrections include compensation for isobaric interferences from elements and polyatomic species. Instrumental drift and suppressions or enhancements of response caused by the sample matrix must be corrected for by the use of internal standards.

The GFAAS method for selenium is based on the introduction of sample material in solution into a resistively-heated graphite furnace. The sample solution is deposited on a graphite platform along with a matrix modifier, and a multistep heating program causes drying, ashing and atomization of the sample. High purity argon is used as the furnace atmosphere, and mixed hydrogen/argon is used in the ash step. Selenium atoms in the gas phase are detected by their absorption of an emission line from a selenium discharge lamp (hollow cathode or electrodeless). Background absorption from molecular species is corrected by the use of the Zeeman technique, in which the emission line from the lamp is split magnetically into components shifted to both higher and lower wavelengths than the original emission line, and the split components are polarized in the process. The Zeeman-shifted lines are not absorbed by the Se atoms, but are absorbed by broad-band absorbing (molecular)

species. The difference in absorption between the shifted and unshifted lines is the net atomic absorption signal from Se. The Zeeman effect will not correct for direct spectral interferences, such as that of Fe at the Se 196.1 nm line. Either another line must be used or the iron content of the samples measured and an interelement correction empirically determined. Suppression or enhancement of the Se signal must be corrected for by the use of the method of standard additions.

3.0 DEFINITIONS

3.1 Calibration Blank

A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP-MS or GFAAS instrument (Sections 7.7.1 and 7.8.1).

3.2 Calibration Standard (CAL)

A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration (Section 7.5).

3.3 Instrument Detection Limit (IDL)

The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of 10 replicate measurements of the calibration blank signal at the selected analytical mass(es) or analytical wavelength, whichever is appropriate.

3.4 Instrument Performance Check (IPC) Solution

A solution of method analytes, used to evaluate the performance of the instrument system with respect to a defined set of method criteria (Section 9.3.4).

3.5 Internal Standard

Pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component (Section 7.6).

3.6 Laboratory Duplicates (LD1 and LD2)

Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.7 Laboratory Fortified Blank (LFB)

An aliquot of LRB to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements (Sections 7.7.3 and 7.8.3).

3.8 Laboratory Fortified Sample Matrix (LFM)

An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentration (Section 9.4.2).

3.9 Laboratory Reagent Blank (LRB)

An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus (Sections 7.7.2 and 7.8.2).

3.10 Linear Dynamic Range (LDR)

The concentration range over which the instrument response to an analyte is linear (Section 9.2).

3.11 Matrix Modifier

A substance added to the graphite furnace along with the sample in order to minimize the interference effects by selective volatilization of either analyte or matrix components.

3.12 Method Detection Limit (MDL)

The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

3.13 Quality Control Sample (QCS)

A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance (Section 7.11).

3.14 Solid Sample

For the purpose of this method, a sample taken from material classified as either food or beverage.

3.15 Standard Addition

The addition of a known amount of analyte to the sample in order to determine the relative response of the detector to an analyte within the sample matrix. The relative response is then used to assess either an operative matrix effect or the sample analyte concentration (Section 11.5).

3.16 Stock Standard Solution

A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source (Section 7.3).

3.17 Total Recoverable Analyte

The concentration of analyte determined to be in a solid sample following treatment by refluxing with hot mineral acid.

3.18 Tuning Solution

A solution which is used to determine acceptable instrument performance prior to calibration and sample analyses (Section 7.9).

4.0 INTERFERENCES

- 4.1 In sample preparation, contamination is of prime concern. Samples will be prepared in a metal-free, Class 100 sample preparation lab. The work area will be periodically cleaned in accordance with ACS SOPs for Use of the Class 100 facility.
- 4.2 Chemical interferences are matrix-dependent and cannot be documented prior to analysis.
- 4.3 All glass and plastic labware to be used in this study will be acid-leached prior to use to remove any traces of metal contamination. A series of laboratory reagent blanks is used to monitor contamination.
- 4.4 Several interference sources may cause inaccuracies in the determination of elements by ICP-MS. These are:

4.4.1 Isobaric Elemental Interferences

Are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer in use. All elements determined by this method except selenium have, at a minimum, one isotope free of elemental isobaric interferences. The analytical isotopes recommended for use with this method are listed in Table 3. Selenium-82 has an isobaric elemental interference (krypton-82, which is often present in the liquid argon used for the plasma gas).

4.4.2 Abundance Sensitivity

Is a property defining the degree to which the wings of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Wing overlap interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them.

4.4.3 Isobaric Polyatomic Interferences

Are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use. These ions commonly form in the plasma or interface system from the plasma gas and/or sample components. Most of these common interferences have been identified and these are listed in Table 4 together with the method elements affected. Such interferences must be recognized and when they cannot be avoided by selection of alternate isotopes, appropriate corrections must be made to the data. Equations for the correction of the data are established in the instrument software at the time of the analytical run.

4.4.4 Physical Interferences

Are associated with the physical processes which govern the transport of sample into the plasma, sample conversion processes in the plasma and the transmission of ions through the plasma-mass spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. High levels of dissolved solids in the sample may contribute to deposits of material on the extraction and skimmer cones, reducing the effective diameter of the orifices and therefore ion transmission. Dissolved solids levels not exceeding 0.2% (w/v) have been recommended to reduce such effects. Internal standardization may be effectively used to compensate for many physical interference effects. Internal standards ideally should have similar analytical behavior to the elements being determined.

4.4.5 Memory Interferences

Result when isotopes of elements in previous samples contribute to the signal measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones, and from the buildup of material in the sample introduction system (autosampler and pump tubing, nebulizer, spray chamber and torch). The site where these effects occur is dependent on the element and can be minimized by flushing the system with a suitable rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element should be estimated prior to analysis. This may be achieved by aspirating a standard containing elements corresponding to 10 times the upper end of the linear range for a normal sample analysis period, followed by analysis of a rinse blank. The length of time required to reduce the rinse blank signal to within a factor of 10 of the MDL should be noted. Memory interferences may also be assessed within an analytical run by using a minimum of three replicate integrations for data acquisition. If the integrated signal drops consecutively, the analyst should be alerted to the possibility of a memory effect and should examine the concentration of the previous sample to see if it was high. If a memory interference is suspected, the sample should be reanalyzed after a long rinse period.

4.5 Several interference sources may cause inaccuracies in the determination of trace elements by GFAAS. These are:

- 4.5.1 Spectral interferences are caused by the resulting absorbance of light by a molecule or atom which is not the analyte of interest or emission from black body radiation.
 - 4.5.1.1 Spectral interferences caused by an element can only occur if there is a spectral overlap between the wavelength of the analyte of interest and the interfering element. This type of interference is uncommon. The use of appropriate furnace

temperatures and high spectral purity lamps as light sources can minimize this interference. A specific element interference for selenium by GFAAS is iron, which has been shown to suppress Se signals, particularly when a continuum background corrector is used. In addition, the use of hydrogen in the purge gas during the dry and char steps can cause a suppression of the Se signal if not purged from the furnace prior to the atomization step. Spectral interference can also be caused by molecules and other broad band absorbing species.

