# Pesticide Analysis in Foodstuff, Stomach Contents, and/or Liver Tissue

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## 1 INTRODUCTION

This procedure is used to identify pesticides that are insecticides or herbicides.

## 2 SCOPE

| | |
|---|---|
| Analyses | ☒ Screening ☒ Confirmation ☐ Quantitation |
| Matrices | Common foodstuffs, stomach contents, liver tissue |
| Analytes | Selected organochlorines, organophosphates, and carbamates |
| Personnel | This document applies to authorized personnel who perform the described tasks, singly or in combination. |

## 3 PRINCIPLE

Most of the pesticides analyzed by this procedure will be classified as an insecticide or herbicide. The presence of chlorine or phosphorus in many of the chemicals within these classes permits the use of a gas chromatograph equipped with selective detectors (electron capture and nitrogen phosphorus). To be detected by this procedure, the pesticide must have some degree of solubility in n-hexane (foodstuffs and stomach contents extraction). The identification of a pesticide is achieved by use of the orthogonal technique of gas chromatography with mass spectrometry operated in the full scan electron ionization mode.

## 4 SPECIMEN CRITERIA

Sample matrices typically comprise common foodstuffs, stomach contents, or liver tissue. Typically, 2.5 grams or 2.5 mL of sample are used for this analysis.

## 5 EQUIPMENT

### 5.1 Equipment

A. Centrifuge
B. Heated sample concentrator
C. Homogenizer or mortar/pestle
D. Rotator
E. Vacuum extraction manifold
F. Vortex Mixer

### 5.2 Consumables

A. Disposable glass pipets
B. Mid-range pH paper
C. Supported Liquid Extraction (SLE) cartridges (Biotage Isolute SLE+2; 2 mL sorbent mass; part number 820-0290-D)

D. Test tubes (16 x 125 mm screw-top, 16x100 mm culture, 12 x 75 mm culture)

### 5.3 Instruments

A. Gas Chromatograph capable of dual simultaneous injection and equipped with a 30-meter Rtx-CLPesticides column or equivalent connected to an electron capture detector (ECD) and a 30-meter Rtx-1701 column or equivalent connected to a nitrogen-phosphorous detector (NPD)

B. Gas Chromatograph/Mass Spectrometer (GC/MS) equipped with a 30-meter DB-5 capillary column or equivalent

### 5.4 Software

| Component | Software | Version |
|---|---|---|
| Operating System | Microsoft Windows | 7 Pro SP 1 |
| GC/MS | Enhanced Chemstation | E.02.02.1431 |
| Autosampler | Gerstel Maestro 1 | 1.5.3.3/3.5 |
| Data Analysis | Xcalibur | 2.07 SP1 |

### 5.5 Chemicals/Reagents

#### *5.5.1 Purchased*

| | |
|---|---|
| A. Acetone | ≥ HPLC grade |
| B. Deionized Water | ≥ 18mΩ |
| C. Dichloromethane | ≥ Optima grade |
| D. N-Hexane | ≥ HPLC grade, ≥95% |
| E. Sulfuric Acid | 5N |
| F. Toluene | ≥ HPLC grade |

### 5.6 Standards/Controls

#### *5.6.1 Purchased*

A. Organochlorine (OC) Pesticides Stock Solution

A hexane:toluene (1:1) solution containing approximately 1 mg/mL each of aldrin, 4,4'-DDT, endrin, endrin aldehyde, and lindane (gamma BHC). Purchased as a combined special order (or individually purchased) from Chemservice, Inc. Store refrigerated in glass. Stable for at least two years, or as determined by manufacturer.

B. Organophosphate (OP) Pesticides Stock Solution

A hexane solution approximately 1 mg/mL each in chlorpyrifos, diazinon, fenchlorphos, parathion (ethyl), and prophos. Purchased as a combined special order (or individually purchased) from Chemservice, Inc. Store refrigerated in glass. Stable for at least two years, or as determined by manufacturer.

C. Carbamate Pesticides Stock Solution

An acetonitrile solution approximately 1 mg/mL each in carbaryl, carbofuran, and propoxur. Purchased as a combined special order (or individually purchased) from Chemservice, Inc. (Solutions prepared in-house are diluted with acetone.) Store refrigerated in glass. Stable for at least six months, or as determined by manufacturer.

D. Hexachlorobenzene (98% or better purity)

Obtained as a solid from Sigma-Aldrich or an equivalent supplier. Storage and stability determined by manufacturer.

E. Triphenylphosphate (98% or better purity)

Obtained as a solid from Sigma-Aldrich or an equivalent supplier. Storage and stability determined by manufacturer.

