PN-ADC925

Supporting Egypt's Processed Foods Export Industry

Pesticide Safeguards on Foods Imported into the USA

Appendix II Protocols & Reporting Forms for Testing Chemicals Through PAM Multiresidue Methods

Volume XI of XI

Prepared By Agriculture-Led Export Businesses (ALEB)

February 2000

Funded by U.S. Agency for International Development
USAID/Egypt
RP 0264
Under Contract No: 263-C-00-99-00011-00
USAID Cognizant Technical Office: USAID/Egypt/EG/ED
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APPENDIX II: PROTOCOLS AND REPORTING FORMS FOR TESTING CHEMICALS THROUGH PAM I MULTIRESIDUE METHODS

INTRODUCTION: MULTIRESIDUE METHOD TESTING

Use of any multiresidue method (MRM) is supported by available information about how potential residues behave through the steps of the method. To provide that support for PAM I MRMs, additional chemicals are continually tested through the method steps and the resulting data compiled in a single database. All PAM I tables in Chapters 3 and 4 and Appendix I are produced from that database. This Appendix provides directions for performing such tests and forms for reporting results.

The effort spent on the testing of MRMs and compilation of results is justified by the advantages such compilations offer the analytical chemist. When analytical behavior data for numerous chemicals through the method in use are known, the analyst is better equipped to identify residues that may be present in a sample of unknown treatment history. In situations where the likelihood of some particular residue is known, the data lists for several methods can be consulted to help choose which method should be used.

Regulatory agencies often must assess the incidence of residue occurrence. This effort is also assisted by compilations of method behavior data. The absence of many chemicals from the sample can be ascertained when it is known that those chemicals could have been detected had they been present.

It has been found advisable to define protocols for developing data on MRM behavior. In order to compile data into usable formats, it is imperative that all contributing laboratories perform the tests uniformly. The goal of this method-testing is not to find the optimum conditions for the one chemical currently being tested, but to be able to describe how the chemical will behave when determined by the precisely defined method.

This Appendix includes one protocol for determining GLC characteristics of chemicals and six protocols for testing their behavior through individual MRMs. Forms for reporting the results of testing by each protocol are also included. Each protocol references the PAM I method(s) involved, the types of chemicals to which it applies, and the PAM I table(s) in which previously collected data are published.

Some PAM I MRMs are applicable to a wide variety of residues, while others are targeted to those with specific chemical structures. A Decision Tree is included in this Appendix to direct the user to the most appropriate protocol(s) for each chemical being tested. Follow the Decision Tree in deciding which protocol(s) to use.

Follow the steps of these protocols, in the order written. Report data on a copy of the appropriate form and send it to: PAM I Editors, HFS-337, Food and Drug Administration, 200 C Street SW, Washington, DC 20204.

Decision Tree for MRM Testing

Do data already exist (Index to Methods, Appendix I)?

No ↓ Yes → Develop only data not yet available.

Does the compound have this structure or characteristic:

N-methylcarbamate? — Yes → Test per Protocol A.

naturally fluorescent? — Yes → Test per Protocol A.

acid or phenol? — Yes → Test per Protocol B.

substituted urea? — Yes → Test per Protocol G.

No ↓

Determine GLC characteristics per Protocol C, Level I.
Does it chromatograph on GLC systems?

Yes ↓ No → Discontinue tests.

Does it chromatograph on at least one Level I system in a reasonable time (0.3<rrt<5.0)?

Yes ↓ No → Report rrts for Level I DG modules tested, then retest using appropriate Level II DG module(s).

Is the product a nonfatty (2% fat) food?

Yes ↓ No → Test per Protocol F for fatty food.

Test per Protocol D.
Is the chemical recovered through Section 302 extraction?

Yes ↓ No → Discontinue tests.

Test per Protocol E for nonfatty food.

SUGGESTIONS FOR PRODUCING QUALITY DATA

The following suggestions were developed in response to data received and to specific questions that have been raised.

Decisions on What Protocols to Follow

- As directed in the first step of the Decision Tree, review existing data on the chemical before performing experiments. Examine PAM I Index to Methods for entries, then review the details in appropriate Chapter 3 or 4 table(s); review Appendix I for available GLC data. If these sources reveal gaps in the data, perform the tests necessary to provide missing data, but do not repeat experiments unless specifically asked to do so or unless current data reflect variability.

- Develop GLC data (Protocol C) with system(s) likely to detect the chemical; *i.e.*, of the DG modules listed, choose at least one whose detector is selective to elements in the molecule. The electron capture detector may not be suitable to examine uncleaned extracts from Section 302 (Protocol D), so DG1, 13, and 18 are often insufficient. If an electron capture detector is the only one that responds to the chemical, apply caution when injecting extracts from Section 302.

- Choose the GLC system that provides the best chromatography and sensitivity for examining solutions from recovery studies; it is not necessary to re-examine the extracts by multiple GLC systems.