This type of interference is far more common in STPGFAAS. The use of matrix modifiers, selective volatilization, and background correctors are all used to eliminate this nonspecific absorbance. The nonspecific component of absorbance can vary considerably from sample type to sample type. Therefore the effectiveness of the background correction may vary depending on the analyte wavelength used and the nature and magnitude of the interference.

- 4.5.1.2 Spectral interferences are also caused by the emission of black body radiation produced during the atomization furnace cycle. This black body emission reaches the photomultiplier tube, producing erroneous results. The magnitude of this interference can be minimized by proper furnace tube alignment and monochromator design. In addition, atomization temperatures which adequately atomize the analyte of interest without producing unnecessary black body radiation can help reduce background emission.
- 4.5.2 Matrix interferences are caused by sample components which inhibit the formation of free atoms of the analyte during the atomization cycle.
- 4.5.2.1 Matrix interferences can be of a chemical or physical nature. In this method the use of a delayed atomization device (the platform) which provides stabilized temperatures is required. These devices provide an environment which is more conducive to the formation of free analyte atoms and therefore minimizes this type of interference. This type of interference can be detected by analyzing the sample plus a sample aliquot fortified with a known concentration of the

analyte. If the determined concentration of the analyte addition is outside a designated acceptable range, a matrix interference should be suspected.

- 4.5.2.2 Nitric acid is preferred for GFAAS analyses in order to minimize vapor state anionic chemical interferences. In this method, only nitric acid is used, so these interferences are minimal. However, chloride ions in the sample matrix may act in this role, so the appropriate matrix modifier, a combination of Pd and Mg nitrate, and furnace atmosphere (5% hydrogen-95% argon except during atomization per Section 4.5.3) will be chosen to minimize this interference.
- 4.5.3 Memory interferences result from analyzing a sample containing a high concentration of an analyte which cannot be removed quantitatively in one set of furnace steps. This typically occurs with an element requiring high atomization temperature. The analyte remaining in the furnace can produce false positive signals for subsequent sample(s). The analyst should establish the analyte concentration which can be injected into the furnace and adequately removed in one furnace cycle. If this concentration is exceeded, the sample should be diluted and a blank analyzed to ascertain that there is no memory effect before reanalysis. It may also be necessary to reanalyze the sample(s) immediately following the high concentration sample.

5.0 SAFETY

5.1 All personnel handling samples known or suspected to have been in contact with human waste should be immunized against known disease causative agents and should have completed the RTI Blood-borne Pathogen Training Course and have such training documented in their training files.

5.2 The acidification of samples must be done in a fume hood.

5.3 The toxicity or carcinogenicity of the reagents used in this method have not been fully established. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. Material

safety data sheets (MSDS) for all chemical reagents should be available to and understood by all personnel using this method. Specifically, concentrated nitric acid is moderately toxic and extremely irritating to eye, skin and mucus membranes. Use this in a hood whenever possible, and if eye or skin contact occurs, flush with large amounts of water, and notify your supervisor immediately. Always wear safety glasses with side shields, gloves and a lab coat. Chemicals should be regarded as potential health hazards and exposure kept as low as reasonably achievable.

5.4 Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection from UV emissions.

5.5 The graphite tube emits intense UV radiation during atomization. Suitable precautions should be taken to prevent personnel from eye exposure to this radiation.

5.6 The use of Ar/hydrogen during dry and char steps may evolve a considerable amount of HCl gas. Adequate ventilation is required.

5.7 It is the responsibility of the user of this method to comply with all relevant disposal and waste regulations.

6.0 EQUIPMENT AND SUPPLIES

6.1 Analytical balance, with the capability to measure to 0.1 mg.

6.2 Single pan balance, with capability of weighing to 0.01 g.

6.3 Microwave digestion system.

6.4 Air displacement micropipettors of various sizes with trace metal free tips.

6.5 Labware

For determination of trace levels of elements, contamination and loss are of prime consideration. Potential sources of contamination include improperly cleaned labware, equipment and general contamination from dust and particles in the environment. A clean laboratory work area designated for trace element sample handling must be used, such as the

Class 100 Clean Sample Preparation Laboratory. Sample containers can introduce positive and negative errors in the determination of trace elements by (1) contributing contaminant through surface desorption or leaching, (2) depleting element concentrations from solution through adsorption processes. All reusable laboratory ware (glass, Pyrex, or plastic), should be sufficiently clean for the task objectives. RTI/ACS SOPs for cleaning labware for trace metals analysis will be followed.

6.5.1 Labware

Volumetric flasks, graduated cylinders, funnels, centrifuge tubes (glass or plastic), various sizes

6.5.2 Assorted Calibrated Pipets

6.5.3 Wash Bottle, Acid Leached Plastic

6.5.4 Narrow Mouth Storage Bottles, Acid Leached, Various Sizes

6.6 Inductively Coupled Plasma Mass Spectrometer

6.6.1 Instrument capable of scanning the mass range 5-250 amu with a minimum capability of 1 amu peak width at 5% peak height. Instrument may be fitted with conventional or extended dynamic range detection system.

6.6.2 Radio-frequency Generator Compliant with FCC Regulations

6.6.3 Argon Gas Supply - High Purity Grade 99.99%

6.6.4 Variable Speed Peristaltic Pump

6.6.5 Mass flow controller on the nebulizer is required. A water cooled spray chamber may be of some benefit in reducing polyatomic oxide species.

6.7 Graphite Furnace Atomic Absorption Spectrophotometer

6.7.1 The GFAAS must be capable of programmed heating of the graphite tube and the associated delayed atomization device. The instrument must be equipped with adequate background correction device capable of removing nonspecific absorbance over the spectral region of interest and provide an analytical condition not subject to the occurrence of interelement spectral overlap interferences. The furnace device must be capable of utilizing an alternate gas

supply during specific cycles of the analysis. The capability to record transient signals (<1 s) and evaluate data on a peak area basis is preferred. In addition a recirculating refrigeration bath is recommended for improved reproducibility of furnace temperatures.

- 6.7.2 Single element hollow cathode or electrodeless discharge lamps for the elements of interest along with the associated power supply.
- 6.7.3 Argon gas supply (high purity-99.99%) for use during the atomization of selenium, for sheathing the furnace tube while in operation and for furnace cleanout.
- 6.7.4 Alternate gas mixture (hydrogen 5%-argon 95%) for use as a continuous gas flow environment during the dry and char cycles.
- 6.7.5 Autosampler capable of adding matrix modifier solutions to the furnace, a single addition of analyte and completing method of standard additions when required.