F. 4-Bromo-3,5-dimethylphenyl-N-methylcarbamate (BDMC) (98% or better purity)

Obtained as a solid from Sigma-Aldrich or an equivalent supplier. Storage and stability determined by manufacturer.

G. OC Pesticides Mix AB#1

Obtained from Restek Corporation (Catalog #32291). This mixture contains twenty common organochlorine pesticides (aldrin, alpha-BHC, beta-BHC, delta-BHC, gamma-BHC, cis-chlordane, trans-chlordane, 4,4'-DDD, 4,4-'DDE, 4,4'-DDT, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide (isomer B), and methoxychlor). Store refrigerated in glass. Stability determined by manufacturer.

H. OP Pesticides Mix B

Obtained from Restek Corporation (Catalog #32278). This mixture contains seven common organophosphate pesticides (tetraethylpyrophosphate (TEPP), sulfotepp, monocrotophos, dimethoate, malathion, parathion, and EPN). Store refrigerated in glass. Stability determined by manufacturer.

### *5.6.2 Prepared*

A. Electron Capture Detector (ECD) Pesticides Testmix Solution

Dilute 25 µL of the OC pesticides stock solution to 50 mL with hexane, yielding a solution approximately 0.5 µg/mL in each component. Store refrigerated in glass. Stable for at least two years. (100 µg/mL stock standards can be individually purchased from Chemservice, Inc. or an equivalent supplier and diluted to an appropriate concentration in house.)

B. Nitrogen Phosphorus Detector (NPD) Pesticides Testmix Solution

Dilute 500 µL of the OP pesticides stock solution to 25 mL in hexane, yielding a solution approximately 20 µg/mL in each component. Store refrigerated in glass.

Stable for at least two years. (100 µg/mL stock standards can be individually purchased from Chemservice, Inc. or an equivalent supplier and diluted to an appropriate concentration in house.)

C. Internal Standards Working Solution

Weigh approximately 25 mg each of hexachlorobenzene, triphenylphosphate, and BDMC into a 25-mL volumetric flask and fill to the mark with toluene. Mix well and store refrigerated in glass. Stable for at least one year.

D. Pesticides GC/MS Testmix Solution

Dilute 100 µL of the internal standards working solution to 10 mL in hexane, yielding a solution approximately 10 µg/mL in each component. Store refrigerated in glass. Stable for at least one year.

E. OC Pesticides Retention Time Calibration Mix

Dilute 20 µL of the OC Pesticide Mix AB#1 to 10 mL in hexane. Store refrigerated in glass. Stable for at least two years. A portion of this calibration mix is analyzed by GC/ECD -with every case batch Maintain a record of these samples with the instrument testmix records.

F. OP Pesticides Retention Time Calibration Mix

Dilute 100 µL of the OP Pesticides Mix B to 500 µL with hexane. Store refrigerated in glass. Stable for at least two years. A portion of this calibration mix is analyzed by GC/NPD with every case batch~~,~~. Maintain a record of these samples with the instrument testmix records.

G. Negative Control

A deionized water blank or a matrix similar to the submitted specimen (if known and available) is used as the Negative Control. A negative control is extracted and analyzed with every assay.

H. Positive Control Foodstuff or Stomach Contents

The suggested Positive Control is a spiked aliquot of the questioned sample. If sample volume is limited, a blank matrix similar to the submitted specimen may be used, or, if that is unobtainable, deionized water may be spiked to create a positive control. Alternatively, if a specific pesticide is being targeted it is acceptable to prepare a 10 µg/mL solution of the targeted pesticide in a blank matrix or within deionized water. To prepare: add 25 µL each of the OC Pesticides Stock Solution (1 mg/mL), the OP Pesticides Stock Solution (1mg/mL), and the Carbamate Pesticides Stock Solution (1mg/mL) to 2.5 mL or 2.5 g of the sample matrix. This will yield a spiked sample that is approximately 10 µg/mL in each of the thirteen control analytes. A Positive Control is extracted and analyzed with every assay.

I. Positive Control Liver Tissue

To prepare: add 50 µL each of the OC Pesticides Stock Solution (1 mg/mL), the OP Pesticides Stock Solution (1mg/mL), and the Carbamate Pesticides Stock Solution

(1mg/mL) to 5 g of the liver homogenate (2.5 g liver tissue: 2.5 g deionized water). This will yield a spiked sample that is approximately 20 µg/g in each of the thirteen control analytes. A Positive Control is extracted and analyzed with every assay.