- The Decision Tree provides basic criteria for making decisions about which methods to test. Where situations exist that make testing or continuation of testing illogical, suspend testing and report the reasons. Examples:

  - If previous studies with radiolabelled chemical clearly show that the residue will partition into the (discarded) water layer of the method, method recovery tests need not be run; however, collection of GLC data should still be attempted.

  - If the only commodity of interest is fatty, do not attempt to perform tests on the product with methods designed for nonfatty foods. If the commodity is meat, use only Section 304 E1 (Protocol F) or, if the chemical is an acid or phenol, Section 402 E1 (Protocol B).

  - Suspend testing if the GLC tests (Protocol C) indicate that even the most sensitive GLC system is insensitive to the compound. As a general rule, suspend testing if the minimum weight of compound that causes 10% full scale deflection (FSD) is equivalent to ≥10 times the tolerance for an injection of the normal mg sample equivalent described in the method.

  - If the method being tested proves not amenable to analysis of a particular commodity, as evidenced by severe emulsions, failure to form distinct phases, *etc.*, suspend testing.

Proper Application of Methods

- Do not combine a GLC column that contains cyano groups with a nitrogen-selective detector.
- Adjust GLC column temperature to establish the correct relative retention time (rrt) of the marker chemical(s). Absolute retention times (min) are provided in DG modules to provide an indication of normal behavior, but the temperature should not be adjusted to achieve this.
- Measure retention times on GLC columns from the solvent front wherever possible for both the compound being tested and the marker compound. If the instrumentation in use precludes measurement from the solvent front, state this fact in the report.
- When submitting chromatograms with reporting forms, label them clearly: indicate how many nanograms (ng) or picograms (pg) are represented by the peak. Do not label a chromatogram of a standard in ppm. For chromatograms that result from recovery studies, indicate on the chromatogram label how much sample weight equivalent (mg) was injected.
- For accurate quantitation, detector response to the reference standard should be within ±25% of the response to the analyte in the sample, based on observable (on-scale) peak heights. Usual GLC linearity does not support quantitation that compares responses differing in size by more than that amount.
- Make sure that chromatograms do not overlap; allow peaks from one injection to elute before the next injection is made.
- When the chromatogram contains multiple peaks representing different components in the standard, report the rrt of each peak >5% FSD.
- It is not necessary to evaporate the solvent from the fortification solution once it has been added to the sample, unless there is some reason to expect it to interfere with the analysis. It is preferable for the fortifying solution to be made from the same solvent used to extract the sample.
- Use only the 60/100 mesh PR grade Florisil specified by Sections 303 and 304 (Protocols E and F). Other grades of commercially available Florisil are likely to result in different elution patterns.
- During tests of elution from Florisil, do not add reference material to the Florisil column in a polar solvent, which will affect elution pattern.
- Test Florisil elution by both ethyl ether/petroleum ether (Sections 303 C1, 304 C1 and C3) and methylene chloride (303 C2, 304 C2 and C4) elution systems when following the steps of Protocols E and F.
- Even when Florisil elution tests with reference standards indicate no elution with later eluants, examine by GLC all the Florisil eluates of the recovery test from the fortified sample. Sometimes the presence of extract changes the Florisil elution pattern.

PROTOCOL A: PROCEDURE FOR TESTING CHEMICALS THROUGH SECTION 401

BACKGROUND

Methods: Section 401 E1 + C1 + DL1 or DL2

Chemical Type: Applicable to chemicals with N-methylcarbamate structure (DL1) and to some chemicals that are naturally fluorescent (DL2).

Commodity Type: Applicable to nonfatty foods and to certain fatty foods such as soybeans and nuts.

PAM I Tables: Tables 401-a, 401-b

DATA DEVELOPMENT

HPLC Analytical Behavior

N-Methylcarbamates: Set up HPLC with post-column fluorescence labeling and fluorescence detector, as described in Section 401 DL1; check for proper operation using system suitability test described.

Fluorescent Pesticides: Set up HPLC and fluorescence detector, as described in Section 401 DL2.

Develop information on test chemical(s) as follows:

- Dissolve reference standard in methanol to prepare stock solutions. Dilute with methanol for HPLC working standards.
- Determine amount (ng) of chemical that causes detector response of 50% full scale deflection (FSD) on recorder or printer/plotter. Note peak shape of response to determine adequacy of chromatography.
- Determine linear response range of detector to chemical.
- Determine stability of chemical in methanol:
  - Short term. Prepare 1 µg/mL methanol solution of chemical. Use actinic glassware. Inject 5-10 µL injections of this solution into HPLC system over 8 hr to measure short term stability of chemical in solution. Report results as peak height (mm) response.
  - Long term. Using same solution as above, inject 10 µL into system once a day for 1-2 wk to measure long term stability of solution. Store solution on laboratory bench during day and in refrigerator overnight. Report results as peak height (mm) response to test chemical, normalized to peak height for carbofuran standard injected on same day.