7.0 REAGENTS AND STANDARDS

7.1 Reagents may contain impurities that might affect the integrity of the analytical data. Owing to the high sensitivity of both the ICP-MS and GFAAS, high purity reagents must be used whenever possible. All acids used for this method must be of ultra-high purity grade. Suitable acids are available commercially or may be prepared by sub-boiling distillation. Nitric acid is preferred for both ICP-MS and GFAAS in order to minimize interferences.

- 7.1.1 Nitric acid, Concentrated (sp. gr. 1.41)

- 7.1.2 Matrix Modifier

Dissolve 300 mg Pd powder in 1 mL concentrated nitric acid, adding 0.1 mL concentrated HCl if necessary. Dissolve 200 mg magnesium nitrate in ASTM Type 1 water. Pour the two solutions together and dilute to 100 mL with ASTM Type 1 water. (Alternatively, the prepared matrix modifier solution may be purchased commercially.)

7.2 Reagent Water

All references to reagent grade water or deionized water in this method refer to ASTM Type 1 water (ASTM D1193). Suitable water may be prepared by passing distilled water through a mixed bed of anion and cation exchange resins.

7.3 Standard Stock Solutions

Stock solutions of single elements may be purchased from a reputable commercial source and should be traceable to NIST standards.

7.4 Multielement Stock Standard Solutions

Care must be taken in the preparation of multielement stock standards that the elements are compatible and stable.

All of the analytes in this method are compatible in solution. Multielement stock standards may also be purchased from a reputable commercial source or blended from the single element stock standards.

7.5 Preparation of Calibration Standards

Fresh multielement calibration standards should be prepared every two weeks or as needed. From a multielement standard containing 10 ppm of each element to be determined, prepare calibration working standards by volumetric dilution using reagent water containing 5% nitric acid. The element concentrations in the working standards should be sufficiently high to produce good precision and accurately define the slope of the response curve. The concentration range should cover approximately 10 - 200 ppb. Internal standards must be added to all working standards for ICP-MS. All calibration standards must be verified initially using a quality control sample. Although the internal standard is present in sample and blank solutions, it is not used in the GFAAS calibration or Se.concentration calculations. The internal standard solution must be analyzed by GFAAS to evaluate any contamination or interference problems, or alternatively, it may be added to all the Se calibration standards and calibration blanks, even though it is not used in the calculation.

7.6 Internal Standards Stock Solution

Mix 1 mL each of 1000 ppm yttrium, indium and bismuth and dilute to 100 mL in 1% nitric acid to give a 10 ppm intermediate stock internal standard solution. This solution is

added to blanks, standards and samples in an appropriate amount to give a final concentrations of 10 ppb internal standards in the solutions to be analyzed. Additional intermediate dilutions may be made as needed. The 10 ppm solution may be stored in a refrigerator in an acid cleaned plastic bottle for 2 months.

7.7 ICP-MS Blanks

Four types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure and to assess spectral background, and the rinse blank is used to flush the instrument between samples in order to reduce memory interferences.

7.7.1 Calibration Blank

Consists of 5% nitric acid in reagent grade water plus the internal standard solution.

7.7.2 Laboratory Reagent Blank (LRB)

Must contain all the reagents in the same volumes as is used in processing the samples. The LRB must be carried through the entire preparation scheme as the samples, including digestion. Internal standard solution is added after the sample preparation is complete.

7.7.3 Laboratory Fortified Blank (LFB)

Consists of the laboratory reagent blank spiked with an intermediate concentration of all analytes. Note that the Se concentration must meet the requirement in Section 7.8.3. The LFB must be carried through the same sample preparation scheme as the samples, including digestion. Internal standard solution, if used, is added after the sample preparation is complete.

7.7.4 Rinse Blank

Consists of 5% nitric acid in reagent grade water.

7.8 GFAAS Blanks

Four types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, the laboratory reagent blank is used to assess possible

contamination from the sample preparation procedure and to assess spectral background, the laboratory fortified blank is used to assess routine laboratory performance, and the rinse blank is used to flush the instrument autosampler system.

7.8.1 Calibration Blank

Consists of 5% nitric acid. Use of internal standard depends on approach to correction as described in Section 7.5.

7.8.2 Laboratory Reagent Blank (LRB)

Must contain all the reagents in the same volumes as are used in processing the samples. The LRB must be carried through the entire preparation scheme as the samples, including digestion. Internal standard solution, if used, is added after the sample preparation is complete.

7.8.3 Laboratory Fortified Blank (LFB)

Consists of the laboratory reagent blank spiked with an intermediate concentration of selenium which will produce an absorbance of approximately 0.1. The LFB must be carried through the same sample preparation scheme as the samples, including digestion. Internal standard solution, if used, is added after the sample preparation is complete.

7.8.4 Rinse Blank

Consists of 5% nitric acid.

7.9 ICP-MS Tuning and Instrument Calibration Solutions

Prepare as per RTI/ACS-SOP-170-010 and RTI/ACS-SOP-170-011. These solutions are used for tuning the instrument and for mass calibration prior to analysis in accordance with the SOP.

7.10 GFAAS Instrument Performance Check Solution (IPC)

The IPC is used to periodically verify instrument performance during analysis. It should contain Se at an intermediate concentration relative to the calibration range in the same acid matrix as the calibration standards.

7.11 Quality Control Sample (QCS)

The QCS must be obtained from an outside source different from the standard stock solutions. The quality control concentration will depend on the sensitivity of the instrument. Internal standards should be added after dilution as needed. The QCS is analyzed as needed to meet data quality objectives. A fresh solution should be prepared quarterly or more frequently as needed.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Food and beverage samples will be collected and stored in accordance with RTI/ACS-AP-211-038. This AP requires freezing of samples after collection and homogenization.

8.2 These are solid samples by definition, therefore no preservation other than freezing is required. There is no established holding time for solid samples.

9.0 QUALITY CONTROL

9.1 Each laboratory determining total recoverable elements is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of LRBs, LFBs and calibration solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data thus generated.

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear range and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to analysis of samples conducted by this method.

9.2.2 Linear calibration ranges (or linear dynamic range (LDR))

The calibration or dynamic range is primarily detector or background corrector limited. The upper limit of the LDR is established for each analyte at each

wavelength or mass used by determining the signal responses from calibration standards. For ICP-MS, a minimum of three different concentration standards must be used, one of which is close to the upper LDR. For GFAAS, a minimum of six different concentrations must be used, two of which are close to the upper limit of the LDR. The calibration range to be used must be judged by the analyst from the resulting data. The upper limit of the LDR should be an observed signal no more than 10% below the level extrapolated from the lower standards. The LDRs should be verified whenever, in the judgement of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate that they be redetermined. Measured sample analyte concentrations that are greater than 90% of the upper LDR limit must be diluted and reanalyzed.