## 6 PROCEDURE

### 6.1 Foodstuff or Stomach Contents

| | Step | Note | Reference/Lot |
|---|---|---|---|
| | **A. Samples** | | |
| | 1. Prepare homogenate | | |
| ☐ | i. Photograph the specimen if its appearance will be grossly altered by homogenizing | | |
| | ii. Liquid Specimens | | |
| ☐ | a. Mix the specimen well | | |
| ☐ | b. Aliquot 2.5 mL into a screw-top tube | | |
| ☐ | c. Add 2.5 mL deionized water | | |
| ☐ | d. Mix/vortex well | | |
| | iii. Solid/Semi-Solid Specimens | | |
| ☐ | a. Homogenize a portion of the specimen 1:1 with deionized water (2.5 g specimen: 2.5 g deionized water) into a screw-top tube | | |
| | **B. Controls** | | |
| ☐ | 1. Prepare Negative Control(s) | | |
| ☐ | 2. Prepare Positive Control(s)<br>i. Refer to 5.6.2-H and document preparation | | |
| | **C. Internal Standard(s)** | | |
| ☐ | 1. Add 25 µL of the appropriate Internal Standard Working Solution | | |
| ☐ | 2. Vortex for 30 seconds | | |
| | **D. pH/Buffer** | | |
| ☐ | 1. Add 70 µL L of 5N sulfuric acid | | |
| ☐ | 2. Vortex for 30 seconds | | |
| ☐ | 3. Check pH < 6 with indicator paper | | |
| | ☐ i. If pH > 6 add more sulfuric acid | | |

| | | | |
|---|---|---|---|
| | **E. Extract** | | |
| | 1. GC/ECD | | |
| ☐ | i. Add 5 mL of n-hexane and extract by rotation for 20 minutes. | | |
| ☐ | ii. Centrifuge for 15 minutes at 3000 rpm | | |
| | a. If a severe emulsion forms, stir the emulsion with a clean wooden stick and re-centrifuge | | |
| ☐ | iii. Transfer the hexane supernatant to a 16x100 mm culture tube | | |
| ☐ | iv. Mix a 200 µL portion of the extract with 800 µL of n-hexane in an ALS vial and cap. | | |
| | 2. GC/NPD and GC/MS | | |
| ☐ | i. Concentrate a 1.5 mL portion of the extract from E-1-iii to ca. 250-300 µL under a stream of nitrogen at ca. 40ºC | | |
| | **F. Instrumental Analysis** | | |
| | 1. GC/ECD | | |
| ☐ | **i.** Analyze 1.0 µL GC/ECD testmix prior to batch analysis | | |
| ☐ | **ii.** Analyze 1.0 µL of the extract from E-1-iv | | |
| | 2. GC/NPD | | |
| ☐ | i. Analyze 2.0 µL GC/NPD textmix prior to batch analysis | | |
| ☐ | ii. Analyze 2.0 µL of the extract from E-2-i | | |
| | 3. GC/MS | | |
| ☐ | i. Analyze 2.0 µL GC/MS textmix prior to batch analysis | | |
| ☐ | ii. Analyze 2.0 µL of the extract from E-2-i | | |

## 6.2 Liver Tissue

| | Step | Note | Reference/Lot |
|---|---|---|---|
| | **A. Samples** | | |
| | 1. Prepare homogenate | | |
| ☐ | i. Place specimen and an equal amount of deionized water (e.g., 2.5 g specimen: 2.5 g deionized water, maintain 1:1 ratio) into a homogenizer | | |
| ☐ | ii. Blend for 2-3 minutes on high | | |
| ☐ | iii. Transfer homogenate to a 16 x 100 mm culture tube with a polypropylene snap-top | | |
| | **B. Controls** | | |
| ☐ | 1. Prepare Negative Control(s) | | |
| ☐ | 2. Prepare Positive Control(s)<br>i. Refer to 5.6.2-I and document preparation<br>ii. An optional control made from the unknown tissue may be prepared by spiking the prepared unknown homogenate | | |
| | **C. Internal Standard(s)** | | |
| ☐ | 1. Add 50 µL of the appropriate Internal Standard Working Solution | | |
| ☐ | 2. Vortex for 30 seconds | | |
| | **D. Prepare Supernatant** | | |
| ☐ | 1. Centrifuge homogenate at high speed for 15 minutes | | |
| ☐ | 2. Transfer supernatant to a clean 12 x 75 mm culture tube with a polypropylene snap-top. | | |
| ☐ | 3. Re-centrifuge supernatant at high speed for 15 minutes | | |
| | i. Repeat above transfer and centrifuge if supernatant contains any homogenate | | |
| | **E. Supported Liquid Extraction (SLE)** | | |
| ☐ | 1. Load supernatant samples onto SLE cartridges by gravity. (A brief application of vacuum will be | | |