- Calculate retention time of chemical relative to carbofuran on the HPLC system.

Recovery of Chemical Through Cleanup Column

Prepare fortification solution by diluting stock solution with methanol.

- Initially determine that charcoal/silanized Celite column has proper elution characteristics as described in Section 401 C1.

- In duplicate, add 25 µg pesticide to newly prepared charcoal/silanized Celite column. Then elute as described in method. After collection of eluate (20 mL methylene chloride + 125 mL toluene/acetonitrile) in round-bottom (r-b) flask, momentarily stop flow, remove bottom flask and replace with second r-b flask. Elute column with additional 100 mL toluene/acetonitrile. Evaporate solvents in both flasks to dryness as described in method. Dissolve residue in first flask to appropriate volume with appropriate solvent. Dissolve residue in second flask with 5 mL solvent. Determine percentage of total added pesticide eluted in each eluate.

- Continue recovery studies with food products only if combined recoveries from charcoal column are >50%. If ≥10% of pesticide elutes in second flask, collect separate additional 100 mL eluting solution in recovery studies with food products.

Recovery Through Complete Method

- Select representative food sample. Analyze by Section 401 to ensure that there are no interferences with chemical being tested. Simultaneously, analyze reagent blank for further information on source of possible interferences.

- Fortify duplicate 150 g portions of chopped food product, while it is in homogenizer, at about 0.05 ppm; analyze as above.

- Fortify duplicate 150 g samples at level near tolerance or, if no tolerance exists, at about 0.25 ppm; analyze as above.

REPORTING RESULTS

Report all results on copy of Reporting Form A. An asterisk (*) appears on form wherever name of tested chemical should be entered.

REPORTING FORM A: BEHAVIOR THROUGH SECTION 401

The following data resulted from testing the chemical * through PAM I Section 401 E1 + C1 + DL1 or DL2, according to Appendix II, Protocol A.

Name: *

Alternative Names:

Reference Standard (source and number):

Molecular Formula:

Structure:

Comments:

Results of HPLC Tests

The following HPLC system was used:

Analytical Column:

Guard Column:

Mobile Phase:

For natural fluorescence, DL2:

* fluoresces at excitation and emission wavelengths of _____ and _____ nm, respectively.

Peak Characteristics:

	DL1	DL2
Peak shape	_____	_____
Retention time (relative to carbofuran)	_____	_____
ng causing 50% FSD	_____	_____
Linear range	_____	_____

Results of Stability in Methanol Studies

Short Term Study		Long Term Study	
Time	Peak Ht (mm)	Day	Peak Ht (mm)
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Recovery Through Charcoal/Silanized Celite Column

µg * added to column: _____

Percent recovered from charcoal/silanized Celite column:

Methylene chloride + toluene/acetonitrile		Additional 100 mL toluene/acetonitrile	
Trial 1	Trial 2	Trial 1	Trial 2
_____	_____	_____	_____

Recovery Through Complete Method

Food sample:

Does sample extract cause any interference with test chemical?

Does reagent blank cause any interference with test chemical?

Determinative step used for recovery test:

Duplicate 150 g samples fortified at _____ ppm and _____ ppm.

Percent * recovered:

_____ ppm		_____ ppm	
Trial 1	Trial 2	Trial 1	Trial 2
_____	_____	_____	_____

Additional Data on Crop Used as Samples

Pesticide residues found:

Unidentified peaks (specify determinative step used and list peaks by retention time relative to appropriate chemical).

Information submitted by:

Address:

Phone: ( )

Date:

PROTOCOL B: PROCEDURE FOR TESTING CHEMICALS THROUGH SECTION 402

BACKGROUND

Method: Section 402

Chemical Type: Applicable to chemicals with acid or phenol structure. Chemicals are methylated before determination by GLC, because esters and ethers are more easily chromatographed than acids and phenols.

Commodity Type: Applicable to wide variety of commodities, both fatty and nonfatty. Different extraction steps are used depending on commodity.

PAM I Tables: Table 402-a

DATA DEVELOPMENT

Gas Chromatography

Because the method being tested includes methylation of the analyte, GLC characteristics of both the acid/phenol and its methylation product are collected.

Perform the following operations:

- Dissolve methyl ester/ether reference standard, if available, in 10% acetone/isooctane (v/v) to prepare stock standard solution. Dilute with isooctane.
- Follow directions in Protocol C for chromatography of methyl ester/ether reference standard. Report results on Reporting Form C.
- Stop work if methyl ester/ether does not cause response on any detector when chromatographed on any appropriate column.
- Dissolve acid/phenol reference standard in acetone to prepare stock standard solution. Dilute as needed with acetone. Also dilute with 50% methylene chloride/hexane to prepare solution suitable for testing recovery through gel permeation chromatography (GPC). (Note: 2,4,5-T will not dissolve in 50% methylene chloride/hexane, so solutions in that solvent mixture must be prepared by diluting acetone stock solution.)
- Follow directions in Protocol C for chromatography of acid/phenol reference standard. Report results on separate copy of Reporting Form C.
- Methylate 100 µg acid/phenol reference standard in acetone solution according to procedure described in Section 402 C1b. Dilute as needed with hexane. If any other methylation procedure is used, describe procedure on Reporting Form B.
- Follow directions in Protocol C for chromatography of methylated reference standard. Report results on separate copy of Reporting Form C (Note: if ester/ether reference standard is available, these results should

verify retention times and response characteristics previously found. Note this on Reporting Form prepared for methyl ether/ester reference standard test results.)