9.2.3 Quality Control Sample (QCS)

When beginning the use of this method, on a quarterly basis or as required to meet data quality needs, verify the calibration standards and instrument performance with a QCS (Section 7.11). If the determined mean concentration from triplicate measurements of the QCS is not within 10% of the stated QCS value, performance of the determinative step of the method is unacceptable. An immediate second analysis of the QCS is recommended. If acceptable performance is not achieved, the source of the problem must be identified and corrected before proceeding. See Table 6.

9.2.4 Method Detection Limits (MDL)

Must be established for all analytes using reagent water (blank) fortified at a concentration of two to five times the Instrument Detection Limit (IDL). The IDL is determined from the aqueous calibration curve and aqueous calibration blank. The IDL is defined as that concentration corresponding to three times the standard deviation of the calibration blank. To determine the MDL, seven replicate aliquots of the fortified reagent water are processed through the entire

analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$\text{MDL} = (t) \times (s)$$

where:

t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom ($t = 3.14$ for seven replicates)

s = standard deviation of the replicate analyses

NOTE: If additional confirmation is desired, reanalyze the seven replicate aliquots on two more nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide a more appropriate MDL estimate. If the relative standard deviation (RSD) from the analyses of the seven aliquots is <10%, the concentration used to determine the analyte MDL may have been inappropriately high for the determination. This could result in an unrealistically low MDL. Concurrently, determination of the MDL in fortified reagent water represents a best case situation, and does not reflect possible matrix effects in real world samples. Successful analyses of laboratory fortified matrix samples can give confidence to the MDLs.

The MDLs must be sufficient to detect analytes at the required levels. MDLs should be determined annually, whenever a new analyst begins work, or whenever in the judgement of the analyst, a change in hardware or operating conditions would dictate that they be redetermined.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory Reagent Blank (LRB)

The laboratory must analyze at least one LRB with each batch of 40 or fewer samples of the same matrix. LRB data are used to assess contamination from the laboratory environment and to characterize spectral background from the reagents used in sample processing. LRB values that exceed the MDL indicate that contamination should be suspected. When LRB values constitute 10% or more of the analyte level determined for a sample or are 2.2 times the analyte MDL, whichever is greater, fresh aliquots of the samples must be prepared

again and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory Fortified Blank(LFB)

The laboratory must analyze at least one LFB with each batch of samples.

Calculate accuracy as percent recovery using the following equation:

$$R = \frac{LFB - LRB}{S} \times 100$$

where:

R = Percent recovery

LFB = Laboratory fortified blank

LRB = Laboratory reagent blank

S = Concentration equivalent of analyte added to fortify the LRB solution

If the recovery of any analyte falls outside the required control limits of 80-120%, that analyte is judged out of control and the source of the problem must be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 80-120%. When sufficient internal performance data on real samples become available (a minimum of 30 analyses) optional control limits can be developed from the mean % recovery data and the standard deviation of the mean % recovery. These limits are established as follows:

UPPER CONTROL LIMIT = $x + 3S$

LOWER CONTROL LIMIT = $x - 3S$

The optional control limits for the LFB must be equal to or better than the required control limits. The standard deviation data should be used to

establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance

For all determinations, the laboratory must analyze an instrument performance check solution (calibration check solution) and a calibration blank immediately following each calibration, after every tenth sample and at the end of the sample run. Analysis of the calibration check solution must verify that the instrument is within 10% of calibration. If not verified, the calibration check and blank should be reanalyzed. If the verification is not confirmed, the instrument must be recalibrated and the samples reanalyzed (all samples after the last successful calibration check must be reanalyzed). The calibration blank in GFAAS should result in a signal less than the IDL, but greater than a negative signal in concentration units equal to the IDL. In other words, the calibration blank must be $<1|IDL|$. Failure of the calibration blank must be handled as the failure of the calibration check solution.

9.4 Assessing Analyte Recovery and Data Quality

- 9.4.1 Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect. Unless otherwise specified by the data user or project director, the following laboratory fortified matrix procedure (Section 9.4.2) is required.
- 9.4.2 The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte is added prior to sample preparation. The level of analyte added should be such that the concentration of analyte is doubled, providing that does not exceed the detector linear range. At a minimum the analyte concentration should be increased by 50%. Over time, samples from all routine sample sources should be fortified.

- 9.4.3 Calculate the percent recovery for each analyte, corrected for background concentrations in the unfortified sample, and compare these values to the designated LFM recovery range of 70-130%. Recovery calculations are not required if the concentration of the added analyte is less than 30% of the background concentration. Percent recovery is calculated in units appropriate to the matrix using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where:

- R = Percent recovery
C_s = fortified sample concentration
C = sample background concentration
s = concentration equivalent of analyte added to fortify the sample

- 9.4.4 If recovery of any analyte falls outside the designated range and laboratory performance for that analyte is shown to be in control, the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The data user will be informed that the result for that analyte in the unfortified matrix is suspect due to the heterogeneous nature of the sample or due to an uncorrected matrix effect. For graphite furnace AAS determinations, if the LFM % recovery is outside the 70-130% range and the background is <1.0 absorbance units, reanalyze the sample using the method of standard additions. In GFAAS determinations, if the background absorbance of the sample (either LFM or unfortified) is >1.0 absorbance units, dilute a portion of the sample (1 + 3) with acidified reagent water. If dilution reduces the background to <1.0 absorbance units, reanalyze the sample by the method of standard additions. If necessary, a larger dilution may be made.
- 9.4.5 If the analysis of an LFM indicates an operative interference (such as a matrix effect), all samples in the batch which are of a similar matrix must be analyzed in

the same manner. Also, the data user must be informed when a matrix effect is so severe it prevents the successful analysis of the analyte or when the heterogeneity of a sample precludes the use of duplicate analyses.

9.4.6 Internal Standard Responses

In ICP-MS, the analyst is expected to monitor the responses from the internal standards in the sample set being analyzed. Ratios of the internal standard responses against each other should be monitored routinely. This information may be used to detect potential problems caused by mass dependent drift, errors incurred in the addition of internal standards, nebulizer clogging and other instrumental problems. The absolute response of any one internal standard in the continuing calibration checks and blanks should not deviate more than 70-130% of the original response in the calibration standards and blank. If deviations greater than these are observed, and the deviations are not consistent with a given type of sample matrix, flush the instrument with the rinse blank and monitor the responses in the calibration blank until the responses are within the limits set above.

