# United States Department of Agriculture

## Food Safety and Inspection Service

# CLG-PST5.10

# Screening for Pesticides by UHPLC/MS/MS and GC/MS/MS

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This method describes the laboratory procedure for the screening of 108 pesticides in bovine, caprine, equine, ovine, porcine, poultry, fish of the order Siluriformes (catfish) muscle, liquid egg products, and powdered egg.

## Notice of Change

The method was reformatted with no substantive changes.

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**Safety Precautions**

The personnel performing the analysis must read the Safety Data Sheets (SDS) for the standards and reagents used in this method. Follow applicable federal, state, and local regulations regarding the disposal of chemicals listed in this method.

# Introduction

Pesticides are used in agriculture to prevent, mitigate, destroy, or repel pests. The Food Safety and Inspection Service (FSIS) uses this CLG-PST5 method to detect pesticides in animal tissue and egg products. Pesticides are used on crops and as insecticides on the animals themselves. This method analyzes for 108 pesticides in 10 pesticides classes: 9 carbamates, 4 conazoles/triazoles, 9 halogenated, 4 neonicotinoids, 20 organochlorines, 19 organophosphates, 28 general pesticides, 9 pyrethroids, 3 substituted benzenes, and 3 triazines.

Per FMIA, PPIA, and EPIA, FSIS collects and tests samples of domestic and imported meat (including *Siluriformes* fish products), poultry, and egg products for pesticides to verify that meat, poultry, and egg products meet tolerances and are safe, wholesome, and accurately labeled. The Environmental Protection Agency (EPA) regulates the approval and use of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act. The Food and Drug Administration (FDA) has the authority to approve and regulate pesticides that are applied to food through the Federal Food, Drug, and Cosmetic Act FSIS works closely with its partner agencies to follow any tolerances set in the Code of Federal Regulations. EPA and FDA set the levels and FSIS collects and samples the product to ensure that any pesticides are below regulated tolerances.

The National Residue Program (NRP) is an interagency program designed to identify, rank, and analyze residues and chemical contaminants in meat, poultry, and egg products. FSIS publishes an Annual Sampling Plan to provide information on the process of sampling meat, poultry, and egg products for pesticides of public health concern. The NRP is monitored and modified annually to set future priorities if data shows trends in detected residues. FSIS uses this multi-residue method to test for pesticides in agency-regulated products.

## Method Overview

The following method describes the laboratory procedure for screening of residues (108 total) from various classes of pesticides and environmental contaminants in muscle tissue from beef, goat, sheep, pork, *Siluriformes* (catfish), liquid egg products and powdered egg.

Pesticide residues are extracted from muscle tissue through an extraction with ethyl acetate and QuEChERS salts containing sodium chloride and magnesium sulfate. The crude material is separated through filtration, resulting in an ethyl acetate extract. A solvent exchange is conducted with acetonitrile, which is then followed by a clean-up through precipitation using ultra-low temperature freezing to separate the fats from the samples. The liquid layer is transferred, and solid-phase extraction (SPE) is then used to further clean up the extracts. The extracts are then separated for gas chromatography with tandem mass spectrometry (GC-MS/MS) analysis (37 pesticides) and liquid chromatography with tandem mass spectrometry (UHPLC/MS/MS) analysis (71 pesticides). Prior to GC-MS/MS analysis, the extract is further cleaned up with anion exchange SPE. Prior to UHPLC-MS/MS analysis, the extract is further cleaned up with dispersive SPE and QuEChERS salts containing an anion exchange sorbent.

**KEY DEFINITIONS**

**Solid Phase Extraction (SPE):** An extraction technique that utilizes a solid support that contains an adsorbing surface or chemical coating that can interact with analyte.

**QuEChERS** (Quick, Easy, Cheap, Effective, Rugged and Safe): A solid phase extraction method for detection of pesticide residues in food.

**Precipitation:** An extraction technique resulting in solid material being left at the bottom of an extraction vessel with the extract or liquid layer containing the analyte. The liquid layer can be separated out for further analysis.

**UHPLC-MS/MS:** an analytical technique where there is a physical separation of target compounds followed by their mass-based detection.

**GC-MS/MS:** An analytical technique that involves separation analytes with gas chromatography followed by analysis of masses of individual atoms or molecules through mass spectrometry.[2]

Both the GC-MS/MS and UHPLC/MS/MS instrumental analyses are independent of one another. In the case that an instrument is not available, samples can continue to undergo through the

respective extraction and clean-up processes with the results for the available system being reported while excluding the results for unavailable instrument. Additionally, this method may be performed using standards or solutions that contain fewer analytes than the method applicability. When that occurs, the excluded analytes would not be included in the reported results.

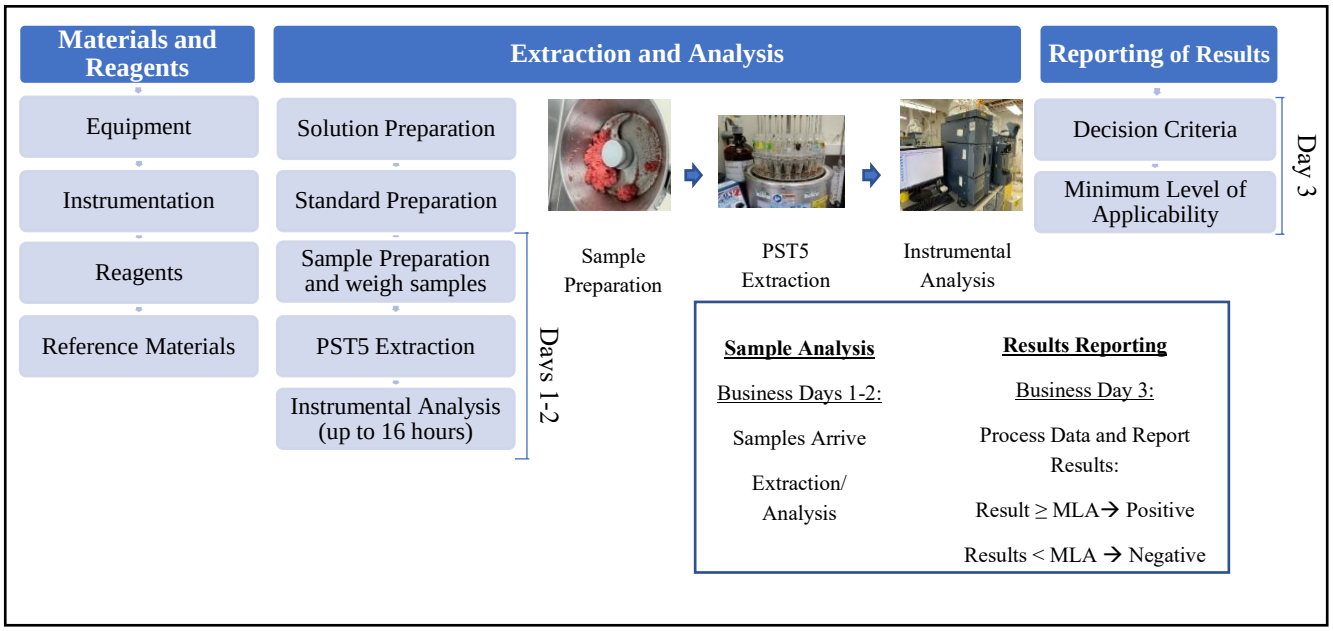


**Figure 1:** Overview and timeframe of Pesticides (PST5). Materials and reagents are obtained and utilized to prepare solutions and standards. The samples arrive at laboratory, are prepared into a homogenized mixture, weighed, extracted, and analyzed by UHPLC-MS/MS on business days 1-2. Screening results are reported on business day 3. This figure represents the best-case scenarios, but analyses may take longer. Photos courtesy of Hue Quach, USDA FSIS and Ryan Matsuda, USDA FSIS.

## Decision Criteria

A sample is considered negative if the results are less than the minimum level of applicability (MLA). A sample is considered a screened positive if the results are greater than or equal to the MLA. Screened positive results will require further analysis through additional methods.

**KEY DEFINITIONS**

**MLA:** Lowest level at which an FSIS method has been successfully validated for a residue in each matrix. Full definition is on the CLG website here.

## Disclosure Statement

FSIS does not specifically endorse any test products listed in this method. FSIS acknowledges that equivalent equipment, reagents, or solutions may be suitable for laboratory use. The FSIS laboratory system uses method performance requirements when evaluating the equivalence of an alternative equipment, reagent, or solution for a given analyte and sample matrix pair. Significant equivalence changes would require FSIS laboratory leadership approval.

# Materials and Reagents

## Equipment

**Table 1: Equipment Required to Perform CLG-PST5**

| Equipment | Supplier and Part Number | Purpose |
|---|---|---|
| **Food Processor** | Robot Coupe USA, Inc | Homogenize sample |
| **Analytical Balance** | General lab supplier | Record weight of standard reagent. Minimum accuracy ±0.0001g |
| **Top Loading Balance** | General lab supplier | Record weight of standard reagent. Minimum accuracy Minimum accuracy ±0.01g |
| **Microcentrifuge** | General lab supplier | Separates the solid sample material from the extraction solution |
| **Centrifuge** | General lab supplier | Separates the solid sample material from the extraction solution Capable of centrifuging 50 mL glass test tubes |
| **Centrifuge tubes, Polypropylene (PP), 50 mL** | SARSTEDT, 62.547.205 | Contain sample material and extraction vessel |
| **Centrifuge tubes, glass, 50 mL** | General lab supplier | Contain extraction solution and extraction vessel, Pyrex tube with stopper |
| **Centrifuge tubes, glass, 15mL** | Kimble Chase, 45166-15 | Contain extraction solution and extraction vessel, Pyrex tube with stopper |
| **Shaker** | General lab supplier | Facilitates extraction of pesticides from the sample |
| **Multi Tube Vortex** | General lab supplier | Facilitates extraction of pesticides from the sample |
| **Freezer, -20 °C** | General lab supplier | Storage of standards and reagents |
| **Nitrogen Evaporator Apparatus with Heated Water Bath** | General lab supplier | Reduces extraction solution down to desired volume |
| **Positive Pressure Manifold** | UCT, LLC, VMFPPM16 | Perform SPE Clean Up |
| **1000 mg C18 SPE Columns** | UCT, LLC, CEC181M6 | Separates out the pesticides from the extraction solution |
| **Fluted filter paper** | VWR, 28333-043 | Filter extracts |
| **Nylon Syringe Filter, 0.2 µm** | VWR International., 28143-242 | Dispersive solid phase extraction |
| **500 mg (Primary Secondary Amine) SPE Columns** | UCT, LLC, CUPSA156 | Clean up sample for GC-MS/MS analysis |

| | | |
|---|---|---|
| **Micro centrifuge tubes with QuEChERS salts (150 mg MgSO4 & 50 mg PSA)** | UCT, LLC, CUMPS2CT | Clean up sample for LC-MS/MS analysis |
| **Dispensers** | General lab supplier | Dispense standards and reagents |
| **Repeating pipettes and tips** | General lab supplier | Dispense standards and reagents |
| **Disposable Pasteur Pipettes** | General lab supplier | Dispense standards and reagents |
| **Disposable Transfer Pipettes** | General lab supplier | Dispense standards and reagents |
| **Syringe, Plastic, 3mL** | Becton Dickenson, 309657 | Filter final extracts |
| **Auto Sampler Vials** | General lab supplier | Store final extractions for analysis |
| **Glassware, Class A** | General lab supplier | Measuring standards and reagents |