- Determine efficiency of methylation by direct comparison to methyl ester/ether reference standard, if available. Report percentage conversion to methylated product on Reporting Form B. If methyl ester/ether reference standard is not available, assume complete methylation of chemical for calculating amount of reference standard causing 50% FSD.

Stop work if acid/phenol cannot be methylated or if there is no GLC response to ester/ether.

Recovery Through GPC and Florisil

Methyl Ester/Ether Reference Standards

- In duplicate, place 1-100 µg methyl ester/ether reference standard, in 1-10 mL hexane, on Florisil column prepared as described in Section 402 C1c. (Use Florisil that has been shown to permit elution of both heptachlor epoxide and endrin by eluant 2 [Section 204].)
- Elute columns with 35 mL eluant 1, 60 mL eluant 2, and 100 mL ethyl ether, and determine percentage recovered in each eluate.
- If total recovery is <30%, report results on Reporting Form B and terminate work.

Acid/Phenol Reference Standards

- Place 100 µg acid/phenol reference standard, dissolved in 50% methylene chloride/hexane, on calibrated GPC column and elute as directed in Section 402 C1a.
- Methylate collected fraction according to C1b.
- Determine percentage recovery by comparison to methyl ester/ether reference standard, if available, or to reference standard previously methylated in laboratory.
- If recoveries are <30%, report results on Reporting Form B and terminate work.

Recovery Through Complete Method

- Select one representative fatty and one nonfatty food. Analyze by Section 402, using extraction module appropriate to commodity, to ensure that there are no interferences with chemical being tested. Simultaneously, analyze reagent blank for further information on source of possible interferences.
- Fortify duplicate portions of food samples with 1-2 mL acetone solution of acid/phenol reference standard at tolerance level or, if no tolerance exists, at 0.05 ppm.

- Fortify duplicate portions of food samples with 1-2 mL acetone solution of acid/phenol reference standard at 10 times tolerance levels or, if no tolerance exists, at 0.5 ppm.

- Perform complete analysis as described in Section 402, using extraction module appropriate to commodity. Calculate percentage recovered against methyl ester/ether reference standard, if available, or reference standard previously methylated in laboratory, if necessary.

REPORTING RESULTS

Report all results on copy of Reporting Form B. An asterisk (*) appears on form wherever name of tested chemical should be entered.

REPORTING FORM B: BEHAVIOR THROUGH SECTION 402

The following data resulted from testing the chemical * through PAM I Section 402, according to Appendix II, Protocol B.

GLC characteristics of this chemical have been reported on Form C for both the acid/phenol and the methyl ester/ether. The ester/ether was:

___ available as reference standard

___ prepared by methylating the acid/phenol

Name: *

Reference Standard (source and number):

Acid/phenol:

Methyl ester/ether:

Methylation

Acid/phenol reference standard dissolved in:

µg * methylated:

Methylation procedure used:

Percent conversion to methyl ester/ether, as measured against methyl ester/ether reference standard: _____

Comments:

Recovery Through GPC

µg acid/phenol reference standard (in 50% methylene chloride/hexane) added to column: ____

Percent found in collect fraction: _____

Recovery Through Florisil

µg methyl ester/ether of * added to column: _____

Percent eluted with eluant 1, eluant 2, and 100 mL ethyl ether:

eluant 1 _____

eluant 2 _____

100 mL ethyl ether _____

Recovery Through Complete Method

Method for Fatty Foods

Fatty food sample:

Does sample extract cause any interference with test chemical?

Does reagent blank cause any interference with test chemical?

Duplicate 100 g samples fortified at _____ ppm and _____ ppm.

Percent recovered using eluant 1, eluant 2, and 100 mL ethyl ether:

	_____ ppm		_____ ppm	
	Trial 1	Trial 2	Trial 1	Trial 2
eluant 1	_____	_____	_____	_____
eluant 2	_____	_____	_____	_____
100 mL ethyl ether	_____	_____	_____	_____

Method for Nonfatty Foods

Nonfatty food sample:

Does sample extract cause any interference with test chemical?

Does reagent blank cause any interference with test chemical?

Duplicate 100 g samples fortified at _____ ppm and _____ ppm.