| | | | |
|---|---|---|---|
| | necessary to start loading.) Do not elute. | | |
| ☐ | 2. Allow to stand for 5 minutes | | |
| ☐ | 3. Apply 3 mL of dichloromethane and allow to absorb | | |
| ☐ | 4. Allow to stand for 5 minutes. Do not apply vacuum | | |
| ☐ | 5. Elute by gravity into 16 x 100 mm culture tubes with 2 x 4 mL dichloromethane. Briefly apply full vacuum to complete elution | | |
| ☐ | 6. Evaporate at approximately 45ºC. When eluent reaches 0.5 – 1 mL, briefly vortex before evaporating to dryness | | |
| ☐ | 7. Reconstitute with 0.1 mL acetone | | |
| | **F. Instrumental Analysis** | | |
| | 1. GC/ECD | | |
| ☐ | i. Transfer 25 µL to properly labeled autosampler vials | | |
| ☐ | ii. Add 225 µL acetone | | |
| ☐ | **iii.** Analyze 1.0 µL GC/ECD testmix prior to batch analysis | | |
| ☐ | **iv.** Analyze 1.0 µL of the extract from F-1-ii | | |
| | 2. GC/MS | | |
| ☐ | i. Transfer remaining extract from E-7 to properly labeled autosampler vials | | |
| ☐ | ii. Analyze 2.0 µL GC/MS textmix prior to batch analysis | | |
| ☐ | iii. Analyze 2.0 µL of the extract from F-2-i | | |

## 7 ANALYTICAL PARAMETERS

### 7.1 Agilent Gas Chromatograph (NPD/ECD)

#### *7.1.1 Oven*

| **Step** | Temperature (°C) | Hold (min) | Ramp (°C/min) |
|---|---|---|---|
| **1** | 125 | 1 | 7 |
| **2** | 280 | 22 | |

#### *7.1.2 Inlet/Carrier/Column*

##### *7.1.2.1 ECD*

| **Inlet** | | Carrier | | Column | |
|---|---|---|---|---|---|
| **Temperature (°C)** | 230 | **Gas** | ultrapure helium | **Type** | Rtx-ClPest |
| **Injection Mode** | Splitless | **Mode** | constant pressure | **Length (m)** | 30 |
| **Splitless Time (min)** | 0.5 | **Pressure (psi)** | 16.85 | **Internal Diameter (mm)** | 0.32 |
| | | | | **Film Thickness (µm)** | 0.50 |

##### *7.1.2.2 NPD*

| **Inlet** | | Carrier | | Column | |
|---|---|---|---|---|---|
| **Temperature (°C)** | 250 | **Gas** | ultrapure helium | **Type** | Rtx-1701 |
| **Injection Mode** | Split | **Mode** | constant pressure | **Length (m)** | 30 |
| **Split Ratio** | 15:1 | **Pressure (psi)** | 13.39 | **Internal Diameter (mm)** | 0.32 |
| | | | | **Film Thickness (µm)** | 0.50 |

#### *7.1.3 Detector Parameters*

##### *7.1.3.1 ECD*

| **Temperature (°C)** | 300 | **Makeup Gas** | nitrogen | **Makeup Flow (mL/min)** | 30 |
|---|---|---|---|---|---|

*7.1.3.2 NPD*

| | | | | | |
|---|---|---|---|---|---|
| **Temperature (°C)** | 250 | **Makeup Gas** | nitrogen | **Makeup Flow (mL/min)** | 30 |
| **Offset** | 10 | **Air Flow (mL/min)** | 60 | **Hydrogen Flow (mL/min)** | 2 |

## 7.2 Agilent Gas Chromatograph (MSD)

*7.2.1 Oven*

| **Step** | Temperature (°C) | Hold (min) | Ramp (°C/min) |
|---|---|---|---|
| **1** | 60 | 3.2 | 35 |
| **2** | 280 | 31 | |

Total run time: 40.5 minutes

*7.2.2 Inlet/Carrier/Column*

| **Inlet** | | Carrier | | Column | |
|---|---|---|---|---|---|
| **Temperature (°C)** | 220 | **Gas** | ultrapure helium | **Type** | DB-5MS |
| **Injection Mode** | Split | **Mode** | constant flow | **Length (m)** | 30 |
| **Split Ratio** | 10:1 | **Flow (mL/min)** | 1.2 | **Internal Diameter (mm)** | 0.25 |
| | | | | **Film Thickness (µm)** | 0.25 |

*7.2.3 Agilent Mass Spectrometer*

| | |
|---|---|
| **Ionization Mode** | Electron Impact (+) |
| **Scan Mode** | Full Scan |
| **Scan Range (m/z)** | 35-500 |
| **Solvent Delay (min)** | 5 |
| **Temperatures (°C)** | |
| **Source** | 230 |
| **Quadrupole** | 150 |
| **Transfer Line** | 280 |

## 8 DATA ANALYSIS

### 8.1 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In most cases, all of the below should be met in order to identify a pesticide within a foodstuff or gastric content sample.

