

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

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Title: Screening for Pesticides by LC/MS/MS and GC/MS/MS		
Revision: .08	Replaces: CLG-PST5.07	Effective: 02/26/2018

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A. INTRODUCTION

1. Summary of Procedure

Various classes of pesticides are extracted from muscle tissue with high speed dispersion in ethyl acetate followed by solvent exchange to acetonitrile and clean-up using ultra-low temperature freezing, centrifugation, and solid phase extraction. Detection of pesticide residues is performed by gas chromatography with tandem mass spectrometry (GC/MS/MS) and liquid chromatography with tandem mass spectrometry (LC/MS/MS).

2. Applicability

This method is suitable for screening the listed pesticides in bovine, caprine, equine, ovine, porcine, poultry, fish of the order Siluriformes (catfish) muscle, liquid egg products, and powdered egg products at levels \geq those listed in Appendix J.3.

Note: Refer to 21CFR for tolerance values set by FDA and 40CFR for tolerance values set by EPA.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus

- a. Food processor - Robot Coupe model RS16Y-1, Robot Coupe USA Inc.
- b. Sample cups - eValue 4.5 oz specimen containers w/caps, Cat. No. C686550, E&K Scientific.
- c. Analytical Balance - Readable to 0.20 g, Model 2000, Mettler.
- d. 50 mL centrifuge tubes - Cat. 62-548-004 PP, Sarstedt, Inc.
- e. Shaker - Model E6010.00, Eberbach Corp.
- f. Freezer capable of -20 °C - Isotemp Freezer, Cat. No. 13-986-149, Fisher Scientific.
- g. Centrifuge - Sorvall RC-4, Thermo Scientific.
- h. Micro Centrifuge - Micro Centrifuge 5424, Cat. No. 22620461, Eppendorf.
- i. Nitrogen Evaporator Apparatus with Heated Water Bath - N-Evap, Cat. No. 11250, Organomation.
- j. Multi Tube Vortex - VWR Signature Multi Tube Vortexer, Cat. No. 14005-826, VWR International.
- k. Freezer capable of -70 °C - Isotemp Freezer Ultra-Low Temperature, Cat. No. 13-990-14, Fisher Scientific.

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- l. Positive Pressure Manifold - Cat. No. VMFPPM16, UCT, LLC.
 - m. 50 mL glass centrifuge tubes - Pyrex Centrifuge Tubes w/ stopper, Cat. No. CLS808450-12EA, Sigma-Aldrich.
 - n. 15 mL glass centrifuge tubes - Kimax Centrifuge Tube w/ glass stopper, Cat. No. 89002-1984, VWR International.
 - o. 1000 mg C18 SPE Columns - Cat No. CEC181M6, UCT LLC.
 - p. Filter paper - Whatman #4, Cat. No. 28460-120, VWR International.
 - q. 0.2 µm Nylon Syringe Filter - Cat. No. 28143-242, VWR International.
 - r. Micro centrifuge tubes (150 mg MgSO₄ & 50 mg PSA) - QuEChERS micro centrifuge tubes (150 mg MgSO₄ & 50 mg PSA), Cat. No. CUMPS2CT, UCT LLC. 6 mL SPE cartridge (500 mg PSA) - Cat. No. CUPSA156, UCT LLC.
 - s. Variable volume dispensers - VWR Digital Easy Calibration Dispenser, Cat. No. 18901-130, VWR International.
 - t. Variable volume pipettors capable of accurately delivering 100 - 2500 µL – Eppendorf.
 - u. Eppendorf adjustable volume pipettor, 500-2500 µL - Cat. No. 022470353, Fisher.
 - v. Disposable Pasteur Pipettes - Cat. No. 13-678-20D, Fisher.
 - w. 3 mL Plastic Syringe - Luer Lok Plastic Disposable Syringes, Becton Dickinson, Cat. No. 301073, VWR International.
 - x. Glass Autosampler Vials & Caps - 2 mL, Cat. No. E251036, caps with septa Cat. No. E416209, E&K Scientific.
 - y. Glass Volumetric Flasks - Class A.
 - z. Graduated cylinders - Class A.
2. Instrumentation
- a. Waters UPLC Acquity Xevo TQ Detector.
 - b. Waters Acquity UPLC HSS/T3, 1.8 µm particle size, 2.1 x 100 mm column, part # 186003539.
 - c. VanGuard HSS T3 pre-column, 1.8 µm particle size, 2.1 x 5mm, part # 186003976.
 - d. Agilent 7890B GC equipped with Agilent 7010 triple quadrupole mass spectrometer. Agilent MassHunter Workstation Software.
 - e. Agilent J&W HP-5ms Ultra Inert GC Column, 15 m, 0.25 mm, 0.25 µm, 7 inch cage, part # 19091S-431UI. Two columns are used in series.

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C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted. The maximum length of time that a working reagent shall be used is 1 year unless the laboratory has produced extension data.