Percent recovered using eluant 1, eluant 2, and 100 mL ethyl ether:

	_____ ppm		_____ ppm	
	Trial 1	Trial 2	Trial 1	Trial 2
eluant 1	_____	_____	_____	_____
eluant 2	_____	_____	_____	_____
100 mL ethyl ether	_____	_____	_____	_____

Information submitted by:

Address:

Phone: ( )

Date:

PROTOCOL C: PROCEDURE FOR DEVELOPING GLC DATA

BACKGROUND

Methods: Section 302 DG1-DG23; GLC systems are used with Sections 302, 303, 304, and 402 methods.

Chemical Type: Applicable to chemicals that can be vaporized at temperatures about 250° C without degradation. Most pesticides and their related chemicals that meet this criterion can be chromatographed and detected by at least one of the GLC systems DG1-DG23.

PAM I Tables: Appendix I (PESTDATA)

DATA DEVELOPMENT

For each GLC DG module tested:

- Dissolve reference standard in pesticide grade solvent to prepare stock standard solution. Isooctane is preferred, but acetone may be required for dissolution.
- Set up GLC system as described in specified DG module (Section 302). Check rrts of marker compounds and adjust column temperature to match conditions specified.
- Inject aliquots of test solution into GLC.
- Calculate retention time (relative to marker compound specified in DG module).
- Calculate ng standard that causes 50% FSD response. Do not inject >1000 ng (1 µg).
- Test chemical on one or more of these systems:

  Level I:

  All chemicals:

  DG 1 100% methyl siloxane (*e.g.*, DB-1), 200° C, EC

  DG13 50% phenyl, 50% methyl siloxane(*e.g.*, DB-17), 200° C, EC

  DG18 50% cyanopropylphenyl, 50% methyl siloxane (*e.g.*, DB-225), 200° C, EC

  Chemicals containing halogen:

  DG 3 100% methyl siloxane (*e.g.*, DB-1), 200° C, ElCD-X

  DG16 50% phenyl, 50% methyl siloxane (*e.g.*, DB-17), 200° C, ElCD-X

◀

Chemicals containing phosphorus:

DG 2 100% methyl siloxane (*e.g.*, DB-1), 200° C, FPD-P

DG14 50% phenyl, 50% methyl siloxane (*e.g.*, DB-17), 200° C, FPD-P

DG19 50% cyanopropylphenyl, 50% methyl siloxane (*e.g.*, DB-225), 200° C, FPD-P

▶

Chemicals containing sulfur:

DG15 50% phenyl, 50% methyl siloxane (*e.g.*, DB-17), 200° C, FPD-S

▶

Chemicals containing nitrogen:

DG 4 100% methyl siloxane (*e.g.*, DB-1), 200° C, ElCD-N

DG 5 100% methyl siloxane (*e.g.*, DB-1), 200° C, N/P

DG17 50% phenyl, 50% methyl siloxane (*e.g.*, DB-17), 200° C, N/P

▶

Chemicals with no heteroatom to which element-selective detectors respond:

DG 6 100% methyl siloxane (*e.g.*, DB-1), 130° C, FID

Level II:

If chemical chromatographs on system described in module(s) of Level I, but rrt is <0.3, rechromatograph at lower column temperature, *e.g.*:

DG 7 100% methyl siloxane (*e.g.*, DB-1), 130° C, EC

DG 8 100% methyl siloxane (*e.g.*, DB-1), 130° C, FPD-P

DG 9 100% methyl siloxane (*e.g.*, DB-1), 130° C, ElCD-X

If chemical chromatographs on system described in module(s) of Level I, but rrt is >5.0, rechromatograph at higher column temperature, *e.g.*:

DG10 100% methyl siloxane (*e.g.*, DB-1), 230° C, EC

DG11 100% methyl siloxane (*e.g.*, DB-1), 230° C, FPD-P

DG12 100% methyl siloxane (*e.g.*, DB-1), 230° C, ElCD-X

REPORTING RESULTS

Report results for each DG module on copy of Reporting Form C. An asterisk (*) appears on form wherever name of tested chemical should be entered.

REPORTING FORM C: GLC DATA

The following GLC data resulted from testing the chemical * on systems described in PAM I Section 302 DG modules, according to directions in Appendix II, Protocol C.

Name: *

Alternative Names:

Reference Standard (source and number):

Molecular Formula:

Structure:

Comments:

Results for DG module (Section 302): DG ___

Standard reference material dissolved in

Brief details about GLC system used:

Column:

Length:

id:

Film thickness:

Carrier gas:

Flow rate:

Makeup gas:

Flow rate:

Retention time (relative to _____) of _____: ◀
(marker compound)

Detector:

Temperature:

Other conditions:

Detector response to ____ ng _____:____% FSD
(marker compound)

Behavior of * :

Retention time (relative to _____):

ng required for 50% FSD:

Information submitted by:

Address:

Phone: ( )

Date:

PROTOCOL D: PROCEDURE FOR TESTING CHEMICALS THROUGH SECTION 302 E1, E2

BACKGROUND

Methods: Section 302 E1, E2; use determinative step found useful for chemical in Protocol C and considered suitable for extract according to Section 302 recommendations.