#### *8.1.1 Chromatography*

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

##### *8.1.1.1 Retention Time*

The retention time of the peak should be within ± 2% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard or an extracted positive control

##### *8.1.1.2 Signal-to-Noise*

To justify the existence of a peak, its baseline signal noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10 fold greater than that for any observed peak at similar retention time in a Negative Control or blank solvent injected just prior to the sample.

#### *8.1.2 Mass Spectrometry*

The mass spectrum of the analyte of interest should compare favorably with that of a reference standard, or an extracted Positive Control. See TOX-104 for further guidance.

## 9 REPORTING

In addition to Level 1 documents, reference TOX-100 and TOX-101 for any reporting considerations.

Care should be taken in the interpretation of pesticide levels in foodstuffs. Some pesticides have a legitimate use on food products and a qualitative identification in the absence of quantitative data could produce confusion in interpreting results. The meaning of the toxicological significance of a negative pesticide finding should be considered in conjunction with its biodegradability. Exercise care in reporting and interpreting all pesticide results.

## 10 CORRECTIVE MEASURES

Refer to TOX-101 for guidance on action steps in the event of a quality control failure.

## 11 PERFORMANCE CHARACTERISTICS

### 11.1 LOD

The limit of detection varies depending on the pesticide of interest, and the matrix being analyzed. All thirteen target analytes contained in the pesticides stock solutions used in this procedure can be detected at levels of at least 10 µg/mL in a wide variety of food matrices.

| Class/Analyte | GC/ECD | GC/MS |
|---|---|---|
| Organochlorines | | |
| Lindane (g-BHC) | 5 µg/g | 5 µg/g |
| Aldrin | 5 µg/g | 5 µg/g |
| Endrin | 5 µg/g | 10 µg/g |
| Endrin Aldehyde | 5 µg/g | 10 µg/g |
| 4,4-'DDT | 5 µg/g | 5 µg/g |
| Carbamates | | |
| Carbaryl | | 5 µg/g |
| Carbofuran | | 5 µg/g |
| Propoxur | | 5 µg/g |
| Organophosphates | | |
| Chlorpyrifos | 10 µg/g | 5 µg/g |
| Diazinon | | 5 µg/g |
| Fenchlorphos | | 5 µg/g |
| Parathion | | 5 µg/g |
| Profos | | 10 µg/g |

### 11.2 Carryover

No significant carryover established during validation.

## 12 LIMITATIONS

### 12.1 Interferences

None known. Grossly decomposed or putrefied samples may affect both detection.

### 12.2 Procedure Driven Analyte Modifications

Carbamate pesticides can undergo chemical breakdown to the corresponding phenolate compounds when subjected to GC injection. The extent of this breakdown depends upon sample matrix, analyte loading, and the age and condition of the GC injection port liner. For any carbamate pesticide, the presence of the phenolate breakdown product may be considered as evidence of the presence of the parent compound if the breakdown product is also observed in a contemporaneously analyzed matrix-matched positive control specimen.

### 12.3 Analyte Suitability

This procedure is not suitable for the detection of aldicarb and its metabolites.

## 13 SAFETY

Take standard precautions for the handling of chemicals and biological materials. It should be noted that many of the pesticides detected by this procedure may be extremely toxic and/or carcinogenic. The utmost caution should be taken in handling reference materials and case specimens containing such pesticides. Refer to the *FBI Laboratory Safety Manual* for guidance.

This procedure utilizes the following P-listed pesticides: aldrin, carbofuran, dimethoate, endosulfan, endrin & metabolites, heptachlor, parathion, and tetraethylpyrophosphate (TEPP). This technical procedure utilizes the following U-listed pesticides: carbaryl, DDD, DDT, hexachlorbenzene, methoxychlor, and propoxur.

## 14 REVISION HISTORY

| Revision | Issued | Changes |
|---|---|---|
| 05 | 07/01/2022 | Complete document reformat |