1. Reagents

- a. QuEChERS Salts Packets (8g MgSO₄ & 2 g NaCl) - Cat. No. ECQUVIN50CT-MP, UCT.
- b. Magnesium sulfate, anhydrous - Cat. No. BDH0246-500G, VWR International.
- c. Acetic acid, ACS grade - Cat. No. EM-AX0073-75, VWR International.
- d. Ethyl acetate, HPLC grade - Cat. No. BJLP100-4, VWR International.
- e. Acetonitrile, HPLC grade - Cat. No. BJLP014-4, VWR International.
- f. Acetone, HPLC grade - Cat. No. BJ010-4, VWR International.
- g. Toluene, HPLC grade - Cat. No. BJLP347-4, VWR International.
- h. Methanol, HPLC grade - Cat. No. BJLP230-4, VWR International.
- i. Ammonium acetate - Cat. No. BDH0204-500G, VWR International.
- j. Formic acid, ACS grade - Cat. No. EM-FX0440-7, VWR International.
- k. Water, HPLC grade - Millipore water (deionized distilled).
- l. Isopropanol, Analytical grade – Cat. No. 323-4, Honeywell Burdick & Jackson.

2. Solutions

- a. 1% Acetic acid/Acetonitrile (by volume)
Using a class A graduated cylinder, measure 20 mL of acetic acid and 1980 mL of acetonitrile into a two liter bottle and mix well. Solution expires one year from preparation date.
- b. LC/MS/MS Mobile Phase A (5 mM ammonium acetate/0.1% formic acid in water by volume)
Dissolve 0.771 g ammonium acetate in a small amount of water and pour into a two liter class A graduated cylinder, add 2 mL of formic acid and bring to volume with water and mix well. Solution expires one year from preparation date.
- c. LC/MS/MS Mobile Phase B (0.1% formic acid in methanol by volume)
Using a class A graduated cylinder measure 2 mL of formic acid into a class A two liter graduated cylinder and bring to volume with methanol and mix well. Solution expires one year from preparation date.

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d. 3:1 v/v Acetone/Toluene

Using a class A graduated cylinder measure 1500 mL of acetone and 500 mL of toluene into a two liter bottle and mix well. Solution expires one year from preparation date.

a. LC/MS/MS Weak Wash (10% methanol in water by volume)

Using a class A graduated cylinder measure 100 mL of methanol and pour into a class A one liter volumetric flask. Fill to line with water and mix well. Solution expires one year from preparation date.

b. LC/MS/MS Strong Wash (0.5% formic acid in 1:1:1:1 acetonitrile : methanol : isopropanol : water)

Using class A graduated cylinders measure 250 mL of each solvent and pour into a one liter bottle. Pipet 0.5 mL formic acid into the bottle and mix well. Solution expires one year from preparation date.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. Purity and counter ions are to be taken into account when calculating standard concentrations. In-house prepared standards shall be assigned an expiration date that is no later than the stability stated in the method. The maximum length of time that an in-house prepared standard shall be used is 1 year unless the laboratory has produced extension data.

1. Standard Information

- a. Trichloronate, 1,000 µg/mL in Ethyl acetate – Accustandard
- b. Ethoprophos, 500 µg/mL in Ethyl acetate – Accustandard
- c. GC Mixed Pesticide Standard - Accustandard

Table 1 – Example GC Mixed Pesticide Standard composition

Cmpd #	Name	CAS #	Spiking Solution (D.2.b) Conc. (µg/mL ethyl acetate)	Stock Solution Conc. (µg/mL ethyl acetate)
1	1-Naphthol	90-15-3	6	60
2	Aldrin	309-00-2	5	50
3	Bifenthrin	82657-04-3	1	10
4	Chlordane cis	5103-71-9	2	20
5	Chlordane trans	5103-74-2	2	20

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Cmpd #	Name	CAS #	Spiking Solution (D.2.b) Conc. (µg/mL ethyl acetate)	Stock Solution Conc. (µg/mL ethyl acetate)
6	Chloroneb	2675-77-6	1.8	18
7	Chlorothalonil	1897-45-6	12	120
8	Chlorpropham	101-21-3	6	60
9	Chlorpyrifos	2921-88-2	1.5	15
10	Chlorpyrifos methyl	5598-13-0	1	10
11	DDD o,p'	53-19-0	10	100
12	DDD p,p' + DDT o,p'	72-54-8 & 789-02-6	10+10	100+100
13	DDE o,p'	3424-82-6	10	100
14	DDE p,p'	72-55-9	10	100
15	DDT p,p'	50-29-3	10	100
16	Dieldrin	60-57-1	5	50
17	Endosulfan I	959-98-8	10	100
18	Endosulfan II	33213-65-9	10	100
19	Endosulfan sulfate	1031-07-8	10	100
20	Fenpropathrin	39515-41-8	5	50
21	Fipronil	120068-37-3	1	10
22	Fipronil desulfinyl	205650-65-3	1	10
23	Fipronil sulfide	120067-83-6	1	10
24	Heptachlor	76-44-8	5	50
25	Heptachlor epoxide (cis&trans) or (B+A)	1024-57-3 & 28044-83-9	5+5	50+50
26	Hexachlorobenzene (HCB)	118-74-1	5	50
27	Lindane (BHC gamma)	58-89-9	8	80
28	MGK-264 (isomers 1&2)	113-48-4	10	100
29	Metolachlor	51218-45-2	2	20
30	Nonachlor cis	5103-73-1	3	30
31	Nonachlor trans	39765-80-5	3	30
32	Oxychlorane	27304-13-8	2	20
33	Pentachloroaniline (PCA)	527-20-8	5	50
34	Pentachlorobenzene	608-93-5	2	20