Chemical Type: Applicable to nonionic pesticides; detection of particular chemicals is dependent on determinative step(s) used to examine extract.

Commodity Type: Applicable to all nonfatty foods, although some commodities contain co-extractives that interfere with some determinative steps.

PAM I Tables: Table 302-a, Appendix I (PESTDATA)

DATA DEVELOPMENT

Prepare fortification solution by diluting stock solution with acetone.

Recovery Through Complete Method Without Cleanup

- Select representative nonfatty food. Analyze by Section 302 to ensure that there are no interferences with chemical being tested. Simultaneously, analyze reagent blank for further information on source of possible interferences.
- Fortify duplicate 100 g portions at 0.05-0.1 ppm. Analyze duplicate fortified samples as described in Section 302 E1 or E2.
- Fortify duplicate 100 g portions at tolerance level for chemical or, if no tolerance exists, at five times level used in first fortification. Analyze duplicate fortified samples.

Recovery Through Florisil

If the chemical being tested can only be determined by electron capture detector, or if that is the only detector available, then the extract must be cleaned up by Florisil chromatography (Section 302 C5 or C1) before determination.

- To determine recovery through Florisil column, use only Florisil that has been shown to permit elution of heptachlor epoxide in 6% ethyl ether/petroleum ether or methylene chloride eluate 2 and elution of endrin in 15% ethyl ether/petroleum ether or eluate 2 (Section 204).
- Add 10-100 µg analyte in 1-10 mL solution to each of two Florisil columns. Elute duplicate columns according to directions of Section 302 C1 or C5. If recovery is <30%, report results, then terminate work.

Recovery Through Complete Method with Florisil Cleanup

- Select representative nonfatty food sample. Analyze by Section 302, with cleanup step being studied, to ensure that there are no interferences with chemical being tested. Simultaneously, analyze reagent blank for further information on source of possible interferences.
- Fortify duplicate 100 g portions at about 0.05 ppm and analyze with method of Section 302, using E1 or E2 with cleanup C1 or C5.
- Fortify duplicate 100 g portions at tolerance level or, if no tolerance exists, at about 0.5 ppm; analyze as above.

REPORTING RESULTS

Report all results on copy of Reporting Form D. An asterisk (*) appears on form wherever name of tested chemical should be entered.

REPORTING FORM D: BEHAVIOR THROUGH SECTION 302

The following data resulted from testing the chemical * through PAM I Section 302 E1/E2 (without cleanup and/or with cleanup C1 or C5), according to Appendix II, Protocol D. GLC characteristics of this chemical have been reported on Reporting Form C.

Name: *

Reference Standard (source and number):

Recovery Through Method Without Cleanup

Nonfatty food sample:

Does sample extract cause any interference with test chemical?

Does reagent blank cause any interference with test chemical?

Duplicate 100 g sample fortified at _____ ppm and _____ ppm.

Percent * recovered:

_____ ppm		_____ ppm	
Trial 1	Trial 2	Trial 1	Trial 2
_____	_____	_____	_____

(Optional) Recovery Through Method with Florisil Column Cleanup

Recovery Through Florisil

Cleanup step tested: Section 302 C ____

µg * added to column: ____

Percent eluted with C1 eluant (50 mL 50% methylene chloride/1.5% acetonitrile/48.5% hexane):

_____

Percent eluted with C5 eluants:

200 mL 15% ethyl ether/petroleum ether (EE/PE) _____

200 mL 50% EE/PE _____

Recovery Through Complete Method

Nonfatty food sample:

Does sample extract cause any interference with test chemical?

Does reagent blank cause any interference with test chemical?

Duplicate _____ g sample fortified at _____ ppm and _____ ppm.

Percent recovered using C1 eluant:

	_____ ppm		_____ ppm	
	Trial 1	Trial 2	Trial 1	Trial 2
	_____	_____	_____	_____

Percent recovered using C5 eluants:

	_____ ppm		_____ ppm	
	Trial 1	Trial 2	Trial 1	Trial 2
15% EE/PE	_____	_____	_____	_____
50% EE/PE	_____	_____	_____	_____

Information submitted by:

Address:

Phone: ( )

Date:

PROTOCOL E: PROCEDURE FOR TESTING CHEMICALS THROUGH SECTION 303

BACKGROUND

Methods: Section 303 E1-E5 + C1 and C2

Chemical Type: Generally applicable to relatively nonpolar chemicals, although many chemicals are recovered through this method. Do not assume too readily that a chemical is too polar to be recovered.

Commodity Type: Applicable to nonfatty foods; with special extractions for samples of low (<75%) moisture foods, high (5-15%) and very high (15-30%) sugar foods, and eggs.

PAM I Tables: Table 303-a, Appendix I (PESTDATA)

DATA DEVELOPMENT

Prepare fortification solution by diluting stock solution with petroleum ether or hexane.