If after flushing, the internal standard responses are still not in control, terminate the analysis and determine the cause of the drift. Possible causes may be partially blocked nebulizer or cones, or a change in the tuning condition of the instrument. If a given sample matrix is consistently outside the internal standard limits, dilute a fresh aliquot of several representative samples by an additional factor of two, if possible, add internal standards and reanalyze. If the internal standards are within limits, and the dilution has not adversely affected the detection of analytes, this matrix should be diluted for further analyses.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Operating Conditions

Because of the diversity of instrument hardware, the analyst is advised to follow the recommended operating conditions provided by the manufacturer. It is the responsibility of the analyst to insure that the instrument configuration and operating conditions satisfy the analytical requirements and to maintain quality control data verifying instrument performance. General operating conditions for the VG PQXR ICP-MS and the Perkin Elmer ZL5100 GFAAS are given in Tables 7 and 8.

10.2 The VG PQXR will be maintained and the instrument calibrated in accordance with RTI/ACS-SOP-170-010 and RTI/ACS-SOP-170-011. Allow a period of not less than 20 minutes for the instrument to warm up prior to beginning an analysis.

10.3 The Perkin-Elmer GFAAS must be optimized following manufacturer's recommendations and maintained in accordance with RTI/ACS-SOP-171-006. Of particular importance is the determination of the charring temperature limit for Se. This limit is the furnace temperature setting where a loss in analyte will occur prior to atomization. This limit must be determined by conducting a char temperature profile for Se in the matrix (or matrices) to be analyzed. The charring temperature selected should minimize background absorbance without loss of analyte. For routine analyses, the char temperature is usually set 100° below the char temperature limit. Once the optimum conditions are determined, they will be recorded and incorporated into this AP.

10.4 For GFAAS, the linear dynamic range for the analyte must be determined using the optimized operating conditions. For all determinations using an electrodeless discharge lamp (EDL), the instrument and lamp must warm up for 30 minutes prior to analysis.

10.5 To meet or achieve lower MDLs in some matrices as needed, preconcentration if the analyte may be required. This is achieved by the use of multiple aliquot injection onto the graphite platform. When using multiple injections, following each injection, the drying cycle is completed before the subsequent injection is made. The matrix modifier is added with each deposition and the total volume of each deposition may not exceed the manufacturer's

recommended capacity for the platform. The minimum number of depositions to achieve the desired analytical result should be used. Use of this technique for a limited number of samples should be accompanied by the method of standard additions for these samples. If a large number of samples require multiple injections, fortified sample recovery and a comparison of the slopes of the normal calibration curve with a standard addition curve should be made to determine if the normal calibration is adequate in order to increase sample throughput.

10.6 For ICP-MS determinations, internal standards must be used in all analyses to correct for instrument drift and physical interferences. The internal standards used in this method will be Y, In and Bi. Internal standards must be present in all samples, standards and blanks at identical levels. The concentration of the internal standard should be sufficiently high that good precision is obtained in the measurement of the isotopes used for data correction and to minimize the possibility of correction errors if the internal standard elements are present naturally in the samples.

10.7 A minimum of three replicate measurements are required for data acquisition. Use the average of the measurements for calculations.

11.0 PROCEDURE

11.1 Sample Preparation for Food and Beverage Matrices

11.1.1 Frozen, homogenized samples will be thawed completely and well-mixed prior to taking aliquots. (Alternatively, aliquots may be taken when samples are first homogenized. If this has been done proceed to Section 11.1.2). Weigh approximately 4 g of food or beverage into a labeled, acid-cleaned 50 mL polypropylene centrifuge tube and record the weight accurately to 0.0001 g. For each batch of samples to be digested, prepare LRBs, LFBs, QCS and LFMAs as required in the same type of centrifuge tube.

11.1.2 To each centrifuge tube, add 4 mL of concentrated nitric acid (Trace Metal Grade or better). Mix the contents gently using a plastic spatula. If the food is not completely wetted by the acid, add a small amount (1 mL) of reagent water.

Replace the original, solid caps for the tubes with acid-cleaned "microwave caps", which have a hole drilled in the center.

Cap tightly and allow to stand for a minimum of 1 hour at room temperature in the Class 100 hood. If the microwave caps are not available, loosely cap with the original caps and allow to stand as above. Do not cap the samples tightly with the original caps, as gases released may overpressurize the tubes and cause them to split. If any samples are observed to react vigorously (immediate rapid charring, extreme heat generated), add reagent water via a squirt bottle to cool the mixture and slow down the reaction. Prepare as described below, recognizing that these diluted samples may require multiple microwave heating steps for complete digestion. At the end of the room temperature period, and with the microwave caps in place, put the tubes into the microwave digestion rack. Disperse the blanks and QCS randomly among the samples in the digestion rack.

- 11.1.3 For the CEM MDS 2000, the unit and computer must be turned on, the temperature reading of the probe checked to insure that it is accurate according to RTI/ACS-SOP-174-020. The samples in the tray are placed in the microwave cavity, the temperature probe is inserted in the Thermowell, which is placed in one of the reagent blanks. The tray must be rotated several times to verify that the probe is placed correctly. Close the microwave door and lower the safety shield into place. Bring up the method PRE FOOD on the computer and create a new data file using the format MMDDYY##, where ## is a sequential run for a given day, e.g., 01,02,03 and so on. Start the program. Upon completion of the program (a multistep heating process), allow the samples to cool in the oven. Detach the temperature probe and place the sample tray in the Class 100 hood.
- 11.1.4 Uncap the tubes in turn (i.e., do not mix up the caps or the samples will be cross-contaminated). To each tube, slowly add 1 mL of concentrated nitric acid (Trace Metal Grade or better) and 1 mL of reagent water, rinsing down the walls of the tube as the liquids are added. Recap the tubes. Return the tray to the

microwave oven, connect the temperature probe and check its position, close the microwave door and put the safety shield in place. Load the method FOOD and create a new data file using the format MMDDYY##. Start the program. Allow tubes to cool to room temperature upon completion of the program, and place the sample tray in the Class 100 hood.

- 11.1.5 Ascertain which tubes contain samples which will require a second dilution. To each of these tubes, add reagent water to the 50 mL mark on the tube, replace caps with the original caps (without the holes), cap tightly, mix well, and store refrigerated until ready for analysis. Samples need to be refrigerated to permit undigested fat to congeal. Allow samples to warm to room temperature prior to analysis or subsequent dilution. For samples which will not require a second dilution (generally beverage samples), the internal standard solution should be added to the 50 mL centrifuge tube before the solution is brought to volume. All analyses should be completed as soon as possible after digestion due to uncertainty in the stability of the analytes in various matrices.
- 11.1.6 If necessary to avoid clogging of the nebulizer or skimmer/sampler cones with undigested fat or particulate material, aliquots of the samples may be filtered. Care must be taken to ensure that no contamination occurs during filtration. If samples are filtered, a reagent blank and a QCS must also be filtered to evaluate loss/gain of analyte during the filtration step. Nalge disposable preassembled filtration units with 0.2 or 0.45 micron cellulose nitrate filters are recommended if filtration is required.

11.2 Sample Analysis

- 11.2.1 Prior to sample analysis, inspect the instrument sample introduction system, furnace, sample tubing and perform all daily maintenance as specified in the applicable instrument maintenance SOP. Perform any needed optimization, tuning or calibration of the instrument operating parameters. Establish the instrument software run procedures for quantitative analysis.

- 11.2.2 For ICP-MS analysis, the isotopes listed in Table 3 will be used for the analytical measurement. A minimum of three replicate measurements are required for data acquisition. Use the average of the measurements for data calculations and reporting.
- 11.2.3 For ICP-MS, masses which might affect data quality must be monitored during the analytical run. The isotopes listed in Table 3 should be monitored in the same scan as the collection of the data. This information is used to correct the data for identified interferences.
- 11.2.4 Samples for ICP-MS analysis should be aspirated for a minimum of 30 seconds prior to collection of the data. The rinse blank should be used to flush the system between samples for a minimum of 1 minute.
- 11.2.5 For GFAAS analysis, after the 30 minute warm-up period, but before calibration, instrument stability must be demonstrated by analyzing a standard solution with a concentration of approximately 20 times the IDL (or mid-range in the calibration) a minimum of five times. The %RSD must be <5%. If the % RSD is > 5%, correct the problem before beginning calibration and analysis.
- 11.2.6 For GFAAS, analyte signals must be collected as peak area measurements. Background absorbances, background corrected analyte signals, and determined analyte concentrations on all solutions must be able to be displayed on a CRT for immediate review by the analyst and be available as hard copy kept on file. Flush the autosampler with rinse solution between each solution injected.
- 11.2.7 For both ICP-MS and GFAAS, the laboratory must comply with the required quality control described in Section 9. For every new or unusual matrix, it is highly recommended that an ICP-AES be used to screen samples for high element concentrations to prevent potential damage to detectors or contamination of the instruments.

11.2.8 Determined sample concentrations by either technique that are 90% or more of the upper limit of calibration must either be diluted with acidified reagent water (and internal standard as appropriate) or determined by another approved analytical procedure which is less sensitive. For GFAAS, samples with background absorbance >1.0 must be appropriately diluted with acidified reagent water and reanalyzed.

11.3 Standard Additions

If the method of standard additions is required, the following procedure should be followed. The standard addition technique involves preparing new standards in the sample matrix by adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interference, which causes a baseline shift. The simplest version of this technique is the single-addition method. The procedure is as follows: Two identical aliquots of the sample solution, each of volume V_x , are taken. To the first (labeled A) is added a small volume V_s of the standard analyte solution of concentration C. To the second (labeled B) is added the same volume V_s of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration C_x is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where:

S_A and S_B = the analytical signals (corrected for the blank) of Solutions A and B, respectively.

V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x , and thus C_s is much greater than C_x , to avoid excess dilution of the sample matrix.

If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results from this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond in the same manner as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 For ICP-MS. Elemental equations recommended for sample calculations are listed in Table 9.

12.2 Sample data should be reported in units of ng/g wet weight (ppb w/w). Do not report element concentrations below the determined MDL.

12.3 For data values less than 10, two significant figures should be used; for data values greater than 10, three significant figures should be used. Do not report more than three significant figures.

12.4 Calculate the concentration using the equation below:

$$\text{Sample Conc. (ng/g)} = \frac{C \times V \times D}{W}$$

where:

C is the solution concentration measured in ppb after correction for the blank

V is the volume of solution in mL (usually 50 mL)

D is the dilution factor if needed

W is the wet weight of the sample in g

12.5 Data values (concentrations in solution) should be corrected for blanks, characterized spectral interferences, and matrix-induced interferences. Chloride interference corrections should be made on all samples, as the chloride ion is common to most sample matrices.

12.6 The QC data obtained during the analyses provide an indication of the quality of the sample data and must be provided along with the sample data.

13.0 METHOD PERFORMANCE

- 13.1 Instrument operating conditions for the VG PQXR ICP-MS are provided in Table 7. Instrument operating conditions for the Perkin-Elmer ZL5100 GFAAS are provided in Table 8.
- 13.2 Typical MDLs for food and beverage matrices are presented in Tables 1 and 2.
- 13.3 Typical regression equations obtained for single laboratory testing of the ICP-MS method for food and beverage matrices for the analytes are presented in Table 10.
- 13.4 Data obtained from single laboratory testing of the ICP-MS method on a Certified Reference Material are presented in Table 11.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that place pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, EPA recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street, NW, Wash., D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The EPA urges laboratories to protect the earth, water and air by minimizing and controlling all releases from hood and bench operations, complying with the letter and spirit of sewer discharge permits and regulations and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Section 14.2.

16.0 REFERENCES

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TABLE 1. ESTIMATED SOLUTION DETECTION LIMITS

	Al (ng/mL)	V (ng/mL)	Cr (ng/mL)	Mn (ng/mL)	Ni (ng/mL)	Cu (ng/mL)	Zn (ng/mL)	As (ng/mL)	Cd (ng/mL)	Ba (ng/mL)	Pb (ng/mL)
Reagent Blank - 1	1.370	2.640	-0.191	0.493	0.280	0.417	13.400	0.097	0.017	0.047	0.336
Reagent Blank - 2	1.390	1.560	-0.152	0.456	0.174	0.316	45.900	0.058	0.026	0.035	0.082
Reagent Blank - 3	0.821	3.330	1.140	0.528	0.136	0.459	3.940	0.025	0.009	0.020	0.179
Reagent Blank - 4	0.915	0.960	2.370	0.511	0.039	0.203	16.700	-0.044	0.018	0.024	0.275
Reagent Blank - 5	0.658	0.764	-1.970	0.432	0.072	0.276	14.400	0.045	0.022	0.016	0.099
Reagent Blank - 6	0.607	0.565	-0.184	0.449	0.041	0.241	4.730	0.032	0.002	0.021	0.086
Average	0.960	1.637	0.169	0.478	0.124	0.319	16.512	0.036	0.016	0.027	0.176
SD	0.34	1.12	1.46	0.04	0.09	0.10	15.33	0.05	0.01	0.01	0.11
%RSD	35.8	68.3	867	8.0	75.8	31.6	92.8	130	54.7	42.9	61.3
MDL [*], ng/mL	1	3	4	0.1	0.3	0.30	46	0.1	0.03	0.03	0.3

MDL [*] = $3 \times SD$ of replicate reagent blanks.

TABLE 2. RECOMMENDED GRAPHITE FURNACE OPERATING CONDITIONS
AND RECOMMENDED MATRIX MODIFIER¹⁻³

Element	Wavelength	Slit	Temperature Char	(C) ⁶ Atom	MDL ⁴ (μ g/L)	MDL ⁵ (μ g/L)
Se ⁷	196.0	2.0	1000	2000	0.6	0.015

¹ Matrix Modifier = 0.015 mg PD + 0.01 mg Mg(NO₃)₂.

² A 5% H₂ in Ar gas mix is used during the dry and char steps at 300 mL/min for all elements.

³ A cool down step between the char and atomization is recommended.

⁴ Obtained using a 20 μ L sample size and stop flow atomization.

⁵ Calculated assuming a 4 g food sample diluted to 100 mL.

⁶ Actual char and atomization temperatures may vary from instrument to instrument and are best determined on an individual basis. The actual drying temperature may vary depending on the temperature of the water used to cool the furnace.

⁷ An electrodeless discharge lamp was used for this element.

TABLE 3. RECOMMENDED ANALYTICAL ISOTOPES AND ADDITIONAL MASSES WHICH MUST BE MONITORED

Isotope	Element of Interest
<u>27</u>	Aluminum
<u>75</u>	Arsenic
<u>135, 137</u>	Barium
<u>106, 108, 111, 114</u>	Cadmium
<u>52, 53</u>	Chromium
<u>63, 65</u>	Copper
<u>206, 207, 208</u>	Lead
<u>55</u>	Manganese
<u>95, 97, 98</u>	Molybdenum
<u>60, 62</u>	Nickel
<u>77, 82</u>	Selenium
<u>51</u>	Vanadium
<u>66, 67, 68</u>	Zinc
47	Titanium ^a
<u>83</u>	Krypton ^a
99	Ruthenium ^a
105	Palladium ^a
118	Tin ^a

NOTE: Isotopes recommended for analytical determination are underlined.

* These isotopes are not analytes but must be measured to insure appropriate correction equations are used.

TABLE 4. COMMON MOLECULAR ION INTERFERENCES IN ICP-MS

Molecular Ion	Mass	Element Interference*
Background Molecular Ions		
NH ⁺	15	
OH ⁺	17	
OH ₂ ⁺	18	
C ₂ ⁺	24	
CN ⁺	26	
CO ⁺	28	
N ₂ ⁺	28	
N ₂ H ⁺	29	
NO ⁺	30	
NOH ⁺	31	
O ₂ ⁺	32	
O ₂ H ⁺	33	
³⁶ ArH ⁺	37	
³⁸ ArH ⁺	39	
⁴⁰ ArH ⁺	41	
CO ₂ ⁺	44	
CO ₂ H ⁺	45	
ArC ⁺ , ArO ⁺	52	Sc
ArN ⁺	54	Cr
ArNH ⁺	55	Cr
ArO ⁺	56	Mn
ArOH ⁺	57	
⁴⁰ Ar ³⁶ Ar ⁺	76	Se
⁴⁰ Ar ³⁸ Ar ⁺	78	Se
⁴⁰ Ar ⁺	80	Se
Bromide¹²		
⁸¹ BrH ⁺	82	Se
⁷⁹ BrO ⁺	95	Mo
⁸¹ BrO ⁺	97	Mo
⁸¹ BrOH ⁺	98	Mo
Ar ⁸¹ Br ⁺	121	Sb

(continued)

TABLE 4. (continued)

Molecular Ion	Mass	Element Interference ^a
Chloride		
$^{35}\text{ClO}^+$	51	V
$^{35}\text{ClOH}^+$	52	Cr
$^{37}\text{ClO}^+$	53	Cr
$^{37}\text{ClOH}^+$	54	Cr
$\text{Ar}^{35}\text{Cl}^+$	75	As
$\text{Ar}^{37}\text{Cl}^+$	77	Se
Sulphate		
$^{32}\text{SO}^+$	48	
$^{32}\text{SOH}^+$	49	
$^{34}\text{SO}^+$	50	V, Cr
$^{34}\text{SOH}^+$	51	V
$\text{SO}_2^+, \text{S}_2^+$	64	Zn
Ar^{32}S^+	72	
Ar^{34}S^+	74	
Phosphate		
PO^+	47	
POH^+	48	
PO_2^+	63	Cu
ArP^+	71	
Group I, II Metals		
ArNa^+	63	Cu
ArK^+	79	
ArCa^+	80	
Matrix Oxides^b		
TiO	62-66	Ni, Cu, Zn
ZrO	106-112	Ag, Cd
MoO	108-116	Cd

- ^a Method elements or internal standards affected by the molecular ions.
- ^b Oxide interferences will normally be very small and will only impact the method elements when present at relatively high concentrations. Some examples of matrix oxides are listed of which the analyst should be aware. It is recommended that Ti and Zr isotopes are monitored in solid waste samples, which are likely to contain high levels of these elements. Mo is monitored as a method analyte.

TABLE 5. INTERNAL STANDARDS AND LIMITATIONS OF USE

Internal Standard	Mass	Possible Limitation
Lithium	6	a
Scandium	45	polyatomic ion interference
Yttrium	89	a, b
Rhodium	103	
Indium	115	isobaric interference by Sn
Terbium	159	
Holium	165	
Lutetium	175	
Bismuth	209	a

- ^a May be present in environmental samples.
- ^b In some instruments. Yttrium may form measurable amounts of YO^+ (105 amu) and YOH^+ (106 amu). If this is the case, care should be taken in the use of the cadmium elemental correction equation.

TABLE 6. ACCEPTANCE LIMITS FOR QC CHECK SAMPLE
METHOD PERFORMANCE

Element	QC Check Sample Conc., ppb	Acceptance Limits, ppb
Aluminum	10	8.5-11.5
Arsenic	10	8.5-11.5
Barium	10	8.5-11.5
Cadmium	10	8.5-11.5
Chromium	10	8.5-11.5
Copper	10	8.5-11.5
Lead	10	8.5-11.5
Manganese	10	8.5-11.5
Nickel	10	8.5-11.5
Selenium	10	8.5-11.5
Vanadium	10	8.5-11.5
Zinc	10	8.5-11.5

TABLE 7. ICP-MS INSTRUMENT OPERATING CONDITIONS

Instrument Parameter	Value
Instrument	Fisons PQXR ICP-MS
Forward RF power	1300-1350 watts
Reflected RF power	<= 2 watts
Argon flow rates:	
Nebulizer	0.80 L min ⁻¹
Coolant	13.75 L min ⁻¹
Auxiliary	0.9 L min ⁻¹
Sample uptake rate	1.0-2.0 mL min ⁻¹
Nebulizer type	Meinhard TR3-A30 glass nebulizer
Spray chamber	Water-cooled at 5°C
Vacuum system	
Expansion chamber P	2 mbar
Intermediate P	1.0 × 10 ⁻⁴ mbar
Analyzer P	2.0 × 10 ⁻⁶ mbar
Ion lens voltages	Optimized with ¹¹³ In
Detector	Electron multiplier
Mode	Peak jump
Dwell time	25 msec
Points/peak	3
DAC step	5
Acquisition time	60 sec
Resolution	0.7-0.8 amu

TABLE 8. GFAAS OPERATING CONDITIONS

Element	Selenium (Se)
Instrument	Perkin-Elmer ZL5100
Furnace Type	THGA with Zeeman
Wavelength	196.0 nm
Slit Width	2.00 nm, low
Source	Electrodeless discharge lamp (EDL)
Signal Measurement	Peak area
Read time	5.0 seconds
Matrix Modifiers	5 µg PD + 3 µg Mg(NO ₃) ₂
Furnace Program	

Step	Temperature (°C)	Ramp (seconds)	Hold (seconds)
1	100	1	10
2	130	5	50
3	1100	10	20
4	2100	0	5
5	2500	1	4

TABLE 9. RECOMMENDED ELEMENTAL EQUATIONS FOR
 DATA CALCULATIONS

Element	Elemental Equation	Note
A1	(1.000) (¹² C)	
Sb	(1.000) (¹²³ C)	
As	(1.000) (⁷⁵ C)-(3.127) [(⁷⁷ C)-(0.815) (⁸² C)]	(1)
Ba	(1.000) (¹³⁷ C)	
Be	(1.000) (⁹ C)	
Cd	(1.000) (¹¹¹ C)-(1.073) [(¹⁰⁸ C)-(0.712) (¹⁰⁶ C)]	(2)
Cr	(1.000) (⁵² C)	(3)
Co	(1.000) (⁵⁹ C)	
Cu	(1.000) (⁶³ C)	
Pb	(1.000) (²⁰⁶ C)+(1.000) [²⁰⁷ C]+(1.000) (²⁰⁸ C)]	(4)
Mn	(1.000) (⁵⁵ C)	
Mo	(1.000) (⁹⁸ C)-(0.146) (⁹⁹ C)	(5)
Ni	(1.000) (⁶⁰ C)	
Se	(1.000) (⁸² C)	(6)
Ag	(1.000) (¹⁰⁷ C)	
Tl	(1.000) (²⁰⁵ C)	
Th	(1.000) (²³² C)	
U	(1.000) (²³⁸ C)	
V	(1.000) (⁵¹ C)-(3.127) [(⁵³ C)-(0.113) (⁵² C)]	(7)
Zn	(1.000) (⁶⁶ C)	
Bi	(1.000) (²⁰⁹ C)	
In	(1.000) (²⁰⁹ C)-(0.016) (¹¹⁸ C)	(8)

(continued)

TABLE 9. (continued)

Element	Elemental Equation	Note
Sc	(1.000) (^{45}C)	
Tb	(1.000) (^{159}C)	
Y	(1.000) (^{89}C)	

- C = Calibration blank subtracted counts at specified mass.
(1) = Correction for chloride interference with adjustment for ^{75}Se . ArCl 75/77 ratio may be determined from the reagent blank. Isobaric mass 82 must be from Se only and not BrH^+ .
(2) = Correction for MoO interference. Isobaric mass 106 must be from Cd only not ZrO^+ . An additional isobaric elemental correction should be made if palladium is present.
(3) = In 0.4% v/v HCl, the background from ClOH will normally be small. However, the contribution may be estimated from the reagent blank. Isobaric mass must be from Cr only not ArC^+ .
(4) = Allowance for isotopic variability of lead isotopes.
(5) = Isobaric elemental correction for ruthenium.
(6) = Some argon supplies contain krypton as an impurity. Selenium is corrected by ^{82}Kr by background subtraction.
(7) = Correction for chloride interference with adjustment for ^{53}Cr . ClO 51/53 ratio may be determined from the reagent blank. Isobaric mass 52 must be from Cr only not ArC^+ .
(8) = Isobaric elemental correction for tin.

TABLE 10. TYPICAL ICP-MS REGRESSION EQUATIONS

Element	Symbol	Mass	a0	a1	a2	Regression*
Aluminum	Al	27	10305.8	16653.1	0.0	0.995325
Vanadium	V	51	-6034.56	17911.2	0.0	0.999550
Chromium	Cr	52	-77205.0	14851.5	0.0	0.997440
Manganese	Mn	55	2226.35	18942.9	0.0	0.999104
Nickel	Ni	60	1850.63	3586.46	0.0	0.998094
Copper	Cu	63	323.618	8657.00	0.0	0.997133
Copper	Cu	65	-176.387	3969.90	0.0	0.996945
Zinc	Zn	66	-19.2287	2524.36	0.0	0.996995
Arsenic	As	75	-209.531	2726.76	0.0	0.999116
Selenium	Se	82	-13.9408	139.692	0.0	0.999018
Cadmium	Cd	111	71.5466	1470.05	0.0	0.999938
Barium	Ba	137	405.331	1706.68	0.0	0.999348
Lead	Pb	208	3507.84	4970.80	0.0	0.998694

*Internal standards: Y 89, In 115, Bi 209 (interpolating).

TABLE 11. ANALYSIS OF CRM BY ICP-MS METHOD IN RTI/ACS-AP-211-138
 OYSTER TISSUE CRM*

	Concentration, ppb (ng/g)										
	Al	V	Cr	Mn	Ni	Cu	Zn	As	Cd	Ba	Pb
Measured	177	1.10	ND	17	0.9	49	760	7.7	2.5	3.8	0.4
Theoretical	300	NC	0.7	20	1.0	60	900	10	3.0	5.0	0.5
% Recovery	59			85	92	81	85	77	82	76	86
	Al	V	Cr	Mn	Ni	Cu	Zn	As	Cd	Ba	Pb
Measured	270	0.07	2.96	18.7	1.2	54.4	889	8.6	2.9	5.0	0.5
Theoretical	300	NC	0.7	20	1.0	60	900	10	3.0	5.0	0.5
% Recovery	90		423	93	124	91	99	86	97	101	103
	Al	V	Cr	Mn	Ni	Cu	Zn	As	Cd	Ba	Pb
Measured	180	0.7	0.3	18.7	1.0	55	876	9.6	2.8	4.9	0.5
Theoretical	300	NC	0.7	20	1.0	60	900	10	3.0	5.0	0.5
% Recovery	60		40	94	100	92	97	96	95	97	100

* Oyster Tissue CRM from High Purity Standards, Matthews, NC. This is a solution containing the elements in a ratio corresponding to the dissolution of NIST Oyster Tissue SRM. Data presented are for CRM spiked with internal standards and analyzed with no digestion or addition of other reagents. Results shown are from three separate analyses on three separate days and are representative of data obtained on a daily basis when 80-90 food digests are analyzed for multiple elements.

ND = not detected

NC = not certified