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Cmpd #	Name	CAS #	Spiking Solution (D.2.b) Conc. (µg/mL ethyl acetate)	Stock Solution Conc. (µg/mL ethyl acetate)
	(PCB)			
35	Permethrin (cis&trans)	52645-53-1	5	50
36	Pronamide	23950-58-5	1	10
37	Tefluthrin	79538-32-2	1	10

a. LC Mixed Pesticide Standard- Accustandard

Table 2- Example LC Mixed Pesticide Standard composition

Cmpd #	Name	CAS #	Spiking Solution (D.2.b) Conc. (µg/mL ethyl acetate)	Stock Solution Conc. (µg/mL ethyl acetate)
38	3-Hydroxycarbofuran	16655-82-6	1	10
39	Acephate	30560-19-1	2	20
40	Acetamiprid	135410-20-7	1	10
41	Alachlor	15972-60-8	1	10
42	Aldicarb	116-06-3	2	20
43	Aldicarb sulfone	1646-88-4	2	20
44	Aldicarb sulfoxide	1646-87-3	5	50
45	Atrazine	1912-24-9	2	20
46	Azinphos methyl	86-50-0	2	20
47	Azoxystrobin	131860-33-8	1	10
48	Benoxacor	98730-04-02	1	10
49	Boscalid	188425-85-6	3	30
50	Buprofezin	69327-76-0	5	50
51	Carbaryl	63-25-2	5	50
52	Carbofuran	1563-66-2	1	10
53	Carfentrazone ethyl	128639-02-1	1	10
54	Clothianidin	210880-92-5	2	20
55	Coumaphos O	321-54-0	2	20
56	Coumaphos S	56-72-4	2	20

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Cmpd #	Name	CAS #	Spiking Solution (D.2.b) Conc. (µg/mL ethyl acetate)	Stock Solution Conc. (µg/mL ethyl acetate)
57	Deethylatrazine	6190-65-4	2	20
58	Diazinon	333-41-5	1	10
59	Dichlorvos (DDVP)	62-73-7	2	20
60	Difenoconazole	119446	3	30
61	Diflubenzuron	35367-38-5	2.5	25
62	Dimethoate	60-51-5	2	20
63	Diuron	330-54-1	16	160
64	Ethion	563-12-12	2	20
65	Ethion monoxon	17356-42-2	2	20
66	Ethofumesate	26225-79-6	4	40
67	Fenoxaprop ethyl	66441-23-4	2	20
68	Fluridone	59756-60-4	5	50
69	Fluroxypyr-1-Methylheptyl-Ester	81406-37-3	1	10
70	Fluvalinate	102851-06-9	1.5	15
71	Hexazinone	51235-04-2	6	60
72	Hexythiazox	78587-05-0	2	20
73	Imazalil	35554-44-0	1	10
74	Imidacloprid	138261-41-3	5	50
75	Indoxacarb	144171-61-9	5	50
76	Linuron	330-55-2	5	50
77	Malathion	121-75-5	8	80
78	Metalaxyl	57837-19-1	2	20
79	Methamidophos	10265-92-6	2	20
80	Methomyl	16752-77-5	6	60
81	Methoxyfenozide	161050-58-4	1	10
82	Metribuzin	21087-64-9	10	100
83	Myclobutanil	88671-89-0	2	20
84	Norflurazon	27314-13-2	2	20
85	Omethoate	1113-02-6	2	20
86	Piperonyl butoxide	51-03-6	4.5	45
87	Pirimiphos methyl	29232-93-7	2	20
88	Prallethrin	23031-36-9	8	80
89	Profenofos	41198-08-7	2	20

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Cmpd #	Name	CAS #	Spiking Solution (D.2.b) Conc. (µg/mL ethyl acetate)	Stock Solution Conc. (µg/mL ethyl acetate)
90	Propachlor	1918-16-7	2	20
91	Propanil	709-98-8	5	50
92	Propetamphos	31218-83-4	1.5	15
93	Propiconazole	60207-90-1	3	30
94	Pyraclostrobin	175013-18-0	10	100
95	Pyrethrin I	8003-34-7	9.2	92
96	Pyrethrin II	8003-34-7	6.2	62
97	Pyridaben	96489-71-3	1.8	18
98	Pyriproxyfen	95737-68-1	4	40
99	Resmethrin (cis& trans)	10453-86-8	10	100
100	Simazine	122-34-9	2	20
101	Sulprofos	34500-43-2	5	50
102	Tebufenozide	112410-23-8	8	80
103	Tetrachlorvinphos	22248-79-9	2	20
104	Tetraconazole	11281-77-3	1	10
105	Thiabendazole	148-79-8	3	30
106	Thiamethoxam	153719-23-4	2	20
107	Thiobencarb	28249-77-6	10	100
108	Trifloxystrobin	141517-21-7	1	10