Recovery Through Cleanup Column

- To determine recovery through Florisil column, use only Florisil that has been shown to permit elution of heptachlor epoxide in 6% ethyl ether/petroleum ether or methylene chloride eluate 2 and endrin in 15% ethyl ether/petroleum ether or eluate 2 (Section 204).
- Add 10-100 µg analyte in 1-10 mL solution to each of four Florisil columns. Elute duplicate columns according to directions of Section 303 C1 and C2, respectively. If recoveries through both elution systems are <30%, report results, then terminate work.

Recovery Through Complete Method

- Select representative nonfatty food sample. Analyze by Section 303 to ensure that there are no interferences with chemical being tested. Simultaneously, analyze reagent blank for further information on source of possible interferences.
- Fortify duplicate 100 g portions at about 0.05 ppm and analyze with method of Section 303, using extraction module appropriate to commodity and cleanup C1.
- Fortify duplicate 100 g portions at tolerance level or, if no tolerance exists, at about 0.5 ppm; analyze as above.
- If chemical is recovered through method, and if it was previously found to be eluted from Florisil using methylene chloride eluants of C2, then repeat recovery experiments through whole method using extraction module appropriate to commodity and cleanup C2.

REPORTING RESULTS

Report all results on copy of Reporting Form E. An asterisk (*) appears on form wherever name of tested chemical should be entered.

REPORTING FORM E: BEHAVIOR THROUGH SECTION 303

The following data resulted from testing the chemical * through PAM I Section 303 E1-E5 + C1 and C2, according to Appendix II, Protocol E. GLC characteristics have been reported on Reporting Form C.

Name: *

Reference Standard (source and number):

Recovery Through Florisil

µg * added to column: _____

Percent eluted with C1 eluants:

6% _____
15% _____
50% _____

Percent eluted with C2 eluants:

1 _____
2 _____
3 _____

Recovery Through Complete Method

Nonfatty food sample: Extraction used: 303 E ___

Does sample extract cause any interference with test chemical?

Does reagent blank cause any interference with test chemical?

Duplicate _____ g sample fortified at _____ ppm and _____ ppm.

Percent recovered using C1 eluants:

	_____ ppm		_____ ppm	
	Trial 1	Trial 2	Trial 1	Trial 2
6%	_____	_____	_____	_____
15%	_____	_____	_____	_____
50%	_____	_____	_____	_____

Percent recovered using C2 eluants:

	_____ ppm		_____ ppm	
	Trial 1	Trial 2	Trial 1	Trial 2
1	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____

Information submitted by:

Address:

Phone: ( )

Date:

PROTOCOL F: PROCEDURE FOR TESTING CHEMICALS THROUGH SECTION 304

BACKGROUND

Methods: Section 304 E1-E5 + C1-C4

Chemical Type: Generally applicable to relatively nonpolar chemicals, although many chemicals can be recovered through this method. Do not assume too readily that a chemical is too polar to be recovered. Very nonpolar chemicals may be only partially recovered through acetonitrile/petroleum ether partitioning steps of C1-C4.

Commodity Type: Applicable to fatty foods, with special extraction steps for removal of fat from different fatty commodities.

PAM I Tables: Table 304-a, Appendix I (PESTDATA)

DATA DEVELOPMENT

Prepare fortification solution by diluting stock solution with petroleum ether or hexane.

Recovery Through Cleanup Column

- To determine recovery through Florisil column, use only Florisil that has been shown to permit elution of heptachlor epoxide in 6% ethyl ether/petroleum ether or eluate 2 and endrin in 15% ethyl ether/petroleum ether or eluate 2 (Section 204).

- Add 10-100 µg analyte in 1-10 mL solution to each of four Florisil columns. Elute duplicate columns according to directions of Section 304 C1 and C2, respectively. If recoveries through both elution systems are <30%, report results, then terminate work.

- If chemical elutes in 6% ethyl ether/petroleum ether (C1) or eluate 1 (C2), rerun Florisil column experiment (duplicates) and elute according to directions of Section 304 C3 and/or C4, *i.e.*, elute with 250 mL petroleum ether before elution with first eluant of each system.

Recovery Through Complete Method

- Select one representative fatty food sample. Analyze by Section 304 to ensure that there are no interferences with chemical being tested. Simultaneously, analyze reagent blank for further information on source of possible interferences.

- Fortify duplicate 100 g portions at about 0.05 ppm and analyze with method of Section 304, using extraction module appropriate to the commodity and cleanup C1.

- Fortify duplicate 100 g portions at tolerance level or, if no tolerance exists, as about 0.5 ppm; analyze as above.

- If chemical is recovered through method, and if it was previously found to be eluted from Florisil using methylene chloride eluants of C2, then repeat recovery experiments through whole method using extraction module appropriate to commodity and cleanup C2.

REPORTING RESULTS

Report all results on copy of Reporting Form F. An asterisk (*) appears on form wherever name of tested chemical should be entered.