## Instrumentation

**Table 2: Instrumentation**

| Instrument | Supplier and Model Number | Purpose |
|---|---|---|
| **Waters UPLC-MS/MS System** | Waters Xevo I-Class LC, Waters Xevo TQ-S micro Mass Spectrometer | Extract analysis |
| **Waters UPLC HSS T3, 2.1 × 100 mm, 1.8µm** | Waters, 186003539 | Extract analysis |
| **Waters VanGuard Pre-column UHPLC HSS T3, 2.1 × 5.0 mm, 1.8 µm** | Waters, 186003976 | Extract analysis |
| **Gas Chromatograph** | Agilent, 7890B | Extract analysis |
| **GC Quadrupole Mass Spectrometer** | Agilent, 7010 | Extract analysis |
| **Agilent, J&W HP-5MS Ultra Inert 15m, 0.25mm, 0.25um 7in cage, two columns are used in series** | Agilent, 19091S-431UI. Two columns are used in a series | Extract analysis |

## Reagents

**Table 3: Reagents**

| Reagent | Supplier and Part Number |
|---|---|
| **QuEChERS Salts Packets (8g MgSO4 & 2 g NaCl)** | UCT, LLC, ECQUVIN50CT-MP |
| **Magnesium sulfate, anhydrous** | General lab supplier |
| **Acetic acid – ACS Grade** | General lab supplier |
| **Ethyl acetate – LC Grade** | General lab supplier |
| **Acetonitrile (ACN) – LC Grade** | General lab supplier |
| **Acetone – LC Grade** | General lab supplier |
| **Toluene – LC Grade** | General lab supplier |
| **Methanol – LC Grade** | General lab supplier |
| **Ammonium Acetate** | General lab supplier |
| **Formic Acid – ACS Grade** | General lab supplier |

| | |
|---|---|
| **Water – Resistivity of > 18 MΩ-cm** | House system |
| **Isopropanol – LC-MS Grade** | General lab supplier |

### Reference Materials

**Table 4: Reference Materials**

| Standard | Supplier | Catalog Number |
|---|---|---|
| **Custom Pesticide Standard (Internal standard Trichloronate and Ethoprophos** | Accustandard | S-23242 |
| **Custom Pesticide Standard** | Accustandard | S-66100-01 |
| **Custom Pesticide Standard** | Accustandard | S-66100-02 |
| **Custom Pesticide Standard** | Accustandard | S-63904-01-R1 |
| **Custom Pesticide Standard** | Accustandard | S-63904-02 |
| **Custom Pesticide Standard** | Accustandard | S-63903-R1-SET |
| **Ethion Monoxon Standard** | EPA | F714 |

Purity and counterions are to be taken into account when calculating standard concentrations. In-house prepared standards are to be assigned an expiration date that is no later than the stability stated in the method.

## Extraction and Analysis

### Solution Preparation

**Table 5: Preparation of Solutions**

| Solution | Procedure |
|---|---|
| **1% Acetic acid/Acetonitrile (by Volume)**<br><br>**Solution expires one year from preparation date.** | 1) Using a graduated cylinder measure 1980 mL of acetonitrile.<br>2) Using a graduated cylinder measure 20 mL of acetic acid.<br>3) Combine and mix in a 2L glass storage container for use. |
| **LC/MS/MS Mobile Phase A (5 mM ammonium acetate/0.1% formic acid in water by volume)**<br><br>**Solution expires one year from preparation date**. | 1) Measure 0.771 g ammonium acetate.<br>2) Dissolve and transfer the ammonium acetate to a 2 L graduated cylinder or volumetric flask with water.<br>3) Measure and add 2 mL of formic acid to same cylinder or volumetric flask.<br>4) Dilute to volume with water.<br>5) Mix well and transfer to glass storage container for use. |
| **LC/MS/MS Mobile Phase B (0.1% formic acid in methanol by volume)**<br><br>**Solution expires one year from preparation date.** | 1) Measure 2 mL of formic acid and add to a 2 L graduated cylinder or volumetric flask.<br>2) Dilute to volume with methanol.<br>3) Mix well and transfer to glass storage container for use. |

| | |
|---|---|
| **3:1 v/v Acetone/Toluene**<br><br>**Solution expires one year from preparation date**. | 1) Measure 500 mL of toluene and add to a 2 L graduated cylinder.<br>2) Measure 1500 mL of acetone and add to same cylinder.<br>3) Mix well for use. |
| **LC/MS/MS Weak Wash (10% methanol in water by volume)**<br><br>**Solution expires one year from preparation date.** | 1) Measure 100 mL of methanol and add to a 1 L glass storage container.<br>2) Measure 900 mL of water and add to same container.<br>3) Mix well for use. |
| **LC/MS/MS Strong Wash (0.5% formic acid in 1:1:1:1 acetonitrile: methanol: isopropanol: water)**<br><br>**Solution expires one year from preparation date**. | 1) Measure 250 mL of acetonitrile and add to a 1 L glass storage container.<br>2) Measure 250 mL of methanol and add to same container.<br>3) Measure 250 mL of isopropanol and add to same container.<br>4) Measure 250 mL of water and add to same container.<br>5) Measure 5.0 mL formic acid and add to same container.<br>6) Mix well for use. |

## Standard Preparation

**Table 6: GC Mixed Pesticide Standard**

| Cmpd # | Pesticide | CAS # | Stock Conc. (µg/mL ethyl acetate) | Spiking Solution Conc. (µg/mL ethyl acetate) |
|---|---|---|---|---|
| 1 | 1-Naphthol | 90-15-3 | 60 | 6 |
| 2 | Aldrin | 309-00-2 | 50 | 5 |
| 3 | Bifenthrin | 82657-04-3 | 10 | 1 |
| 4 | Chlordane cis | 5103-71-9 | 20 | 2 |
| 5 | Chlordane trans | 5103-74-2 | 20 | 2 |
| 6 | Chloroneb | 2675-77-6 | 18 | 1.8 |
| 7 | Chlorothalonil | 1897-45-6 | 120 | 12 |
| 8 | Chlorpropham | 101-21-3 | 60 | 6 |
| 9 | Chlorpyrifos | 2921-88-2 | 15 | 1.5 |
| 10 | Chlorpyrifos methyl | 5598-13-0 | 10 | 1 |
| 11 | DDD o,p' | 53-19-0 | 100 | 10 |
| 12 | DDD p,p' + DDT o,p' | 72-54-8 & 789-02-6 | 100+100 | 10+10 |
| 13 | DDE o,p' | 3424-82-6 | 100 | 10 |

| Cmpd # | Pesticide | CAS # | Stock Conc. (µg/mL ethyl acetate) | Spiking Solution Conc. (µg/mL ethyl acetate) |
|---|---|---|---|---|
| 14 | DDE p,p' | 72-55-9 | 100 | 10 |
| 15 | DDT p,p' | 50-29-3 | 100 | 10 |
| 16 | Dieldrin | 60-57-1 | 50 | 5 |
| 17 | Endosulfan I | 959-98-8 | 100 | 10 |
| 18 | Endosulfan II | 33213-65-9 | 100 | 10 |
| 19 | Endosulfan sulfate | 1031-07-8 | 100 | 10 |
| 20 | Fenpropathrin | 39515-41-8 | 50 | 5 |
| 21 | Fipronil | 120068-37-3 | 10 | 1 |
| 22 | Fipronil desulfinyl | 205650-65-3 | 10 | 1 |
| 23 | Fipronil sulfide | 120067-83-6 | 10 | 1 |
| 24 | Heptachlor | 76-44-8 | 50 | 5 |
| 25 | Heptachlor epoxide (cis&trans) or (B+A) | 1024-57-3 & 28044-83-9 | 50+50 | 5+5 |
| 26 | Hexachlorobenzene (HCB) | 118-74-1 | 50 | 5 |
| 27 | Lindane (BHC gamma) | 58-89-9 | 80 | 8 |
| 28 | MGK-264 (isomers 1&2) | 113-48-4 | 100 | 10 |
| 29 | Metolachlor | 51218-45-2 | 20 | 2 |
| 30 | Nonachlor cis | 5103-73-1 | 30 | 3 |
| 31 | Nonachlor trans | 39765-80-5 | 30 | 3 |
| 32 | Oxychlordane | 27304-13-8 | 20 | 2 |
| 33 | Pentachloroaniline (PCA) | 527-20-8 | 50 | 5 |
| 34 | Pentachlorobenzene (PCB) | 608-93-5 | 20 | 2 |
| 35 | Permethrin (cis&trans) | 52645-53-1 | 50 | 5 |
| 36 | Pronamide | 23950-58-5 | 10 | 1 |
| 37 | Tefluthrin | 79538-32-2 | 10 | 1 |

**Table 7: LC Mixed Pesticide Standard**

| Cmpd # | Pesticide | CAS # | Stock Conc. (µg/mL ethyl acetate) | Spiking Solution Conc. (µg/mL ethyl acetate) |
|---|---|---|---|---|
| 38 | 3-Hydroxycarbofuran | 16655-82-6 | 10 | 1 |
| 39 | Acephate | 30560-19-1 | 20 | 2 |
| 40 | Acetamiprid | 135410-20-7 | 10 | 1 |
| 41 | Alachlor | 15972-60-8 | 10 | 1 |
| 42 | Aldicarb | 116-06-3 | 20 | 2 |
| 43 | Aldicarb sulfone | 1646-88-4 | 20 | 2 |
| 44 | Aldicarb sulfoxide | 1646-87-3 | 50 | 5 |
| 45 | Atrazine | 1912-24-9 | 20 | 2 |
| 46 | Azinphos methyl | 86-50-0 | 20 | 2 |
| 47 | Azoxystrobin | 131860-33-8 | 10 | 1 |
| 48 | Benoxacor | 98730-04-02 | 10 | 1 |
| 49 | Boscalid | 188425-85-6 | 30 | 3 |
| 50 | Buprofezin | 69327-76-0 | 50 | 5 |
| 51 | Carbaryl | 63-25-2 | 50 | 5 |
| 52 | Carbofuran | 1563-66-2 | 10 | 1 |
| 53 | Carfentrazone ethyl | 128639-02-1 | 10 | 1 |
| 54 | Clothianidin | 210880-92-5 | 20 | 2 |
| 55 | Coumaphos O | 321-54-0 | 20 | 2 |
| 56 | Coumaphos S | 56-72-4 | 20 | 2 |
| 57 | Desethylatrazine | 6190-65-4 | 20 | 2 |
| 58 | Diazinon | 333-41-5 | 10 | 1 |
| 59 | Dichlorvos (DDVP) | 62-73-7 | 20 | 2 |
| 60 | Difenoconazole | 119446 | 30 | 3 |
| 61 | Diflubenzuron | 35367-38-5 | 25 | 2.5 |
| 62 | Dimethoate | 60-51-5 | 20 | 2 |
| 63 | Diuron | 330-54-1 | 160 | 16 |
| 64 | Ethion | 563-12-12 | 20 | 2 |
| 65 | Ethion monoxon | 17356-42-2 | 20 | 2 |
| 66 | Ethofumesate | 26225-79-6 | 40 | 4 |

| Cmpd # | Pesticide | CAS # | Stock Conc. (µg/mL ethyl acetate) | Spiking Solution Conc. (µg/mL ethyl acetate) |
|---|---|---|---|---|
| 67 | Fenoxaprop ethyl | 66441-23-4 | 20 | 2 |
| 68 | Fluridone | 59756-60-4 | 50 | 5 |
| 69 | Fluroxypyr-1-Methylheptyl-Ester | 81406-37-3 | 10 | 1 |
| 70 | Fluvalinate | 102851-06-9 | 15 | 1.5 |
| 71 | Hexazinone | 51235-04-2 | 60 | 6 |
| 72 | Hexythiazox | 78587-05-0 | 20 | 2 |
| 73 | Imazalil | 35554-44-0 | 10 | 1 |
| 74 | Imidacloprid | 138261-41-3 | 50 | 5 |
| 75 | Indoxacarb | 144171-61-9 | 50 | 5 |
| 76 | Linuron | 330-55-2 | 50 | 5 |
| 77 | Malathion | 121-75-5 | 80 | 8 |
| 78 | Metalaxyl | 57837-19-1 | 20 | 2 |
| 79 | Methamidophos | 10265-92-6 | 20 | 2 |
| 80 | Methomyl | 16752-77-5 | 60 | 6 |
| 81 | Methoxyfenozide | 161050-58-4 | 10 | 1 |
| 82 | Metribuzin | 21087-64-9 | 100 | 10 |
| 83 | Myclobutanil | 88671-89-0 | 20 | 2 |
| 84 | Norflurazon | 27314-13-2 | 20 | 2 |
| 85 | Omethoate | 1113-02-6 | 20 | 2 |
| 86 | Piperonyl butoxide | 51-03-6 | 45 | 4.5 |
| 87 | Pirimiphos methyl | 29232-93-7 | 20 | 2 |
| 88 | Prallethrin | 23031-36-9 | 80 | 8 |
| 89 | Profenofos | 41198-08-7 | 20 | 2 |
| 90 | Propachlor | 1918-16-7 | 20 | 2 |
| 91 | Propanil | 709-98-8 | 50 | 5 |
| 92 | Propetamphos | 31218-83-4 | 15 | 1.5 |
| 93 | Propiconazole | 60207-90-1 | 30 | 3 |
| 94 | Pyraclostrobin | 175013-18-0 | 100 | 10 |
| 95 | Pyrethrin I | 8003-34-7 | 56 (92) | 5.6 (9.2) |

| Cmpd # | Pesticide | CAS # | Stock Conc. (µg/mL ethyl acetate) | Spiking Solution Conc. (µg/mL ethyl acetate) |
|---|---|---|---|---|
| 96 | Pyrethrin II | 8003-34-7 | 62 | 6.2 |
| 97 | Pyridaben | 96489-71-3 | 18 | 1.8 |
| 98 | Pyriproxyfen | 95737-68-1 | 40 | 4 |
| 99 | Resmethrin (cis& trans) | 10453-86-8 | 100 | 10 |
| 100 | Simazine | 122-34-9 | 20 | 2 |
| 101 | Sulprofos | 34500-43-2 | 50 | 5 |
| 102 | Tebufenozide | 112410-23-8 | 80 | 8 |
| 103 | Tetrachlorvinphos | 22248-79-9 | 20 | 2 |
| 104 | Tetraconazole | 11281-77-3 | 10 | 1 |
| 105 | Thiabendazole | 148-79-8 | 30 | 3 |
| 106 | Thiamethoxam | 153719-23-4 | 20 | 2 |
| 107 | Thiobencarb | 28249-77-6 | 100 | 10 |
| 108 | Trifloxystrobin | 141517-21-7 | 10 | 1 |

**Table 8: Ethion monoxon solutions**

| | |
|---|---|
| Individual ethion monoxon stock solutions (~1.0 mg/mL in ethyl acetate):<br><br>This standard is stable for 1 year when stored at < -10°C. | 1) Weigh approximately 10 mg of ethion monoxon, into a 10 mL volumetric flask. Weigh this amount to the nearest 0.1 mg. Determine final concentration of ethion monoxon based on purity of stock standard.<br>2) Dilute to volume with ethyl acetate.<br>3) Mix well and transfer to glass storage container. |
| Intermediate standard solution of ethion monoxon (100 µg/mL)<br><br>This standard is stable for 1 year when stored at < -10°C. | 1) Calculate amount of ethion monoxon stock solution to prepare 10 mL of 100 µg/mL solution of ethion monoxon and pipette that amount into a 10 mL volumetric flask.<br>2) Dilute to volume with ethyl acetate.<br>3) Mix well and transfer to glass storage container. |

**Table 9: Mixed Pesticides spiking solution in Ethyl Acetate**

| | |
|---|---|
| **Mixed Pesticide Spiking Solution:**<br><br>**Spiking solutions are stored at ≤ -10 °C and expire one year from the preparation date**. | 1) Measure 5 mL of each pesticide standard and add to a 50 mL volumetric flask.<br>2) Dilute to volume with ethyl acetate.<br>3) Mix well and transfer to glass storage container. |

**Table 10: Preparation of Internal Standard**

| | |
|---|---|
| **Internal Standard Spiking Solution (20 µg/mL Trichloronate & 10 µg/mL Ethoprofos):**<br><br>**Spiking solutions are stored at ≤ -10 °C and expire one year from the preparation date.** | 1) Measure 1.0 mL of the pesticide internal standard and add to a 50 mL volumetric flask.<br>2) Dilute to volume with ethyl acetate.<br>3) Mix well and transfer to glass storage container. |

**Table 11: Preparation of Injection Standard for LC and GC compounds**

| | |
|---|---|
| **Muscle Injection Standard for LC compounds:**<br><br>**Injection standards are stored at ≤ -10 °C and expire in one month.** | 1) Measure 200 µL of internal standard spiking solution and add to a 10 mL volumetric flask.<br>2) Measure 200 µL of mixed pesticide spiking solution and add to the same flask.<br>3) Dilute to volume with acetonitrile.<br>4) Mix well and transfer to glass storage container. |
| **Egg Injection Standard for LC compounds:**<br><br>**Injection standards are stored at ≤ -10 °C and expire in one month.** | 1) Measure 33.3 µL of internal standard spiking solution and add to a 10 mL volumetric flask.<br>2) Measure 33.3 µL of mixed pesticide spiking solution and add to the same the flask.<br>3) Dilute to volume with acetonitrile.<br>4) Mix well and transfer to glass storage container. |
| **Muscle Injection Standard for GC compounds:**<br><br>**Injection standards are stored at ≤ -10 °C and expire in one month.** | 1) Measure 200 µL of internal standard spiking solution and add to a 10 mL volumetric flask.<br>2) Measure 200 µL of mixed pesticide and add to the same flask.<br>3) Dilute to volume with toluene.<br>4) Mix well and transfer to glass storage container. |
| **Egg Injection Standard for GC compounds:**<br><br>**Injection standards are stored at ≤ -10 °C and expire in one month**. | 1) Measure 33.3 µL of internal standard spiking solution and add to 10 mL volumetric flask.<br>2) Measure 33.3 µL of mixed pesticide spiking solution and add to the same flask.<br>3) Dilute to volume with toluene.<br>4) Mix well and transfer to glass storage container. |

## Table 12: Concentration of GC and LC Injection Standard

| Cmpd # | Name | Muscle Injection Standard Conc. (µg of pest./mL of solution) | Egg Injection Standard Conc. (µg of pest./mL of solution) |
|---|---|---|---|
| | GC Mixed Standard | | |
| 1 | 1-Naphthol | 0.12 | 0.0200 |
| 2 | Aldrin | 0.1 | 0.0167 |
| 3 | Bifenthrin | 0.02 | 0.00333 |
| 4 | Chlordane cis | 0.04 | 0.00666 |
| 5 | Chlordane trans | 0.04 | 0.00666 |
| 6 | Chloroneb | 0.036 | 0.00599 |
| 7 | Chlorothalonil | 0.24 | 0.0400 |
| 8 | Chlorpropham | 0.12 | 0.0200 |
| 9 | Chlorpyrifos | 0.03 | 0.00500 |
| 10 | Chlorpyrifos methyl | 0.02 | 0.00333 |
| 11 | DDD o,p' | 0.2 | 0.0333 |
| 12 | DDD p,p' + DDT o,p' | 0.2 + 0.2 | 0.0333 + 0.0333 |
| 13 | DDE o,p' | 0.2 | 0.0333 |
| 14 | DDE p,p' | 0.2 | 0.0333 |
| 15 | DDT p,p' | 0.2 | 0.0333 |
| 16 | Dieldrin | 0.1 | 0.0167 |
| 17 | Endosulfan I | 0.2 | 0.0333 |
| 18 | Endosulfan II | 0.2 | 0.0333 |
| 19 | Endosulfan sulfate | 0.2 | 0.0333 |
| 20 | Fenpropathrin | 0.10 | 0.0167 |
| 21 | Fipronil | 0.02 | 0.00333 |
| 22 | Fipronil desulfinyl | 0.02 | 0.00333 |
| 23 | Fipronil sulfide | 0.02 | 0.00333 |
| 24 | Heptachlor | 0.1 | 0.0167 |
| 25 | Heptachlor epoxide (cis&trans) or (B+A) | 0.1 + 0.1 | 0.0167 + 0.0167 |
| 26 | Hexachlorobenzene (HCB) | 0.1 | 0.0167 |
| 27 | Lindane (BHC gamma) | 0.16 | 0.0266 |
| 28 | MGK-264 (isomers 1&2) | 0.2 | 0.0333 |
| 29 | Metolachlor | 0.04 | 0.00666 |
| 30 | Nonachlor cis | 0.06 | 0.00999 |

| Cmpd # | Name | Muscle Injection Standard Conc. (µg of pest./mL of solution) | Egg Injection Standard Conc. (µg of pest./mL of solution) |
|---|---|---|---|
| 31 | Nonachlor trans | 0.06 | 0.00999 |
| 32 | Oxychlordane | 0.04 | 0.00666 |
| 33 | Pentachloroaniline (PCA) | 0.1 | 0.0167 |
| 34 | Pentachlorobenzene (PCB) | 0.04 | 0.00666 |
| 35 | Permethrin (cis&trans) | 0.1 | 0.0167 |
| 36 | Pronamide | 0.02 | 0.00333 |
| 37 | Tefluthrin | 0.02 | 0.00333 |
| | LC Mixed Standard | D.2.c | D.2.d |
| 38 | 3-Hydroxycarbofuran | 0.02 | 0.00333 |
| 39 | Acephate | 0.04 | 0.00666 |
| 40 | Acetamiprid | 0.02 | 0.00333 |
| 41 | Alachlor | 0.02 | 0.00333 |
| 42 | Aldicarb | 0.04 | 0.00666 |
| 43 | Aldicarb sulfone | 0.04 | 0.00666 |
| 44 | Aldicarb sulfoxide | 0.1 | 0.0167 |
| 45 | Atrazine | 0.04 | 0.00666 |
| 46 | Azinphos methyl | 0.04 | 0.00666 |
| 47 | Azoxystrobin | 0.02 | 0.00333 |
| 48 | Benoxacor | 0.02 | 0.00333 |
| 49 | Boscalid | 0.06 | 0.00999 |
| 50 | Buprofezin | 0.1 | 0.0167 |
| 51 | Carbaryl | 0.1 | 0.0167 |
| 52 | Carbofuran | 0.02 | 0.00333 |
| 53 | Carfentrazone ethyl | 0.02 | 0.00333 |
| 54 | Clothianidin | 0.04 | 0.00666 |
| 55 | Coumaphos O | 0.04 | 0.00666 |
| 56 | Coumaphos S | 0.04 | 0.00666 |
| 57 | Deethylatrazine | 0.04 | 0.00666 |
| 58 | Diazinon | 0.02 | 0.00333 |
| 59 | Dichlorvos (DDVP) | 0.04 | 0.00666 |
| 60 | Difenoconazole | 0.06 | 0.00999 |
| 61 | Diflubenzuron | 0.05 | 0.00833 |

| Cmpd # | Name | Muscle Injection Standard Conc. (µg of pest./mL of solution) | Egg Injection Standard Conc. (µg of pest./mL of solution) |
|---|---|---|---|
| 62 | Dimethoate | 0.04 | 0.00666 |
| 63 | Diuron | 0.32 | 0.0533 |
| 64 | Ethion | 0.04 | 0.00666 |
| 65 | Ethion monoxon | 0.04 | 0.00666 |
| 66 | Ethofumesate | 0.08 | 0.0133 |
| 67 | Fenoxaprop ethyl | 0.04 | 0.00666 |
| 68 | Fluridone | 0.1 | 0.0167 |
| 69 | Fluroxypyr-1-Methylheptyl-Ester | 0.02 | 0.00333 |
| 70 | Fluvalinate | 0.03 | 0.00500 |
| 71 | Hexazinone | 0.12 | 0.0200 |
| 72 | Hexythiazox | 0.04 | 0.00666 |
| 73 | Imazalil | 0.02 | 0.00333 |
| 74 | Imidacloprid | 0.1 | 0.0167 |
| 75 | Indoxacarb | 0.1 | 0.0167 |
| 76 | Linuron | 0.1 | 0.0167 |
| 77 | Malathion | 0.16 | 0.0266 |
| 78 | Metalaxyl | 0.04 | 0.00666 |
| 79 | Methamidophos | 0.04 | 0.00666 |
| 80 | Methomyl | 0.12 | 0.0200 |
| 81 | Methoxyfenozide | 0.02 | 0.00333 |
| 82 | Metribuzin | 0.2 | 0.0333 |
| 83 | Myclobutanil | 0.04 | 0.00666 |
| 84 | Norflurazon | 0.04 | 0.00666 |
| 85 | Omethoate | 0.04 | 0.00666 |
| 86 | Piperonyl butoxide | 0.09 | 0.0150 |
| 87 | Pirimiphos methyl | 0.04 | 0.00666 |
| 88 | Prallethrin | 0.16 | 0.0266 |
| 89 | Profenofos | 0.04 | 0.00666 |
| 90 | Propachlor | 0.04 | 0.00666 |
| 91 | Propanil | 0.1 | 0.0167 |
| 92 | Propetamphos | 0.03 | 0.00500 |
| 93 | Propiconazole | 0.06 | 0.00999 |

| Cmpd # | Name | Muscle Injection Standard Conc. (µg of pest./mL of solution) | Egg Injection Standard Conc. (µg of pest./mL of solution) |
|---|---|---|---|
| 94 | Pyraclostrobin | 0.2 | 0.0333 |
| 95 | Pyrethrin I | 0.184 | 0.0306 |
| 96 | Pyrethrin II | 0.124 | 0.0206 |
| 97 | Pyridaben | 0.036 | 0.00599 |
| 98 | Pyripoxyfen | 0.08 | 0.0133 |
| 99 | Resmethrin (cis&trans) | 0.2 | 0.0333 |
| 100 | Simazine | 0.04 | 0.00666 |
| 101 | Sulprofos | 0.1 | 0.0167 |
| 102 | Tebufenozide | 0.16 | 0.0266 |
| 103 | Tetrachlorvinphos | 0.04 | 0.00666 |
| 104 | Tetraconazole | 0.02 | 0.00333 |
| 105 | Thiabendazole | 0.06 | 0.00999 |
| 106 | Thiamethoxam | 0.04 | 0.00666 |
| 107 | Thiobencarb | 0.2 | 0.0333 |
| 108 | Trifloxystrobin | 0.02 | 0.00333 |
| | Internal Standards | | |
| 109 | Trichloronate | 0.4 | 0.0666 |
| 110 | Ethoprophos | 0.2 | 0.0333 |

## Sample Preparation

### Preparation of Samples and Quality Controls

Samples must be kept cold before and during shipping to the laboratory. Once received at the laboratory, muscle samples must be frozen (≤ -10 °C) prior to grinding if they cannot be prepared on the day of receipt. Once frozen, temper (partially thaw) while keeping it as cold as possible. As shown in Figure 2, trim away fat and connective tissue. Grind tissue in blender or vertical cutter-mixer until homogeneous, as shown in Figure 3. Store samples frozen (≤ -10 °C) prior to analysis.

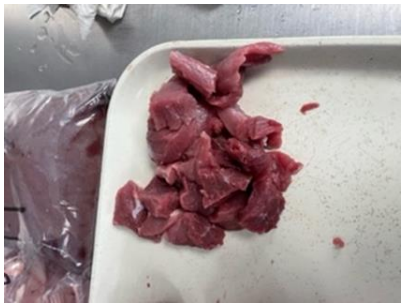

**Figure 2**: Prepared lean muscle sample with connective tissue removed. Photo courtesy of Hue Quach, USDA FSIS.

**Figure 3**: Homogenized sample. Photo courtesy of Hue Quach, USDA FSIS.

Both liquid and powdered egg products require no sample preparation. An example of a liquid egg product is shown in Figure 4.

**Figure 4**: Liquid egg samples. Photo courtesy of Hue Quach USDA FSIS.

## Pesticides Extraction

### Samples

Weigh 20.0 ± 0.20 g of homogenized muscle sample, 5.0 ± 0.04 g of liquid egg product, or 2.5 ± 0.04 g powdered egg product into a 50 mL polypropylene centrifuge tube, as shown in Figure 5. Make sure the sample is all the way down in the tube.

**QUALITY CONTROL**

1. Weigh three 20.0 ± 0.2 g portions of blank muscle tissue, 5.0 ± 0.04 g portions for liquid egg, and 2.5 ± 0.04 portions for powdered egg into 50 mL polypropylene centrifuge tubes. One for the blank (negative control), one for the decision level control, and one for the positive control. Weigh one additional portion for a check sample, if applicable.
2. Prepare decision level and recovery control by fortifying the sample with 100 µL for muscle and 25 µL for the liquid and powdered egg of the appropriate fortification standard.
3. Allow the sample to dry about five minutes (min) before continuing to the extraction.


**Figure 5**: Weighed controls and samples. Photo courtesy of Ryan Matsuda, USDA-FSIS

### Extraction

1. Add 30 mL of ethyl acetate to each sample as shown in Figure 6.
2. Fortify each sample and each control with 100 µL, for muscle, or 25 µL, for eggs, of the internal standard spiking solution and cap centrifuge tube. Invert, vortex, or shake tubes to homogenize (or shred) tissue and ensure solvent reaches the entire sample.
3. Place samples on the shaker for one minute to mix.

**Figure 6**: Ethyl acetate added to controls and samples Photo courtesy of Ryan Matsuda USDA-FSIS

4. Add 8 g of $MgSO_4$ and 2 g NaCl (pre-weighed QuEChERS salts) to each sample and cap tube as shown in Figure 7. Invert, vortex, or shake tubes to homogenize (or shred) tissue and ensure solvent reaches the entire sample as shown in Figure 8.

**Figure 8**: Samples with QuEChERS salts. Photo courtesy of Jason Stone USDA-FSIS

**Key Fact:**

*Make sure the solvent interacts well with the entire sample and the crystalline agglomerates are broken up sufficiently.*

5. Shake vigorously for five min on the shaker.
6. Place samples into the ≤ -20 °C freezer for 30 min.
7. Remove samples from freezer and centrifuge at 3000 RCF for 8 min.
8. As shown in Figure 9, decant more than 18 mL of the ethyl acetate layer into a 50 mL graduated glass centrifuge tube using a funnel and filter paper.
9. Adjust the volume of muscle samples to 18 mL and of egg samples to 12 mL, discarding the excess.
10. Concentrate the extract under nitrogen in a 65 ± 5 °C water bath until the volume remains constant. This volume is typically 0.5 mL to 2.0 mL, as shown in Figure 10.
11. Dilute to 15 mL with acetonitrile, cap glass tube and vortex for one minute.
12. Place samples in ≤ -70 °C freezer for 30 min.
13. After removing samples from the freezer, let them sit until the stopper can be removed from each sample tube and recap them. This will prevent pressure from building up while in the centrifuge and tubes breaking. Centrifuge the extract while frozen for 3.5 min at 1050 RCF.

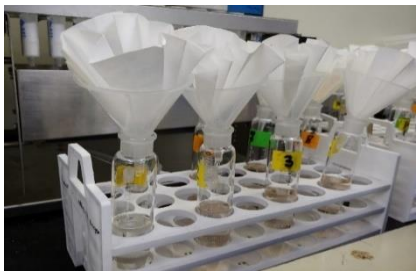

**Figure 9**: Samples undergoing filtration. Photo courtesy of Ryan Matsuda USDA-FSIS

**Figure 10**: Samples undergoing evaporation. Photo courtesy of Ryan Matsuda USDA-FSIS

**Key Fact:**

*Acetonitrile will thaw during centrifugation.*

14. To minimize disruption of the pellet, transfer 10 mL to another vessel prior to SPE.

15. Prepare a solid phase extraction (SPE) column containing 1000 mg $C_{18}$ by adding approximately 2 g anhydrous $MgSO_4$ to the top of the $C_{18}$ layer.

**Key Fact:**

*To save time during the analysis, prepare SPE columns containing $MgSO_4$ ahead of time and store in a desiccator.*

16. Using a positive pressure SPE manifold (PPM), condition the SPE cartridge with 5 mL of 1% acetic acid/acetonitrile and elute to waste, as shown in Figure 11.
17. Place properly labeled 15 mL graduated glass tubes in the collection rack below SPE cartridges.
18. Transfer the 10 mL of sample extract into the SPE column from the previous vessel and pass the extract through the column using a regulated flow pressure of not greater than 35 psi.

**Figure 11**: Solid phase extraction manifold. Photo courtesy of Ryan Matsuda USDA-FSIS

**Key Fact:**

*Be careful not to overfill the SPE columns.*

19. After the extract has completely passed through the column, add two aliquots of 2.5 mL of 1% acetic acid/acetonitrile to elute the sample from the column. (Change gas flow to full flow for approximately one minute to completely elute the extract from the column.)

**Optional Stopping Point:**

*This is an optional stopping point. If stopping overnight, samples should be capped and stored at ≤ -20 °C.*

20. As shown in Figure 12, concentrate each sample to less than 2 mL (final sample volume) under nitrogen in a 65 ± 5 °C water bath. Adjust all samples to 2 mL with acetonitrile.

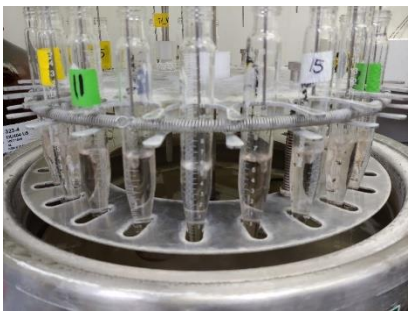

**Figure 12**: Samples undergoing evaporation. Photo courtesy of Ryan Matsuda USDA-FSIS

## Prepare Extract for UHPLC/MS/MS Analysis

1. Transfer 1 mL of the extract from step 20. to a 2 mL mini-centrifuge tube that contains 50 mg PSA (primary secondary amine) and 150 mg of $MgSO_4$.
2. Vortex the mini-centrifuge tubes for 1 min.
3. As shown in Figure 13, centrifuge the mini-centrifuge tubes for two min at 10,000 RCF.
4. Transfer the sample extract to a 3 mL plastic syringe with a 0.2 µm Nylon syringe filter and filter extract into a labeled autosampler vial. Analyze vial by UHPLC/MS/MS.

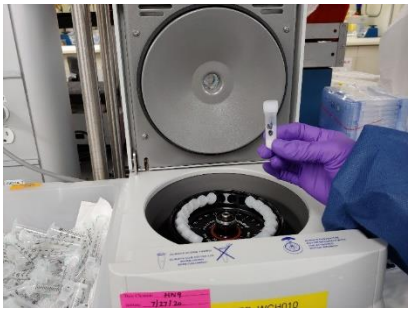

**Figure 13**: Samples undergoing centrifugation. Photo courtesy of Ryan Matsuda USDA-FSIS

## Prepare Extract for GC/MS/MS Analysis

1. Using a PPM, condition a 500 mg PSA SPE column with 4 mL of 3:1 v/v acetone/toluene and elute to waste.
2. Place properly labeled 15 mL graduated glass tubes in the collection rack below SPE columns.
3. Using a Pasteur pipette, transfer the remainder of the sample extract from step 20. to the SPE column, as illustrated in Figure 14.
4. Elute the extract through the column using a regulated flow pressure of not greater than 35 psi with 4 mL of 3:1 v/v acetone/toluene.

**Figure 14**: Samples undergoing SPE cleanup for GC-MS/MS Analysis. Photo courtesy of Ryan Matsuda USDA-FSIS

5. Collect the eluate while washing the SPE column two times with 4 mL of 3:1 v/v acetone/toluene (eluant). Do not allow the SPE column to go dry.
6. After the last 4 mL portion of eluant has passed through the column move the switch of the PPM from “Regulated Flow” to “Full Flow/Dry” and dry the column for one minute.
7. Evaporate the sample to less than 0.5 mL under nitrogen in a 65 ± 5 °C water bath, as shown in Figure 15.
8. Add 3 mL of toluene to centrifuge tube and vortex.
9. Evaporate again to less than 0.5 mL to ensure all other solvents have been removed.

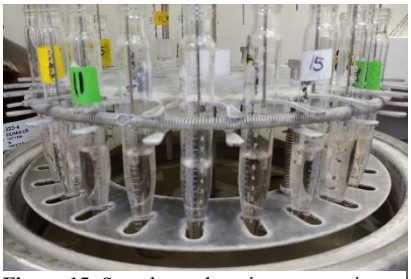

**Figure 15**: Samples undergoing evaporation for final toluene step. Photo courtesy of Ryan Matsuda USDA-FSIS

10. Bring the volume to 1.0 mL with toluene and vortex to mix.
11. Transfer the sample to a labeled autosampler vial. Analyze by GC/MS/MS.

## Instrumental Analysis

An example of a UHPLC/MS-MS instrument and a GC/MS-MS instrument are shown in Figure 16 and Figure 17, respectively.

### UHPLC/MS/MS Instrumental Settings

### Chromatographic Parameters

1) Mobile phases for pesticide analysis
   a) Mobile Phase A – 5 mM ammonium acetate/0.1% formic acid in water
   b) Mobile Phase B – 0.1% formic acid in methanol
2) Flow rate: 0.5 mL/min
3) Run time: 12 min
4) Gradient Program

**Table 13 UHPLC Gradient Program**

| Time (min) | % Mobile Phase A | % Mobile Phase B | Gradient |
|---|---|---|---|
| Initial | 90% | 10% | none |
| 0.25 | 90% | 10% | none |
| 7.75 | 2% | 98% | linear |
| 10.50 | 2% | 98% | linear |
| 10.55 | 90% | 10% | none |
| 12 | 90% | 10% | linear |

5) Autosampler program
   a) Run time: 12 min
   b) Injection needle: 15 µL
   c) Sample injection mode: Flow through needle
   d) Injection volume: 1 µL for muscle samples, 2 µL for egg samples
   e) Weak Wash: 10% methanol in water
   f) Strong Wash: 0.5% formic acid in 1:1:1:1 acetonitrile: methanol: isopropanol: water
6) Column manager
   a) Column valve position: To match column location
   b) Column manager temperature: 50 °C

**Instrumental Note:**

Autosampler parameters can be modified or optimized if needed to ensure that all chromatographic peaks are present.

**Mass Spectrometry Parameters**

1. Type: MS/MS
2. Electrospray Source Parameters
   a) Capillary (kV): 3.5
   b) Multiplier: - 640V
   c) Dwell time: varied from 0.025-0.2s
   d) Cone (V): Variable - analyte dependent
   e) Extractor (V): 3.0
   f) RF (V): 0.10
   g) Source Temperature (°C): 150
   h) Desolvation Temperature (°C):
   i) Cone Gas Flow (L/hr): 25
   j) Desolvation Gas Flow (L/hr):
   k) Collision Gas Flow (mL/min): 0.25
3. Analyzer Parameters
   a) LM1 Resolution 3.5
   b) HM 1 Resolution: 15
   c) Ion Energy 1: -0.8
   d) MSMS Mode Entrance: -5
   e) MSMS Mode Collision Energy: Variable – analyte dependent
   f) MSMS Mode Exit: 1
   g) LM 2 Resolution: 12.50
   h) HM 2 Resolution: 12.50
   i) Ion Energy 2: 0.2
4. MS Method Parameters:
   a) Type: MRM
   b) Ion Mode: ES+
   c) MRM Transitions:

**Instrumental Note:**

Mass spectrometry parameters are optimized and adjusted as needed during annual preventative maintenance and calibration.

**Figure 16**: Example of a UHPLC-MS/MS instrument. Photo courtesy of Ryan Matsuda USDA-FSIS

## Table 14 – LC MRM Transitions

| Cmpd # | Pesticide | RT (min) | Cone (V) | First transition (m/z) | Coll En (V) | Second transition (m/z) | Coll En (V) | Quant Ion |
|---|---|---|---|---|---|---|---|---|
| 1 | 3-Hydroxycarbofuran | 3.57 | 15 | 255.2 < 163 | 18 | 255.2 < 181 | 15 | 163 |
| 2 | Acephate | 1.53 | 20 | 184.1 < 125 | 16 | 184.1 < 143 | 12 | 143 |
| 3 | Acetamiprid | 3.57 | 40 | 223 < 56 | 16 | 223 < 126 | 16 | 126 |
| 4 | Alachlor | 6.61 | 27 | 269.8 < 161.9 | 19 | 269.8 < 237.8 | 11 | 237.8 |
| 5 | Aldicarb | 4.23 | 12 | 190.8 < 88.7 | 13 | 190.8 < 115.8 | 5 | 115.8 |
| 6 | Aldicarb sulfone | 2.16 | 23 | 223 < 76 | 7 | 223 < 86 | 12 | 86 |
| 7 | Aldicarb sulfoxide | 1.98 | 16 | 207 < 89 | 14 | 207 < 132 | 10 | 89 |
| 8 | Atrazine | 5.49 | 35 | 216.1 < 104 | 26 | 216.1 < 174.1 | 18 | 174.1 |
| 9 | Azinphos methyl | 5.82 | 22 | 317.7 < 124.8 | 35 | 317.7 < 131.9 | 30 | 131.9 |
| 10 | Azoxystrobin | 6.01 | 30 | 404.1 < 344.2 | 26 | 404.1 < 372.1 | 14 | 372.1 |
| 11 | Benoxacor | 5.86 | 22 | 259.7 < 133.8 | 29 | 259.7 < 148.9 | 17 | 148.9 |
| 12 | Boscalid | 6.17 | 22 | 342.8 < 271.3 | 33 | 342.8 < 306.7 | 19 | 306.7 |
| 13 | Buprofezin | 7.52 | 22 | 306 < 115.9 | 15 | 306 < 201 | 11 | 201 |
| 14 | Carbaryl | 5.04 | 20 | 202.2 < 127 | 28 | 202.2 < 145 | 15 | 145 |
| 15 | Carbofuran | 4.87 | 25 | 222.2 < 123 | 23 | 222.2 < 165 | 13 | 123 |
| 16 | Carfentrazone ethyl | 6.83 | 37 | 412 < 345.7 | 23 | 412 < 365.6 | 17 | 345.7 |
| 17 | Clothianidin | 3.23 | 25 | 250.1 < 132.1 | 29 | 250.1 < 168.6 | 15 | 168.6 |
| 18 | Coumaphos O | 5.91 | 45 | 347 < 211 | 34 | 347 < 291 | 22 | 291 |
| 19 | Coumaphos S | 6.95 | 40 | 363 < 227 | 24 | 363 < 307 | 16 | 227 |
| 20 | Desethylatrazine | 3.89 | 35 | 187.9 < 104 | 28 | 187.9 < 146 | 20 | 146 |
| 21 | Diazinon | 6.97 | 36 | 305.1 < 153.1 | 22 | 305.1 < 169.1 | 18 | 169.1 |
| 22 | Dichlorvos | 4.7 | 32 | 220.7 < 108.8 | 19 | 220.7 < 144.8 | 11 | 108.8 |
| 23 | Difenoconazole | 7.16 | 42 | 406 < 250.8 | 25 | 406 < 336.8 | 17 | 250.8 |
| 24 | Diflubenzuron | 6.65 | 23 | 311 < 141.1 | 32 | 311 < 158.2 | 15 | 158.2 |
| 25 | Dimethoate | 3.5 | 17 | 230 < 125 | 20 | 230 < 199 | 10 | 199 |
| 26 | Diuron | 5.6 | 25 | 233 < 72.1 | 15 | 233 < 160 | 28 | 72.1 |
| 27 | Ethion | 7.6 | 22 | 384.7 < 142.8 | 25 | 384.7 < 198.8 | 11 | 198.8 |
| 28 | Ethion monoxon | 6.71 | 27 | 368.7 < 170.7 | 17 | 368.7 < 198.8 | 11 | 198.8 |
| 29 | Ethofumesate | 6.01 | 13 | 304.1 < 121.1 | 20 | 304.1 < 161.2 | 25 | 121.1 |
| ISTD | Ethoprofos | 6.57 | 23 | 243.1 < 173 | 22 | | | 173 |
| 30 | Fenoxaprop ethyl | 7.43 | 12 | 361.9 < 243.7 | 25 | 361.9 < 287.7 | 19 | 287.7 |

| Cmpd # | Pesticide | RT (min) | Cone (V) | First transition (m/z) | Coll En (V) | Second transition (m/z) | Coll En (V) | Quant Ion |
|---|---|---|---|---|---|---|---|---|
| 31 | Fluridone | 5.9 | 22 | 330 < 258.9 | 45 | 330 < 309.2 | 33 | 309.2 |
| 32 | Fluroxypyr-1methylheptyl-ester | 7.76 | 20 | 367 < 209 | 22 | 367 < 255 | 10 | 255 |
| 33 | Fluvalinate | 8.14 | 27 | 502.8 < 180.7 | 27 | 502.8 < 207.8 | 13 | 207.8 |
| 34 | Hexazinone | 4.89 | 32 | 252.9 < 70.9 | 33 | 252.9 < 170.8 | 17 | 170.8 |
| 35 | Hexythiazox | 7.72 | 30 | 353 < 168.1 | 26 | 353 < 228.1 | 14 | 228.1 |
| 36 | Imazalil | 5.24 | 30 | 297 < 159 | 36 | 297 < 255 | 20 | 159 |
| 37 | Imidacloprid | 3.2 | 25 | 256.1 < 175 | 18 | 256.1 < 209 | 14 | 209 |
| 38 | Indoxacarb | 7.2 | 25 | 528 < 150.1 | 22 | 528 < 203.2 | 35 | 150.1 |
| 39 | Linuron | 5.99 | 28 | 249 < 160 | 18 | 249 < 182 | 17 | 160 |
| 40 | Malathion | 6.26 | 22 | 330.7 < 126.8 | 11 | 330.7 < 284.7 | 7 | 126.8 |
| 41 | Metalaxyl | 5.65 | 18 | 280.1 < 192.2 | 18 | 280.1 < 220.1 | 13 | 220.1 |
| 42 | Methamidophos | 1.21 | 22 | 142 < 94 | 14 | 142 < 125 | 13 | 94 |
| 43 | Methomyl | 2.51 | 13 | 163.1 < 88 | 9 | 163.1 < 106 | 9 | 88 |
| 44 | Methoxyfenozide | 6.32 | 15 | 369.1 < 91.1 | 47 | 369.1 < 149.2 | 18 | 149.2 |
| 45 | Metribuzin | 4.8 | 32 | 214.8 < 83.8 | 21 | 214.8 < 186.7 | 19 | 186.7 |
| 46 | Myclobutanil | 6.32 | 28 | 289.1 < 70.1 | 18 | 289.1 < 125.1 | 30 | 70.1 |
| 47 | Norflurazon | 5.66 | 30 | 304.1 < 160.1 | 40 | 304.1 < 284.1 | 32 | 284.1 |
| 48 | Omethoate | 1.79 | 20 | 214 < 155 | 14 | 214 < 183 | 12 | 183 |
| 49 | Piperonyl butoxide | 7.63 | 12 | 356 < 118.9 | 37 | 356 < 176.8 | 13 | 176.8 |
| 50 | Pirimiphos methyl | 7.08 | 12 | 305.9 < 107.8 | 33 | 305.9 < 163.9 | 21 | 107.8 |
| 51 | Prallethrin | 7.22 | 22 | 301.1 < 132.9 | 11 | 301.1 < 168.9 | 9 | 132.9 |
| 52 | Profenofos | 7.43 | 35 | 374.8 < 304.9 | 18 | 374.8 < 346.8 | 14 | 304.9 |
| 53 | Propachlor | 5.58 | 17 | 211.8 < 105.8 | 25 | 211.8 < 169.7 | 17 | 169.7 |
| 54 | Propanil | 6.01 | 32 | 217.8 < 126.8 | 27 | 217.8 < 161.7 | 15 | 161.7 |
| 55 | Propetamphos | 6.34 | 17 | 281.9 < 137.8 | 19 | 281.9 < 155.7 | 11 | 137.8 |
| 56 | Propiconazole | 6.95 | 27 | 341.8 < 68.9 | 21 | 341.8 < 158.8 | 27 | 68.9 |
| 57 | Pyraclostrobin | 7.01 | 40 | 387.8 < 163.7 | 12 | 387.8 < 194.1 | 10 | 194.1 |
| 58 | Pyrethrin I | 7.87 | 22 | 329.2 < 143 | 15 | 329.2 < 161 | 9 | 161 |
| 59 | Pyrethrin II | 7.25 | 27 | 373.2 < 133 | 17 | 373.2 < 161 | 11 | 161 |
| 60 | Pyridaben | 8.09 | 25 | 365.2 < 147.1 | 28 | 365.2 < 309 | 13 | 147.1 |
| 61 | Pyriproxyfen | 7.62 | 12 | 322 < 95.8 | 15 | 322 < 184.8 | 23 | 95.8 |
| 62 | Resmethrin | 8.15 | 12 | 356.2 < 127.9 | 41 | 356.2 < 170.8 | 15 | 170.8 |

| Cmpd # | Pesticide | RT (min) | Cone (V) | First transition (m/z) | Coll En (V) | Second transition (m/z) | Coll En (V) | Quant Ion |
|---|---|---|---|---|---|---|---|---|
| 63 | Simazine | 4.85 | 35 | 202 < 124.1 | 20 | 202 < 132 | 20 | 132 |
| 64 | Sulprofos | 7.72 | 27 | 322.9 < 218.7 | 17 | 322.9 < 246.8 | 13 | 218.7 |
| 65 | Tebufenozide | 6.73 | 12 | 353.1 < 105 | 50 | 353.1 < 133.1 | 22 | 133.1 |
| 66 | Tetrachlorvinphos | 6.77 | 27 | 366.5 < 126.7 | 17 | 366.5 < 240.6 | 17 | 126.7 |
| 67 | Tetraconazole | 6.5 | 37 | 371.9 < 69.8 | 23 | 371.9 < 158.7 | 33 | 158.7 |
| 68 | Thiabendazole | 3.25 | 45 | 202.1 < 131 | 33 | 202.1 < 175 | 24 | 175 |
| 69 | Thiamethoxam | 2.63 | 23 | 292 < 181 | 18 | 292 < 211 | 13 | 211 |
| 70 | Thiobencarb | 7.1 | 25 | 257.9 < 100.1 | 10 | 257.9 < 125.1 | 20 | 125.1 |
| 71 | Trifloxystrobin | 7.24 | 25 | 409 < 145 | 44 | 409 < 186 | 20 | 186 |

## GC/MS/MS Instrumental Settings

### GC Chromatographic Parameters

**Instrumental Note:**

GC chromatographic parameters are optimized and adjusted as needed during annual preventative maintenance and calibration.

1) Carrier Gas: Helium
2) Column 1 Flow Rate: 1.4 mL/min
3) Column 2 Flow Rate: 1.2 mL/min
4) Injector temperature: 280 ºC
5) Injection volume: 1 µL for muscle samples, 2 µL for egg
6) Injection Mode: splitless
7) Temperature program
   a) Initial temp: 60 ºC
   b) Initial hold time: 1 min
   c) Program rate up to 120 ºC: 40 ºC/min
   d) Program rate up to 292: 5 ºC/min
   e) Post-run time: 2 min
   f) Total Run time: 36.9 min

**Figure 17**: Example of a GC-MS/MS instrument. Photo courtesy of Ryan Matsuda USDA-FSIS

## GC Mass Spectrometry Parameters

1) Ionization: Positive Electron Impact
2) Detector EMV: 1352 V
3) Collision Gas: Nitrogen @ 1.5 mL/Min
4) Collision Energy: Optimized for each compound.
5) MS Source temperature: 300 ºC
6) Transfer line temperature: 300 ºC
7) Solvent delay: 7.0 min
8) Autotune the instrument as needed.
9) MRM Transitions

**Instrumental Note:**

GC mass spectrometer parameters are optimized and adjusted as needed during annual preventative maintenance and calibration.

**Table 15 – GC MRM Transitions**

| Cmpd # | Pesticide | RT (min) | First transition (m/z) | Coll En (V) | Second transition (m/z) | Coll En (V) | Third transition (m/z) | Coll En (V) | Quant Ion |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1-Naphthol | 9.479 | 144 > 115 | 25 | 115 > 89 | 20 | | | 115 |
| 2 | Aldrin | 19.54 | 263 > 193 | 55 | 263 > 228 | 35 | 263 > 191 | 55 | 193 |
| 3 | Bifenthrin | 31.76 | 181 > 165 | 10 | 181 > 166 | 20 | 165 > 115 | 40 | 165 |
| 4 | Chlordane cis | 23.77 | 373 > 266 | 25 | 373 > 337 | 20 | 373 > 264 | 25 | 266 |
| 5 | Chlordane trans | 23.08 | 373 > 266 | 15 | 373 > 337 | 10 | 373 > 264 | 20 | 265.9 |
| 6 | Chloroneb | 9.101 | 191 > 113 | 15 | 191 > 141 | 10 | | | 113 |
| 7 | Chlorothalonil | 15.34 | 266 > 133 | 30 | 266 > 168 | 60 | 266 > 231 | 20 | 132.9 |
| 8 | Chlorpropham | 11.88 | 213 > 127 | 20 | 213 > 171 | 5 | | | 127 |
| 9 | Chlorpyrifos | 19.57 | 316 > 260 | 15 | 314 > 166 | 40 | 314 > 286 | 5 | 260 |
| 10 | Chlorpyrifos methyl | 17.16 | 286 > 93 | 35 | 286 > 271 | 35 | 286 > 208 | 25 | 93 |
| 11 | DDD o,p' | 25.57 | 237 > 165 | 20 | 235 > 199 | 10 | 199 > 164 | 20 | 165 |
| 12 | DDD p,p' + DDT o,p' | 27.66 | 235 > 165 | 20 | 199 > 164 | 20 | 235 > 199 | 15 | 165 |
| 13 | DDE o,p' | 23.32 | 246 > 176 | 30 | 318 > 248 | 15 | 318 > 246 | 15 | 176 |
| 14 | DDE p,p' | 25.12 | 246 > 176 | 30 | 318 > 248 | 15 | 318 > 246 | 15 | 176 |
| 15 | DDT p,p' | 29.71 | 235 > 165 | 20 | 235 > 199 | 15 | 199 > 164 | 15 | 165 |

| Cmpd # | Pesticide | RT (min) | First transition (m/z) | Coll En (V) | Second transition (m/z) | Coll En (V) | Third transition (m/z) | Coll En (V) | Quant Ion |
|---|---|---|---|---|---|---|---|---|---|
| 16 | Dieldrin | 25.32 | 277 > 241 | 5 | 263 > 193 | 60 | 272 > 237 | 10 | 241 |
| 17 | Endosulfan I | 23.75 | 241 > 206 | 20 | 339 > 160 | 20 | | | 205.9 |
| 18 | Endosulfan II | 27.32 | 241 > 206 | 20 | 339 > 160 | 20 | | | 206 |
| 19 | Endosulfan sulfate | 29.58 | 272 > 237 | 15 | 272 > 235 | 30 | 272 > 143 | 30 | 237 |
| 20 | Fenpropathrin | 32.1 | 181 > 152 | 25 | 265 > 210 | 10 | | | 152 |
| 21 | Fipronil | 22.12 | 367 > 213 | 60 | 367 > 255 | 35 | | | 213 |
| 22 | Fipronil desulfinyl | 17.74 | 388 > 333 | 20 | 333 > 231 | 60 | | | 333 |
| 23 | Fipronil sulfide | 21.59 | 351 > 255 | 20 | 420 > 351 | 10 | | | 255 |
| 24 | Heptachlor | 17.7 | 272 > 237 | 15 | 337 > 266 | 15 | | | 237 |
| 25 | Heptachlor epoxide (cis&trans) or (B+A) | 22.04 | 183 > 119 | 25 | 272 > 237 | 20 | 353 > 282 | 25 | 119 |
| 26 | Hexachlorobenzene (HCB) | 12.92 | 284 > 249 | 15 | 282>247 | 60 | 250 > 142 | 45 | 249 |
| 27 | Lindane (BHC gamma) | 14.36 | 181 > 145 | 15 | 219 > 183 | 5 | 219 > 109 | 35 | 145 |
| 28 | MGK – 264 1 | 20.8 | 164 > 98 | 10 | 164 > 67 | 5 | 164 > 80 | 35 | 98 |
| | MGK – 264 2 | 21.54 | 164 > 67 | 15 | 164 > 98 | 10 | 164 > 80 | 35 | 67 |
| 29 | Metolachlor | 19.46 | 238 > 162 | 10 | 162 > 133 | 15 | | | 162 |
| 30 | Nonachlor cis | 27.46 | 409 > 109 | 15 | 409 > 302 | 20 | | | 108.9 |
| 31 | Nonachlor trans | 23.9 | 409 > 302 | 25 | 409 > 109 | 40 | 409 > 263 | 40 | 302 |
| 32 | Oxychlordane | 21.69 | 187 > 123 | 10 | 187 > 85 | 30 | | | 123 |
| 33 | Pentachloroaniline (PCA) | 16.54 | 265 > 192 | 25 | 265 > 228 | 35 | | | 192 |
| 34 | Pentachlorobenzene (PCB) | 9.348 | 250 > 142 | 35 | 250 > 179 | 30 | | | 142 |
| 35 | Permethrin (cis&trans) | 34.21 | 183 > 153 | 15 | 183 > 165 | 10 | 183 > 127 | 45 | 153 |
| 36 | Pronamide | 14.71 | 173 > 145 | 15 | 173 > 109 | 55 | | | 145 |
| 37 | Tefluthrin | 15.35 | 177 > 127 | 15 | 177 > 137 | 20 | 177 > 87 | 60 | 127 |
| ISTD | Trichloronate | 20.38 | 297 > 269 | 10 | 299 > 271 | 10 | | | 269 |

**Instrumental Note:**

Retention time windows, collision energies, and selected masses for precursor and product ions were set and utilized at time of method validation.

- Retention time windows may be adjusted to account for aging of UHPLC or GC columns or for improved separation to ensure that all chromatographic peaks are present.
- Collision energies may be adjusted and optimized for improved mass spectrometry detection.
- Target masses for precursor and product ions can be optimized to a *m/z* value that falls within the unit mass resolution of the exact mass, but not to exceed the next integer value (e.g., if the exact mass is 787.5, an allowable target mass range includes 787.0-787.9).

**Sample Set**

The injection sequence below can be modified, as needed, but must include required controls.

System Suitability is to be demonstrated prior to sample set injection.

1) Injection Standard
2) Decision Level
3) Positive Control (Recovery)
4) Solvent Blank
5) Negative Control (Blank)
6) Intra-Laboratory Check Sample (if applicable)
7) Samples, up to a maximum of 18
8) Re-injection of the positive control (recovery) (for system suitability)

**INTRA-LABORATORY CHECK SAMPLE**

Defined on the CLG website here.

## Reporting of Results

### Decision Criteria

Screening

1) The quantitative ion and all other ions listed for the analyte in Table 14 and 15 must be present.
2) All ions must have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection.
3) The internal standard response for the sample must be > 50% of the internal standard response of the recovery (positive control). If the internal standard response of the sample exceeds 200% of the internal standard response of the recovery (positive control), that sample will be investigated.
4) Retention time for the recovery and samples must match the retention time of the decision level recovery within ±5% for LC, ±0.5% for 1-naphthol and chloroneb, ±1% for all other single peak GC compounds, and ±5% for multipeak compounds for GC.
5) All quantitative ion peak areas in the blank must be < 10% of the decision level recovery.

6) The sample is screen positive if the following criteria are met:
   a) The fortified recovery of the analyte must exceed 10% of the decision level recovery.
   b) The sample response equals or exceeds the recovery level.

## QUALITY CONTROL

**Quality Control Procedures**

1) For set acceptance, 95% (for LC and for GC) of the monitored analytes in the recovery (positive control) must meet screening criteria. For sample reporting purposes, screen positive analytes must meet screening criteria in the recovery (positive control), or else further testing is warranted.
2) For set acceptance, 95% (for LC and for GC) of the monitored analytes in the blank (negative control) must not meet the screening criteria. The blank (negative control) must be negative using the criteria in screening criteria.for samples containing corresponding presumptive positive analytes.
3) The internal standard response for the recovery (positive control) and blank (negative control) must be 50-150% of the internal standard response of the decision level.

**Intra-laboratory Check Samples (If applicable)**

1) Acceptability criteria.
   a. 95% of the monitored analytes in a fortified Intra-Laboratory Check must meet screening criteria.
   b. 95% of the monitored analytes in an unfortified Intra-Laboratory Check must be negative using the screening criteria.
   c. FSIS Field Service Laboratories are to refer to internal FSIS Quality Control Procedures when unacceptable values are obtained:
      i. Refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.

**Calculations**

1) Relative Response Factor (RRF)

   This is the internal standard corrected analyte response.

   $A = B / C$

   where

   A = Relative Response Factor (unitless)

   B = Quant Ion Peak Area of Analyte (counts)

   C = Quant Ion Peak Area of Internal Standard (counts)

2) Estimated Amount Found

   This is a quantitative estimate calculated for comparison to the MLA. It is based on a one-point calibration with the recovery (positive control) as the reference. Program the MS instruments to automatically calculate this.

D = E * A sample / A pos. ctrl.

where

D = Estimated Amount Found in the Sample (ppb)

E = Recovery (positive control) Fortification Level (ppb)

A sample = Relative Response Factor in the Sample (unitless)

A pos. ctrl. = Relative Response Factor in the recovery (positive control) (unitless)

## Minimum Level of Applicability

### Table 16 - Minimum Level of Applicability for Screening Level per species

| # | Pesticides | Porcine (ppb) | Bovine (ppb) | Poultry (ppb) | Ovine (ppb) | Caprine (ppb) | Equine (ppb) | Catfish (ppb) | Liquid Eggs (ppb) | Powder Eggs (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| LC Compounds | | | | | | | | | | |
| Carbamates | | | | | | | | | | |
| 1 | 3-Hydroxycarbofuran | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 2 | Aldicarb | 10 | 10 | 10 | 10 | 10 | 10 | 10 | N/App | N/App |
| 3 | Aldicarb sulfone | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 4 | Aldicarb sulfoxide | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 5 | Carbaryl | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 6 | Carbofuran | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 7 | Methomyl | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 60 |
| 8 | Thiobencarb | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| Conazole / Triazole | | | | | | | | | | |
| 9 | Difenoconazole | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 30 |
| 10 | Myclobutanil | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 11 | Propiconazole | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 30 |
| 12 | Tetraconazole | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| Halogenated Pesticides | | | | | | | | | | |
| 13 | Alachlor | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 14 | Boscalid | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 30 |
| 15 | Carfentrazone ethyl | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 16 | Diflubenzuron | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 25 |
| 17 | Linuron | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 18 | Norflurazon | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 19 | Propachlor | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 20 | Propanil | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| Neonicotinoids | | | | | | | | | | |
| 21 | Acetamiprid | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |

| # | Pesticides | Porcine (ppb) | Bovine (ppb) | Poultry (ppb) | Ovine (ppb) | Caprine (ppb) | Equine (ppb) | Catfish (ppb) | Liquid Eggs (ppb) | Powder Eggs (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| 22 | Clothianidin | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 23 | Imidacloprid | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 24 | Thiamethoxam | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| | Organophosphates | | | | | | | | | |
| 25 | Acephate | 10 | 10 | 10 | 10 | 10 | 10 | 10 | N/App | N/App |
| 26 | Azinphos methyl | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 27 | Coumaphos O | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 28 | Coumaphos S | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 29 | Diazinon | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 30 | Dichlorvos (DDVP) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 31 | Dimethoate | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 32 | Ethion | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 33 | Ethion monoxon | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 34 | Malathion | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 80 |
| 35 | Methamidophos | 10 | 10 | 10 | 10 | 10 | 10 | 10 | N/App | N/App |
| 36 | Omethoate | 10 | 10 | 10 | 10 | 10 | 10 | 10 | N/App | N/App |
| 37 | Pirimiphos methyl | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 38 | Profenofos | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 39 | Propetamphos | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 15 |
| 40 | Sulprofos | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 41 | Tetrachlorvinphos | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| | General Pesticides | | | | | | | | | |
| 42 | Azoxystrobin | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 43 | Benoxacor | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 44 | Buprofezin | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 45 | Diuron | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 160 |
| 46 | Ethofumesate | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 40 |
| 47 | Fenoxaprop ethyl | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 48 | Fluridone | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 49 | Fluroxypyr-1-Methylheptyl-Ester | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 50 | Hexazinone | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 60 |
| 51 | Hexythiazox | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 52 | Imazalil | 5 | 5 | 5 | 5 | 5 | 5 | 5 | N/App | N/App |
| 53 | Indoxacarb | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |

| # | Pesticides | Porcine (ppb) | Bovine (ppb) | Poultry (ppb) | Ovine (ppb) | Caprine (ppb) | Equine (ppb) | Catfish (ppb) | Liquid Eggs (ppb) | Powder Eggs (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| 54 | Metalaxyl | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 55 | Methoxyfenozide | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 56 | Metribuzin | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| 57 | Piperonyl butoxide | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 45 |
| 58 | Pyraclostrobin | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| 59 | Pyridaben | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 18 |
| 60 | Pyriproxyfen | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 40 |
| 61 | Tebufenozide | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 80 |
| 62 | Thiabendazole | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 30 |
| 63 | Trifloxystrobin | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| | Pyrethroids | | | | | | | | | |
| 64 | Fluvalinate | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 15 |
| 65 | Prallethrin | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 80 |
| 66 | Pyrethrin I | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 56 |
| 67 | Pyrethrin II | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 62 |
| 68 | Resmethrin (cis&trans) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| | Triazine | | | | | | | | | |
| 69 | Atrazine | 10 | 10 | 10 | 10 | 10 | 10 | 10 | N/App | N/App |
| 70 | Desethylatrazine | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 71 | Simazine | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| | GC Compounds | | | | | | | | | |
| | Carbamate | | | | | | | | | |
| 1 | Chlorpropham | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 60 |
| | Halogenated | | | | | | | | | |
| 2 | Pronamide | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| | Organochlorine | | | | | | | | | |
| 3 | Aldrin | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 4 | Chlordane cis | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 5 | Chlordane trans | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 6 | DDD o,p’ | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| 7 | DDD p,p’ + DDT, o,p' | 50 +50 | 50+50 | 50+50 | 50+50 | 50+50 | 50+50 | 50+50 | 50+50 | 100 + 100 |
| 8 | DDE o,p’ | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| 9 | DDE p,p’ | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |

| # | Pesticides | Porcine (ppb) | Bovine (ppb) | Poultry (ppb) | Ovine (ppb) | Caprine (ppb) | Equine (ppb) | Catfish (ppb) | Liquid Eggs (ppb) | Powder Eggs (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | DDT p,p' | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| 11 | Dieldrin | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 12 | Endosulfan I | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| 13 | Endosulfan II | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| 14 | Endosulfan sulfate | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| 15 | Heptachlor | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 16 | Heptachlor epoxide (cis+ trans) or (B+A) | 25+25 | 25+25 | 25+25 | 25+25 | 25+25 | 25+25 | 25+25 | 25+25 | 50+50 |
| 17 | Hexachlorobenzene (HCB) | 25 | 25 | 25 | 25 | 25 | 25 | 25 | N/App | N/App |
| 18 | Lindane (BHC gamma) | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 80 |
| 19 | Nonachlor cis | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 30 |
| 20 | Nonachlor trans | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 30 |
| 21 | Oxychlordane | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 22 | Pentachlorobenzene (PCB) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| | Organophosphate | | | | | | | | | |
| 23 | Chlorpyrifos | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 15 |
| 24 | Chlorpyrifos methyl | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| | General Pesticides | | | | | | | | | |
| 25 | 1-Naphthol | 30 | 30 | 30 | 30 | 30 | 30 | 30 | N/App | N/App |
| 26 | Fipronil | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 27 | Fipronil desulfinyl | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 28 | Fipronil sulfide | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| 29 | Metolachlor | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 |
| 30 | MGK-264 (isomers 1 & 2) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 |
| | Pyrethroids | | | | | | | | | |
| 31 | Bifenthrin | 5 | 5 | 5 | N/App | 5 | 5 | N/App | 5 | 10 |
| 32 | Fenpropathrin | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |
| 33 | Permethrin (cis&trans) | 25 | 25 | 25 | 25 | 25 | 25 | N/App | N/App | N/App |
| 34 | Tefluthrin | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 10 |
| | Substituted Benzenes | | | | | | | | | |
| 35 | Chloroneb | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 18 |
| 36 | Chlorothalonil | 60 | 60 | 60 | 60 | 60 | 60 | 60 | N/App | 120 |

| # | Pesticides | Porcine (ppb) | Bovine (ppb) | Poultry (ppb) | Ovine (ppb) | Caprine (ppb) | Equine (ppb) | Catfish (ppb) | Liquid Eggs (ppb) | Powder Eggs (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| 37 | Pentachloroaniline (PCA) | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 50 |

## References

The Environmental Protection Agency (EPA) regulates the approval and use of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act.

21CFR 556 for Tolerance values set by FDA.

## Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

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*This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.*

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