2. Preparation of Standard Solution(s)

a. Internal Standard Spiking Solution (20 µg/mL Trichloronate & 10 µg/mL Ethoprofos):

Pipet 1.0 mL of the 1000 µg/mL Trichloronate and 500 µg/mL Ethoprofos mixed stock solution into a 50 mL class A volumetric flask and dilute to volume with ethyl acetate. Mix well. All spiking solutions are stored at ≤ -10 °C and expire one year from the preparation date.

b. Mixed Pesticide Spiking Solution:

Pipet 5 mL of LC compound stock solution and 5 mL of GC compound stock solution into a 50 mL class A volumetric flask, and dilute to volume with ethyl acetate. All spiking solutions are stored at ≤ -10 °C and expire one year from the preparation date.

c. Muscle Injection Standard for LC compounds:

Pipet 200 µL of internal standard spiking solution (D.2.a) and 200 µL of mixed

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pesticide spiking solution (D.2.b) into a 10 mL class A volumetric flask, and dilute to volume with acetonitrile. Injection standards are stored at ≤ -10 °C and expire in one month.

d. Egg Injection Standard for LC compounds:

Pipet 33.3 μ L of internal standard spiking solution (D.2.a) and 33.3 μ L of mixed pesticide spiking solution (D.2.b) into a 10 mL class A volumetric flask, and dilute to volume with acetonitrile. Injection standards are stored at ≤ -10 °C and expire in one month.

e. Muscle Injection Standard for GC compounds:

Pipet 200 μ L of internal standard spiking solution (D.2.a) and 200 μ L of mixed pesticide spiking solution (D.2.b) into a 10 mL class A volumetric flask, and dilute to volume with toluene. Injection standards are stored at ≤ -10 °C and expire in one month.

f. Egg Injection Standard for GC compounds:

Pipet 33.3 μ L of internal standard spiking solution (D.2.a) and 33.3 μ L of mixed pesticide spiking solution (D.2.b) into a 10 mL class A volumetric flask, and dilute to volume with toluene. Injection standards are stored at ≤ -10 °C and expire in one month.

Table 3 - Concentration of GC and LC injection standard.

Cmpd #	Name	Muscle Injection Standard Conc. (μ g of pest./mL of solution)	Egg Injection Standard Conc. (μ g of pest./mL of solution)
	GC Mixed Standard	D.2.e	D.2.f
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

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Cmpd #	Name	Muscle Injection Standard Conc. (µg of pest./mL of solution)	Egg Injection Standard Conc. (µg of pest./mL of solution)
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
31	Nonachlor trans	0.06	0.00999
32	Oxychlorane	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

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Cmpd #	Name	Muscle Injection Standard Conc. (µg of pest./mL of solution)	Egg Injection Standard Conc. (µg of pest./mL of solution)
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
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-Methylhepyl- 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

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Cmpd #	Name	Muscle Injection Standard Conc. (µg of pest./mL of solution)	Egg Injection Standard Conc. (µg of pest./mL of solution)
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
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	Pyriproxyfen	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		

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Cmpd #	Name	Muscle Injection Standard Conc. (µg of pest./mL of solution)	Egg Injection Standard Conc. (µg of pest./mL of solution)
109	Trichloronate	0.4	0.0666
110	Ethoprophos	0.2	0.0333

E. SAMPLE PREPARATION

1. Chop 0.5 -1 lb of muscle tissue into small pieces and homogenize with an equal amount of dry ice in a large food processor. The resulting sample homogenate will be a frozen powder.
2. Transfer a portion of the homogenized sample into a loosely capped sample cup until the dry ice has sublimed. Excess sample from step E.1 may be discarded.
3. Tighten the caps and store sample cups at ≤ -10 °C.
4. No sample preparation is required for egg products.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls
 - a. Weigh 20 ± 0.20 g of blank homogenized muscle sample, 5.0 ± 0.04 g of blank liquid egg product, or 2.5 ± 0.04 g blank powdered egg product.
 - b. Prepare one sample each for a blank (negative control), a decision level recovery, a recovery (positive control), and a check sample if necessary.
 - c. Prepare recoveries by fortifying each recovery with the mixed pesticide spiking solution (D.2.b): for muscle, add 100 µL; for egg, add 25 µL. Allow the sample to dry (about five minutes) before continuing to step F.2.b.
2. Extraction Procedure
 - a. 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. Make sure the sample is all the way down in the tube.
 - b. Add 30 mL of ethyl acetate to each sample.
 - c. Fortify each sample and each control with 100 µL, for muscle, and 25 µL, for eggs, of the internal standard spiking solution (D.2.a.) and cap centrifuge tube. Invert or vortex tubes so solvent reaches entire sample as needed.
 - d. Place samples on the shaker for one minute to mix.

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- e. Add 8 g of MgSO₄ and 2 g NaCl (pre-weighed QuEChERS salts) to each sample and cap tube. Invert or vortex tubes so salt interacts with entire sample as needed.
Note: Make sure the solvent interacts well with the entire sample and the crystalline agglomerates are broken up sufficiently.
- f. Shake vigorously for five minutes on the shaker.
- g. Place samples into the ≤ -20 °C freezer for 30 minutes.
- h. Remove samples from freezer and centrifuge at 3500 RPM for 8 minutes.
- i. Decant more than 18 mL of the ethyl acetate layer into a 50 mL graduated glass centrifuge tube using a funnel and filter paper.
- j. Adjust the volume of muscle samples to 18 mL and of egg samples to 12 mL, discarding the excess.
- k. 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.
- l. Dilute to 15 mL with acetonitrile, cap glass tube and vortex for one minute.
- m. Place samples in ≤ -70 °C freezer for 30 minutes.
- n. 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 minutes at 1050 RCF.
Note: Acetonitrile will thaw during centrifugation.
- o. Prepare a solid phase extraction (SPE) column containing 1000 mg C₁₈ by adding approximately 2 g anhydrous MgSO₄ to the top of the C₁₈ layer.
Note: SPE columns containing MgSO₄ may be prepared ahead of time and stored in a desiccator.
- p. Using a positive pressure SPE manifold (PPM), condition the SPE cartridge with 5 mL of 1% acetic acid/acetonitrile and elute to waste.
- q. Place properly labeled 15 mL graduated glass tubes in the collection rack below SPE cartridges.
- r. Transfer 10 mL of sample extract into the SPE column and pass the extract through the column using a regulated flow pressure of approximately 35 psi.
- s. 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.)

Note: Be careful not to overfill the SPE columns.

Note: This is an optional stopping point. If stopping overnight, samples should be

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capped and stored at ≤ -20 °C.

- t. 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.
3. Extract preparation for LC/MS/MS analysis
 - a. Transfer 1 mL of the extract from step F.2.t to a 2 mL mini-centrifuge tube that contains 50 mg PSA (primary secondary amine) and 150 mg of $MgSO_4$.
 - b. Vortex the mini-centrifuge tubes for one minute.
 - c. Centrifuge the mini-centrifuge tubes for two minutes at 10,000 RCF.
 - d. 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 LC/MS/MS.
 4. Extract preparation for GC/MS/MS analysis
 - a. Using a PPM, condition a 500 mg PSA SPE column with 4 mL of 3:1 v/v acetone/toluene and elute to waste.
 - b. Place properly labeled 15 mL graduated glass tubes in the collection rack below SPE columns.
 - c. Using a Pasteur pipette, transfer the remainder of the sample extract from step F.2.t. to the SPE column.
 - d. Elute the extract through the column using a regulated flow pressure of 35 psi with 4 mL of 3:1 v/v acetone/toluene.
 - e. 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.
 - f. 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.
 - g. Evaporate the sample to approximately 0.5 mL under nitrogen in a 65 ± 5 °C water bath.
 - h. Add 3 mL of toluene to centrifuge tube and vortex.
 - i. Evaporate again to less than 0.5 mL to ensure all other solvents have been removed.
 - j. Bring the volume to 1.0 mL with toluene and vortex to mix.
 - k. Transfer the sample to a labeled autosampler vial. Analyze by GC/MS/MS.
 5. LC/MS/MS Instrumental Settings

Note: The instrument parameters may be optimized to ensure system suitability.

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a. UPLC Conditions:

Aqueous Mobile Phase: 5 mM ammonium acetate/0.1% formic acid in water

Organic Mobile Phase: 0.1% formic acid in methanol

Weak Wash: 10% methanol in water

Strong Wash: 0.5% formic acid in 1:1:1:1 acetonitrile : methanol : isopropanol : water

Flow rate: 0.5 mL/min

Column Temperature: 50 °C

Injection Volume: 1 µL for muscle samples, 2 µL for egg samples

Run Time: 10 minutes.

b. UPLC Mobile Phase Gradient:

Table 4 – UPLC gradient

Time (minute)	% Aqueous	% Organic
initial	90%	10%
0.25	90%	10%
7.75	2%	98%
10.50	2%	98%
10.55	90%	10%
12	90%	10%

c. Interface Conditions

Ion Mode: ES+

Source Temperature: 150 °C

Desolvation Temperature: 450 °C

Cone Gas Flow: 25 L/hr

Desolvation Gas Flow: 850 L/hr

Collision Gas Flow: 0.25 mL/min

d. Multiple Reaction Monitoring (MRM) parameters

Table 5 – LC MRM parameters

Cmpd #	Name	RT (min)	Cone (V)	First transition (m/z)	Coll En (V)	Second transition (m/z)	Coll En (V)	Quant Ion
38	3-Hydroxycarbofuran	3.57	15	255.2 < 163	18	255.2 < 181	15	163
39	Acephate	1.53	20	184.1 < 125	16	184.1 < 143	12	143

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40	Acetamiprid	3.57	40	223 < 56	16	223 < 126	16	126
41	Alachlor	6.61	27	269.8 < 161.9	19	269.8 < 237.8	11	237.8
42	Aldicarb	4.23	12	190.8 < 88.7	13	190.8 < 115.8	5	115.8
43	Aldicarb sulfone	2.16	23	223 < 76	7	223 < 86	12	86
44	Aldicarb sulfoxide	1.98	16	207 < 89	14	207 < 132	10	89
45	Atrazine	5.49	35	216.1 < 104	26	216.1 < 174.1	18	174.1
46	Azinphos methyl	5.82	22	317.7 < 124.8	35	317.7 < 131.9	30	131.9
47	Azoxystrobin	6.01	30	404.1 < 344.2	26	404.1 < 372.1	14	372.1
48	Benoxacor	5.86	22	259.7 < 133.8	29	259.7 < 148.9	17	148.9
49	Boscalid	6.17	22	342.8 < 271.3	33	342.8 < 306.7	19	306.7
50	Buprofezin	7.52	22	306 < 115.9	15	306 < 201	11	201
51	Carbaryl	5.04	20	202.2 < 127	28	202.2 < 145	15	145
52	Carbofuran	4.87	25	222.2 < 123	23	222.2 < 165	13	123
53	Carfentrazone ethyl	6.83	37	412 < 345.7	23	412 < 365.6	17	345.7
54	Clothianidin	3.23	25	250.1 < 132.1	29	250.1 < 168.6	15	168.6
55	Coumaphos O	5.91	45	347 < 211	34	347 < 291	22	291
56	Coumaphos S	6.95	40	363 < 227	24	363 < 307	16	227
57	Deethylatrazine	3.89	35	187.9 < 104	28	187.9 < 146	20	146
58	Diazinon	6.97	36	305.1 < 153.1	22	305.1 < 169.1	18	169.1
59	Dichlorvos	4.7	32	220.7 < 108.8	19	220.7 < 144.8	11	108.8
60	Difenoconazole	7.16	42	406 < 250.8	25	406 < 336.8	17	250.8
61	Diflubenzuron	6.65	23	311 < 141.1	32	311 < 158.2	15	158.2
62	Dimethoate	3.5	17	230 < 125	20	230 < 199	10	199
63	Diuron	5.6	25	233 < 72.1	15	233 < 160	28	72.1
64	Ethion	7.6	22	384.7 < 142.8	25	384.7 < 198.8	11	198.8
65	Ethion monoxon	6.71	27	368.7 < 170.7	17	368.7 < 198.8	11	198.8
66	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
67	Fenoxaprop ethyl	7.43	12	361.9 < 243.7	25	361.9 < 287.7	19	287.7
68	Fluridone	5.9	22	330 < 258.9	45	330 < 309.2	33	309.2
69	Fluroxypyr-1methylheptyl-ester	7.76	20	367 < 209	22	367 < 255	10	255
70	Fluvalinate	8.14	27	502.8 < 180.7	27	502.8 < 207.8	13	207.8
71	Hexazinone	4.89	32	252.9 < 70.9	33	252.9 < 170.8	17	170.8
72	Hexythiazox	7.72	30	353 < 168.1	26	353 < 228.1	14	228.1
73	Imazalil	5.24	30	297 < 159	36	297 < 255	20	159
74	Imidacloprid	3.2	25	256.1 < 175	18	256.1 < 209	14	209

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75	Indoxacarb	7.2	25	528 < 150.1	22	528 < 203.2	35	150.1
76	Linuron	5.99	28	249 < 160	18	249 < 182	17	160
77	Malathion	6.26	22	330.7 < 126.8	11	330.7 < 284.7	7	126.8
78	Metalaxyl	5.65	18	280.1 < 192.2	18	280.1 < 220.1	13	220.1
79	Methamidophos	1.21	22	142 < 94	14	142 < 125	13	94
80	Methomyl	2.51	13	163.1 < 88	9	163.1 < 106	9	88
81	Methoxyfenozide	6.32	15	369.1 < 91.1	47	369.1 < 149.2	18	149.2
82	Metribuzin	4.8	32	214.8 < 83.8	21	214.8 < 186.7	19	186.7
83	Myclobutanil	6.32	28	289.1 < 70.1	18	289.1 < 125.1	30	70.1
84	Norflurazon	5.66	30	304.1 < 160.1	40	304.1 < 284.1	32	284.1
85	Omethoate	1.79	20	214 < 155	14	214 < 183	12	183
86	Piperonyl butoxide	7.63	12	356 < 118.9	37	356 < 176.8	13	176.8
87	Pirimiphos methyl	7.08	12	305.9 < 107.8	33	305.9 < 163.9	21	107.8
88	Prallethrin	7.22	22	301.1 < 132.9	11	301.1 < 168.9	9	132.9
89	Profenofos	7.43	35	374.8 < 304.9	18	374.8 < 346.8	14	304.9
90	Propachlor	5.58	17	211.8 < 105.8	25	211.8 < 169.7	17	169.7
91	Propanil	6.01	32	217.8 < 126.8	27	217.8 < 161.7	15	161.7
92	Propetamphos	6.34	17	281.9 < 137.8	19	281.9 < 155.7	11	137.8
93	Propiconazole	6.95	27	341.8 < 68.9	21	341.8 < 158.8	27	68.9
94	Pyraclostrobin	7.01	40	387.8 < 163.7	12	387.8 < 194.1	10	194.1
95	Pyrethrin I	7.87	22	329.2 < 143	15	329.2 < 161	9	161
96	Pyrethrin II	7.25	27	373.2 < 133	17	373.2 < 161	11	161
97	Pyridaben	8.09	25	365.2 < 147.1	28	365.2 < 309	13	147.1
98	Pyriproxyfen	7.62	12	322 < 95.8	15	322 < 184.8	23	95.8
99	Resmethrin	8.15	12	356.2 < 127.9	41	356.2 < 170.8	15	170.8
100	Simazine	4.85	35	202 < 124.1	20	202 < 132	20	132
101	Sulprofos	7.72	27	322.9 < 218.7	17	322.9 < 246.8	13	218.7
102	Tebufenozide	6.73	12	353.1 < 105	50	353.1 < 133.1	22	133.1
103	Tetrachlorvinphos	6.77	27	366.5 < 126.7	17	366.5 < 240.6	17	126.7
104	Tetraconazole	6.5	37	371.9 < 69.8	23	371.9 < 158.7	33	158.7
105	Thiabendazole	3.25	45	202.1 < 131	33	202.1 < 175	24	175
106	Thiamethoxam	2.63	23	292 < 181	18	292 < 211	13	211
107	Thiobencarb	7.1	25	257.9 < 100.1	10	257.9 < 125.1	20	125.1
108	Trifloxystrobin	7.24	25	409 < 145	44	409 < 186	20	186

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e. MS parameters

Dwell time: varied from 0.025 – 0.2 s

Capillary: 1.4 kV

Multiplier: -640 V

6. GC/MS/MS Instrumental Settings

Note: The instrument parameters may be optimized to ensure system suitability.

a. Gas Chromatograph Parameters:

Carrier Gas	Helium
Column 1 Flow Rate	1.4 mL/min
Column 2 Flow Rate	1.2 mL/min
Injector Temperature	280 °C
Injection Volume:	1 µL for muscle samples, 2 µL for egg samples
Injection Mode	splitless
Temperature Program:	
Initial temp	60 °C
Initial hold time	1 min
Program rate up to 120 °C	40 °C/min
Program rate up to 292 °C	5 °C/min
Post-run time	2 minutes
Total Run time	36.9 min

b. Mass Spectrometer Parameters:

Ionization	Positive Electron Impact
Detector EMV	1352 V
Collision Gas	Nitrogen @ 1.5 mL/Min
Collision Energy	Optimized for each compound
MS Source temperature	300 °C
Transferline temperature	300 °C
Solvent delay	7.0 min

Note: Autotune the instrument as needed.

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c. Summary of MRM transitions and parameters selected for each compound:

Table 6 – GC MRM Parameters

Cmpd #	Name	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
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	32.10	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	17.70	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	282 > 247	60	250 > 142	15	282 > 214	30	247
27	Lindane (BHC gamma)	14.36	181 > 145	15	219 > 183	5	219 > 109	35	145
28	MGK – 264 1	20.80	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	23.90	409 > 302	25	409 > 109	40	409 > 263	40	302

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32	Oxychlorthane	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

7. Injection sequence / Sample Set
 - a. Injection Standard
 - b. Decision Level Recovery
 - c. Recovery (Positive Control)
 - d. Solvent Blank
 - e. Blank (Negative Control)
 - f. Intra-laboratory check sample (if needed)
 - g. Samples, up to a maximum of 18
 - h. Re-injection of the recovery (positive control) (for system suitability)

Note: Placing additional solvent blanks in the sample injection sequence is optional. Additionally, one may want to include an additional injection standard or recovery within the sample injection sequence to verify retention time and instrument response stability.

G. CALCULATIONS / IDENTIFICATION

1. Calculations
 - a. Relative Response Factor (RRF)

This is the internal standard corrected analyte response. The MS instruments can be programmed to automatically do this calculation.

$$A = B / C$$
 where
 - A = Relative Response Factor (unitless)
 - B = Quant Ion Peak Area of Analyte (counts)
 - C = Quant Ion Peak Area of Internal Standard (counts)
 - b. Estimated Amount Found

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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. The MS instruments can be programmed to automatically do this calculation.

$$D = E * A_{\text{sample}} / A_{\text{pos. ctrl.}}$$

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)

2. Screening Criteria

- a. The quantitative ion and all other ions listed for the analyte in Table 5 and 6 must be present.
- b. All ions must have a signal-to-noise ratio ≥ 3 . This may be verified by visual inspection.
- c. 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.
- d. Retention time for the recovery and samples must match the retention time of the decision level recovery within $\pm 5\%$ for LC, $\pm 0.5\%$ for 1-naphthol and chloroneb, $\pm 1\%$ for all other single peak GC compounds, and $\pm 5\%$ for multippeak compounds for GC.
- e. All quantitative ion peak areas in the blank must be $< 10\%$ of the decision level recovery.
- f. The sample is screen positive if the following criteria are met:
 - i. The fortified recovery of the analyte must exceed 10% of the decision level recovery.
 - ii. The sample response equals or exceeds the recovery level.

H. **SAFETY INFORMATION AND PRECAUTIONS**

1. Required Protective Equipment — Safety glasses, laboratory coat and gloves.

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2. Hazards

<i>Procedure Step</i>	<i>Hazard</i>	<i>Recommended Safe Procedures</i>
Organic solvents (Ethyl acetate, Acetonitrile, Methanol)	Flammable, vapors are corrosive to the skin, eyes and respiratory system.	Use only in an efficient chemical fume hood, away from any electrical or heating devices.
Toluene	Flammable, carcinogen, mutagen, vapors are corrosive to the skin, eyes and respiratory system.	Use only in an efficient chemical fume hood, away from any electrical or heating devices.
Pesticides	Persistent, bioaccumulative, carcinogenic, irritation to eyes, skin and respiratory system.	Avoid direct contact, work in properly vented areas and use appropriate personal protective equipment.

3. Disposal Procedures

Follow local, state, and federal guidelines for disposal.

I. **QUALITY ASSURANCE PLAN**

1. Screening Criteria

- a. For set acceptance, 95% 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.
- b. For set acceptance, 95% 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 Section G for samples containing corresponding presumptive positive analytes.
- c. 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.

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2. Critical Control Points and Specifications

	<u>Record</u>	<u>Acceptable Control</u>
a. Grinding muscle samples with dry ice		Ground samples in powder form.
b. Sample weight		20.0 ± 0.2 g for muscle, 5.0 ± 0.04 g for liquid eggs, or 2.5 ± 0.04 g for powdered egg products
c. Addition of QuEChERS salts		Make sure the salts mix thoroughly with the sample
d. Evaporation steps		Do not let the sample evaporate to dryness at any of the evaporation steps

3. Intralaboratory Check Samples

- a. System, minimum contents.
 - i. Frequency: One per week per analyst when samples analyzed.
 - ii. Records are to be maintained.
- b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

 - i. Investigate following established procedures.
 - ii. Take corrective action as warranted.

4. Sample Condition Upon Receipt: Cold or Frozen

J. **APPENDIX**

1. References

- a. Waters Application Note: Minimizing the Impact of the Sample Matrix During Routine Pesticide Residue Analysis in Food, July 2010.
- b. USDA, AMS, MET-100 Pesticide Method, WI-MET100-01 & WI-MET100-02 Work Instructions.
- c. CLG-PST2.01, Screen and Confirmation of Pesticides by HPLC-MS-MS.

2. Chromatograms/spectra

Reserved

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#	Name	Porcine (ppb)	Bovine (ppb)	Poultry (ppb)	Ovine (ppb)	Caprine (ppb)	Equine (ppb)	Catfish (ppb)	Liq Eggs (ppb)	Powd. Eggs (ppb)
90	Propachlor	10	10	10	10	10	10	10	10	20
91	Propanil	25	25	25	25	25	25	25	25	50
92	Propetamphos	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	15
93	Propiconazole	15	15	15	15	15	15	15	15	30
94	Pyraclostrobin	50	50	50	50	50	50	50	50	100
95	Pyrethrin I	46	46	46	46	46	46	46	46	92
96	Pyrethrin II	31	31	31	31	31	31	31	31	62
97	Pyridaben	9	9	9	9	9	9	9	9	18
98	Pyriproxyfen	20	20	20	20	20	20	20	20	40
99	Resmethrin (cis&trans)	50	50	50	50	50	50	50	50	100
100	Simazine	10	10	10	10	10	10	10	10	20
101	Sulprofos	25	25	25	25	25	25	25	25	50
102	Tebufenozide	40	40	40	40	40	40	40	40	80
103	Tetrachlorvinphos	10	10	10	10	10	10	10	10	20
104	Tetraconazole	5	5	5	5	5	5	5	5	10
105	Thiabendazole	15	15	15	15	15	15	15	15	30
106	Thiamethoxam	10	10	10	10	10	10	10	10	20
107	Thiobencarb	50	50	50	50	50	50	50	50	100
108	Trifloxystrobin	5	5	5	5	5	5	5	5	10

K. APPROVALS AND AUTHORITIES

1. Approvals on file.
2. Issuing Authority: Director, Laboratory Quality Assurance Staff.