REPORTING FORM F: BEHAVIOR THROUGH SECTION 304

The following data resulted from testing the chemical * through PAM I Section 304 E1-E5 + C1-C4, according to Appendix II, Protocol F. GLC characteristics have been reported on Reporting Form C.

Name: *

Reference Standard (source and number):

Recovery Through Florisil

µg * added to column: _____

Percent eluted with C1 eluants:

6% _____
15% _____
50% _____

Percent eluted with C2 eluants:

1 _____
2 _____
3 _____

For chemicals that elute in 6% ethyl ether/petroleum ether (C1) or Eluant 1 (C2) only:

µg * added to column: _____

Percent eluted with C3 and C4 eluants:

PE _____
6% _____

PE _____
1% _____

Recovery Through Complete Method

Fatty food sample: Extraction used: 304 E ___

Does sample extract cause any interference with test chemical?

Does reagent blank cause any interference with test chemical?

Duplicate _____ g sample fortified at _____ ppm and _____ ppm.

Percent recovered using C1 eluants:

	_____ ppm		_____ ppm	
	Trial 1	Trial 2	Trial 1	Trial 2
6%	_____	_____	_____	_____
15%	_____	_____	_____	_____
50%	_____	_____	_____	_____

Percent recovered using C2 eluants:

	_____ ppm		_____ ppm	
	Trial 1	Trial 2	Trial 1	Trial 2
1	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____

Information submitted by:

Address:

Phone: ( )

Date:

PROTOCOL G: PROCEDURE FOR TESTING CHEMICALS THROUGH SECTION 403

BACKGROUND

Methods: Section 403 E1 + C1 + DL3

Chemical Type: Applicable to chemicals with phenylurea structure; it may also be applicable to ureas substituted with other constituents that can be degraded photolytically to form primary amines.

Commodity Type: Applicable to nonfatty foods.

PAM I Tables: Table 403-a

DATA DEVELOPMENT

HPLC Analytical Behavior

Phenylureas: Set up HPLC with post-column photodegradation, derivatization, and fluorescence detection as described in Section 403 DL3. Check for proper operation using system suitability test described.

Develop information on test chemical(s) as follows:

- Dissolve reference standard in methanol to prepare 1 mg/mL stock solution. Dilute with methanol for HPLC working standards. Dilute with methylene chloride to 20 µg/mL, then dilute 1 mL to 5 mL, to perform recovery through Florisil column.
- Determine amount (ng) of chemical that causes detector response of 50% full scale deflection (FSD) on recorder or printer/plotter. Note peak shape and measure asymmetry (Section 602 C).
- Determine linear response range of detector to chemical.
- Calculate retention time of chemical relative to diuron on HPLC system.

Recovery of Chemical Through Florisil Cleanup Column

- Prepare duplicate Florisil columns as directed in method (Section 403 C1).
- After washing columns with 30 mL methylene chloride, transfer 20 µg chemical in 5 mL methylene chloride to each column.
- Elute columns as described and proceed with HPLC determination.
- Continue with recovery through entire procedure only if recovery from Florisil column is >50%.

Recovery Through Complete Method

- Select appropriate nonfatty food sample. Analyze by Section 403 to ensure that there are no interferences with chemical being tested. Simultaneously, analyze reagent blank for further information on source of possible interferences.
- Fortify duplicate 50 g portions of chopped food product, while it is in homogenizer, at about 0.05 ppm; analyze as above.
- Fortify duplicate 50 g portions at level near tolerance or, if no tolerance exists, at about 0.25 ppm; analyze as above.

REPORTING RESULTS

Report all results on copy of Reporting Form G. An asterisk (*) appears on form wherever name of tested chemical should be entered.

REPORTING FORM G: BEHAVIOR THROUGH SECTION 403

The following data resulted from testing the chemical * through PAM I Section 403 E1 + C1 + DL3, according to Appendix II, Protocol G.

Name: *

Alternative Names:

Reference Standard (source and number):

Molecular Formula:

Structure:

Comments:

Results of HPLC Tests

The following HPLC system was used:

Analytical column:

Guard column:

Mobile phase:

Detector type:

Detector excitation wavelength:

Detector emission wavelength:

Peak characteristics:

Peak shape (asymmetry):

Retention time relative to diuron:

ng causing 50% FSD response:

Linear range:

Recovery Through Florisil Column

µg * added to column: _____

Percentage recovered from Florisil column:

Trial 1	Trial 2
_____	_____

Recovery Through Complete Method

Food sample:

Does sample extract cause any interference with test chemical?

Does reagent blank cause any interference with test chemical?

Duplicate 50 g samples fortified at 0.05 ppm.

Percent recovered:

Trial 1	Trial 2
_____	_____

Duplicate 50 g samples fortified at _____ ppm.

Percent recovered:

Trial 1	Trial 2
_____	_____

Additional Data on Crop Used as Samples:

Unidentified peaks: rrt relative to diuron.

Any additional residues detected:

Information submitted by:

Address:

Phone: ( )

Date: