Analysis of Pesticide Residues in Rice with Bond Elut QuEChERS Extraction Kits and Agilent J&W HP-5ms Ultra Inert GC Column

Application Note

Food Safety

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Abstract

This application note describes the use of a quick, easy, effective, rugged, and safe (QuEChERS) sample preparation approach described in the European Committee (EN) for extraction and cleanup of 57 GC-amendable multiple pesticide class residues in rice. The method involves initial extraction in an aqueous/acetonitrile system, an extraction/partitioning step after the addition of salt, and a cleanup step using dispersive solid phase extraction (dispersive SPE). The target pesticides in the rice extracts were then separated by an Agilent J&W HP-5ms Ultra Inert Capillary GC column and analyzed by gas chromatography/mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode. With Agilent RTL pesticides library, the GC/MS files were quickly screened and analyzed for pesticide residues identification. The method was validated in terms of recovery and reproducibility. The spiked levels for the recovery experiments were 50, 100, and 200 ng/g. Most of the recoveries ranged between 80 and 110%, with an average RSD of 5.53%.

Introduction

The QuEChERS method for pesticide analysis was first introduced by USDA scientists in 2003 [1]. The method was modified to address some problematic pesticides by including a buffered extraction system [2]. The EN method 15662:2007 is an European variation to the QuEChERS method [3,4]. The method uses acetonitrile extraction, followed by the salting out of water from the sample using anhydrous magnesium sulfate ($MgSO_4$), NaCl and buffering citrate salts to induce liquid-liquid partitioning. A dispersive SPE is conducted for cleanup using a combination of primary secondary amine (PSA) to remove fatty acid s among other components and anhydrous $MgSO_4$ to reduce the remaining water in the extract. After mixing and centrifugation, the upper layer is ready for analysis.

GC/MS is a good tool for reliable detection and separation of pesticides. Retention Time Locking (RTL) is a simple technique for GC and GC/MS that allows a chromatographer to reproduce retention times on any Agilent GC instrument [5]. Agilent has developed GC and GC/MS RTL databases (p/n G1672AA) that include nine hundred sixty-two pesticides, metabolites, and suspected endocrine disrupters [6]. It is much easier to identify pesticides in complex food extracts by locking the method to this database.

GC Conditions

Column	Agilent J&W HP-5ms Ultra Inert, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI)
Inlet temperature	250 °C
Carrier gas	Helium, constant pressure mode
Retention time locking	Chropyrifos-methyl locked to 16.596 min
Injection mode	Splitless, purge flow 50 mL/min at 0.75 min
Injection volume	1 µL
Oven	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C/min to 200 °C, 8 °C/min to 280 °C (10 min), postrun: 320 °C (5 min)

MS Conditions

Solvent delay	4 min
MS temp	230 °C (Source); 150 °C (Quad)
Transfer line	280 °C
MS libraries	Agilent RTL Pesticide Library (G1672AA) and NIST08 Mass Spec Library
MS	EI, SIM/Scan
Scan mode	mass range (50–550 amu)

For other parameters, see Table 1

Table 1. Pesticides, CAS Number, Molecular Form and Target Ion

Compound	CAS no.	Mol form	Target ion
Phenanthrene-d10*	1517-22-2	C14D10	188
Triphenyl phosphate*	115-86-6	C18H15O4P	326
Methamidophos	10265-9-26	C2H8NO2PS	94
Dichlorvos	62-73-7	C4H7Cl2O4P	109
Omethoate	1113-02-6	C5H12NO4PS	156
Monocrotophos	6923-22-4	C7H14NO5P	127
BHC alpha isomer	319-84-6	C6H6Cl6	181
Hexachlorobenzene	118-74-1	C6Cl6	284
Dimethoate	60-51-5	C5H12NO3PS2	87
BHC beta isomer	319-85-7	C6H6Cl6	219
Lindane	58-89-9	C6H6Cl6	181
Pentachloronitrobenzene	82-68-8	C6Cl5NO2	237
Diazinon	333-41-5	C12H21N2O3PS	179
BHC Delta isomer	319-86-8	C6H6Cl6	181
Propanil	709-98-8	C9H9Cl2NO	161
Methyl parathion	298-00-0	C8H10NO5PS	263
Chlorpyrifos methyl	5598-13-0	C7H7Cl3NO3PS	286
Vinclozolin	50471-44-8	C12H9Cl2NO3	212
Heptachlor	76-44-8	C10H5Cl7	272
Metalaxyl	57837-19-1	C15H21NO4	206
Fenitrothion	122-14-5	C9H12NO5PS	277
Pirimiphos-methyl	29232-93-7	C11H20N3O3PS	290
Aldrin	309-00-2	C12H8Cl6	263
Malathion	121-75-5	C10H19O6PS2	173
Fenthion	55-38-9	C10H15O3PS2	278
Chlorpyrifos	2921-88-2	C9H11Cl3NO3PS	197
Parathion	56-38-2	C10H14NO5PS	291
Triadimefon	43121-43-3	C14H16ClN3O2	57
Heptachlor exo-epoxide	1024-57-3	C10H5Cl7O	353
Pendimethalin	40487-42-1	C13H19N3O4	252
Isofenphos	25311-71-1	C15H24NO4PS	213
Quinalphos	13593-03-8	C12H15N2O3PS	146
Triadimenol	55219-65-3	C14H18ClN3O2	112
Methidathion	950-37-8	C6H11N2O4PS3	145
Butachlor	23184-66-9	C17H26ClNO2	176
Dieldrin	60-57-1	C12H8Cl6O	79
Isoprothiolane	50512-35-1	C12H18O4S2	118
p,p'-DDE	72-55-9	C14H8Cl4	246
Endrin	72-20-8	C12H8Cl6O	263
Myclobutanil	88671-89-0	C15H17ClN4	179
p,p'-DDD	72-54-8	C14H10Cl4	235
o,p'-DDT	789-02-6	C14H9Cl5	235
Ethion	563-12-2	C9H22O4P2S4	231
p,p'-DDT	50-29-3	C14H9Cl5	235

Iprodione	36734-19-7	C13H13Cl2N3O3	187
Phosmet	732-11-6	C11H12NO4PS2	160
Bifenthrin	82657-04-3	C23H22ClF3O2	181
Fenpropathrin	64257-84-7	C22H23NO3	97
Tetradifon	116-29-0	C12H6Cl4O2S	159
Phosalone	2310-17-0	C12H15ClNO4PS2	182
Cyhalothrin(lambda)	68085-85-8	C23H19ClF3NO3	181
Permethrin I	52645-53-1	C21H20Cl2O3	183
Permethrin II	999046-03-6	C21H20Cl2O3	183
Cypermethrin I	52315-07-8	C22H19Cl2NO3	181
Cypermethrin II	65731-84-2	C22H19Cl2NO3	181
Fenvalerate	51630-58-1	C25H22ClNO3	167
Deltamethrin	52918-63-5	C22H19Br2NO3	181

*Internal standard

Experimental

The experiments were performed on an Agilent 7890 Gas Chromatograph equipped with 5975C inert MSD, and Agilent 7683 Automatic Liquid Sampler (ALS). The split/splitless inlets were fitted with long-lifetime septa (p/n 5183-4761) and splitless deactivated liner (p/n 5181-3316). Separation of the compounds was achieved on an Agilent J&W HP-5ms ultra inert GC column (30 m × 0.25 mm, 0.25 µm). Extraction and cleanup were achieved with an Agilent Bond Elut QuEChERS EN Extraction kit (p/n 5982-5650) and a Bond Elut QuEChERS EN Dispersive SPE kit (p/n 5982-5156). Injections were made using 5 µL syringe (p/n 5181-1273). The instrument conditions are listed below.

Chemicals and standards

All pesticides standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents and solvents were HPLC or analytical grade.

Sample preparation

Organically grown, pesticide-free rice was purchased from a local supermarket. The rice was placed into a clean plastic bag and frozen at –20 °C overnight. The following day, the required amount of frozen rice was removed and thoroughly blended. Dry rice was added while comminuting, when possible; samples were comminuted thoroughly to get the best sample homogeneity. It was verified that no pieces of rice were visible in the final sample.

A 5 g (± 0.1g) amount of preciously homogenized sample was placed into a 50 mL centrifuge tube. The QC sample was fortified with 100 µL of appropriate QC spiking solution. 100 µL of IS spiking solution was added to all the samples except the control blank. Tubes were capped and vortexed for 1 min. Five mL of water were added to each tube using the dispenser and let sit for 30 min. Tubes were capped and vortexed for 1 min. A 10 mL aliquot of ACN was added to each tube using the dispenser. Tubes were capped and shaken by hand for 1 min. An Agilent Bond Elut QuEChERS EN extraction salt packet, containing 4 g anhydrous $MgSO_4$, 1 g NaCl, 1 g Na_3Citrate, and 0.5 g Na_2H Citrate sesquihydrate, was added directly to each tube. The salt bag was massaged carefully to loosen any clumped salts before pouring. No powders were left in the threads or rims of the tubes. Tubes were sealed tightly and shaken vigorously for 1 min by hand to ensure that the solvent interacted well with the entire sample and crystalline agglomerates were broken up sufficiently. Sample tubes were centrifuged at 4,000 rpm for 5 min.

A 6 mL aliquot of upper ACN layer was transferred into Bond Elut QuEChERS EN dispersive SPE 15 mL tube (p/n 5982-5156). The tubes were capped tightly and vortexed for 1 min. The tubes were centrifuged with a standard centrifuge at 4,000 rpm for 5 min. A 500 µL aliquot from the extract was transferred into an autosampler vial, and analyzed by GC/MS.

Table 2 shows the QuEChERS EN sample preparation procedure.

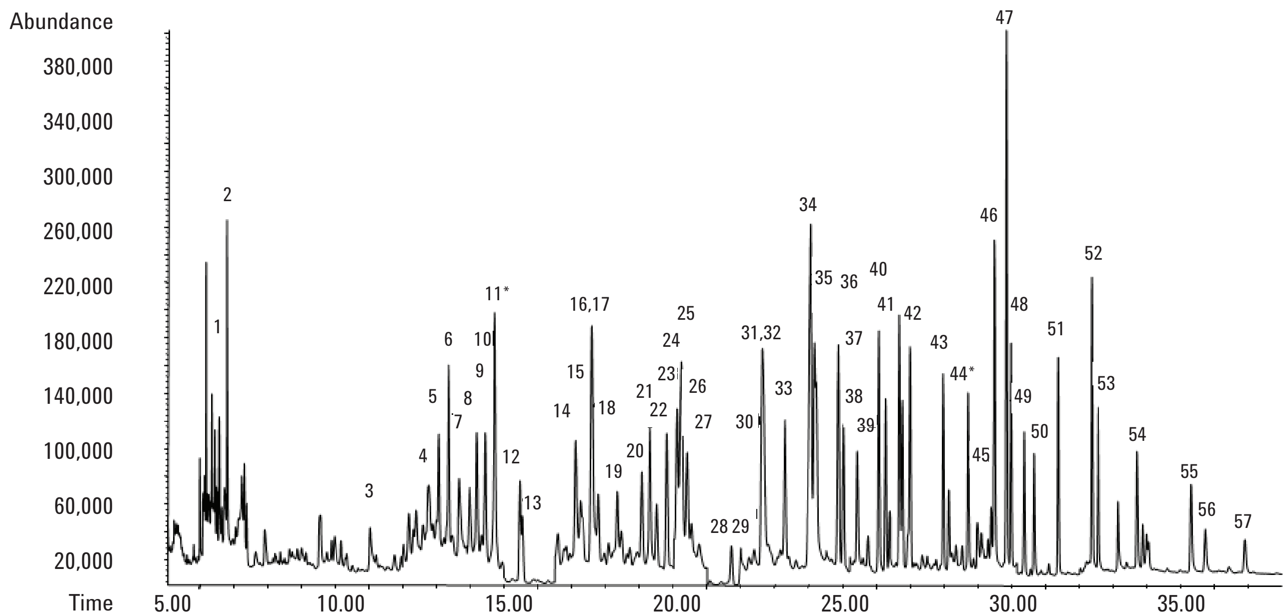
Table 2. The Agilent Bond Elut QuEChERS EN Extraction Procedure

1) Weigh 5 g comminuted rice in a 50 mL centrifuge tube and add 5 mL water.
2) Add IS solution, and GC spike solution if necessary, then vortex 1 min.
3) Add 10 mL of ACN, vortex 1 min..
4) Add Bond Elut EN QuEChERs Extraction salt packet.
5) Cap and shake vigorously for 1 min.
6) Centrifuge at 4,000 rpm for 5 min.
7) Transfer 6 mL of upper ACN layer to Bond Elut EN Dispersive-SPE 15 mL tube.
8) Vortex 1 min, then centrifuge at 4,000 rpm for 5 min.
9) Tranfer 0.5 mL of extract to a sample vial.
10) Analyze sample with GC-MS.

Results and Discussion

The total ion chromatogram (TIC) of the 57 pesticides at 200 ng/mL is shown in Figure 1. These pesticides include organochlorine, organophosphate and pyrethroid pesticides. Figure 2 shows that all target pesticides can be well separated by Agilent J&W HP-5MS Ultra Inert Capillary GC column.

The GC/MS system was retention time locked (RTL) to Chropyrifos-methyl. Retention time of each compound is listed in Table 3. Agilent 30 m × 0.25 mm × 0.25 µm HP-5ms was specified in G1672AA GC and GC/MS RTL Pesticide and Disruptor database, but HP-5ms Ultra Inert Capillary GC column exhibited the same selection selectivity of HP5 ms with same dimensions (see Table 3).



1 Methamidophos
2 Dichlorvos
3 Omethoate
4 Monocrotophos
5 BHC alpha isomer
6 Hexachlorobenzene
7 Dimethoate
8 BHC beta isomer
9 Lindane
10 Pentachloronitrobenzene
11 Phenanthrene-d10*
12 Diazinon
13 BHC delta isomer
14 Propanil
15 Methyl parathion
16 Chlorpyrifos Methyl
17 Vinclozolin
18 Heptachlor
19 Metalaxyl
20 Fenitrothion
21 Pirimiphos-methyl
22 Aldrin
23 Malathion
24 Fenthion
25 Chlorpyrifos
26 Parathion
27 Triadimefon
28 Heptachlor exo-epoxide
29 Pendimethalin
30 Isofenphos
31 Quinalphos
32 Triadimenol
33 Methidathion
34 Butachlor
35 Dieldrin
36 Isoprothiolane
37 p,p'-DDE
38 Myclobutanil
39 Endrin
40 p,p'-DDD
41 o,p'-DDT
42 Ethion
43 p,p'-DDT
44 Triphenyl phosphate*
45 Iprodione
46 Phosmet
47 Bifenthrin
48 Fenpropathrin
49 Tetradifon
50 Phosalone
51 lambda.-Cyhalothrin
52 Permethrin I
53 Permethrin II
54 Cypermethrin I
55 Fenvalerate
56 Cypermethrin II
57 Deltamethrin

* Internal standard

Figure 1. Total ion chromatogram (TIC) of pesticides at 200 ng/mL.

Linearity and Recovery Tests for Target pesticides in Rice

Linearity was determined using calibration curves spiked into the rice matrix. Calibration curves were constructed from data obtained by 1 µL injections of standards at, 50, 100, 200, 300, 400 ng/mL. Each standard solution contains 200 ng/mL of internal standards (ISTDs). All the pesticides of interest have excellent linearity with calibration coefficients (R^2) greater than 0.991, with an average R^2 of 0.9990 across 57 pesticides.

Different levels of target pesticides were spiked into rice before sample preparation to evaluate purifying effect of Bond Elut QuEChERS Extraction Kits. The GC/MS TIC for the rice extract and matrix spiked extract is illustrated in Figure 2. The spiked samples were treated according to the procedure described in the sample preparation. The recovery data for spiked samples are listed in Table 3. All data were based on five replicates of matrix spikes at each level. The levels were 0.05, 0.1, 0.2 µg/mL. Good recoveries were achieved for most of the compounds, ranged from 80-110%, and an average % relative standard deviation (RSD) of 5.35%.

Conclusion

Agilent Bond Elut QuEChERS EN extraction and dispersive SPE kits provide a simple, fast and effective method for the purification and enrichment of representative volatile to semi-volatile pesticide residues in rice. J&W HP-5ms ultra inert column with GC/MS provide a good method for separation and detection. With Agilent DRS software and RTL pesticides library, fifty-five pesticides were identified and analyzed quickly and accurately. The impurities and matrix effects from rice did not interfere with the quantitation of target compounds. Since the selected pesticides represented a broad variety of different classed and properties, the Agilent Bond Elut QuEChERs kits is an excellent choice for other pesticides in similar food matricies.

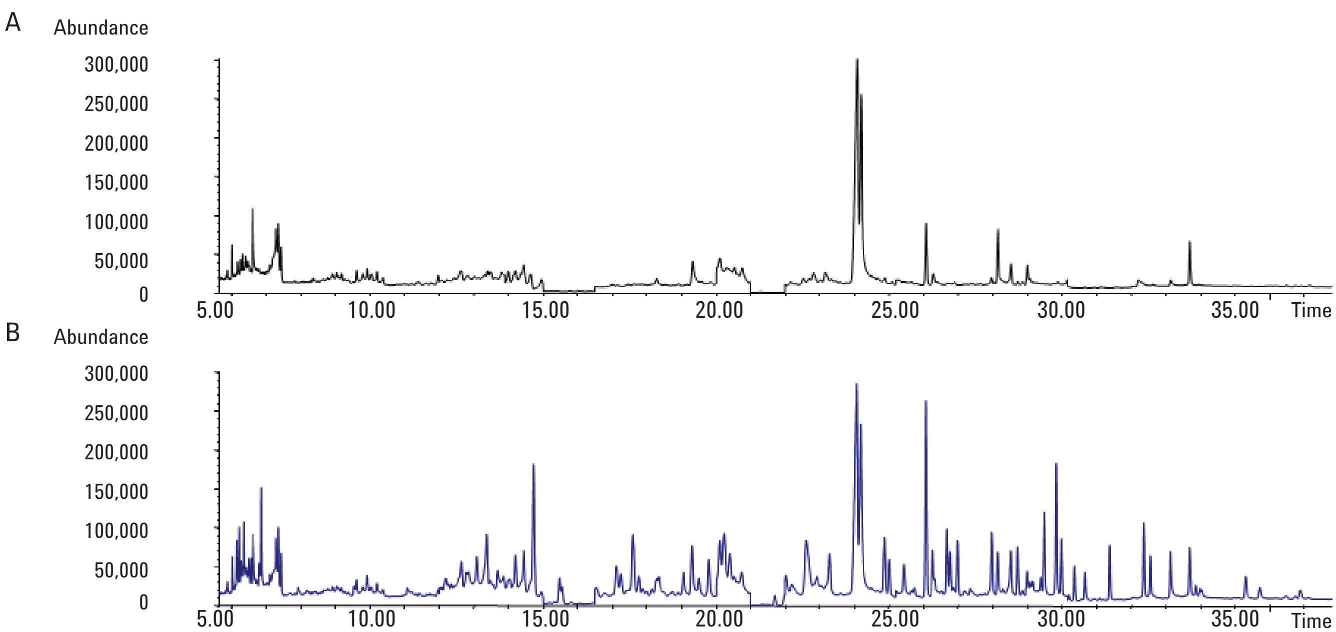


Figure 2. TIC of the rice extract (A) and matrix spiked extract 100 ng/mL (B) using Agilent GC/MS System and Agilent J&W HP-5MS Ultra inert 30 m × 0.25 mm × 0.25 µm column.

Table 3. Recoveries of 57 Pesticides in Real Sample

Compound	Retention time (min)	0.05 µg/mL (n = 5) Recovery%	RSD%	0.10 µg/mL (n = 5) Recovery%	RSD%	0.20 µg/mL (n = 5) Recovery%	RSD%
Methamidophos	5.70	70.8	7.6	76.3	5.3	78.9	7.9
Dichlorvos	5.83	112.3	5.1	103.3	7.9	109.8	5.2
Omethoate	10.04	78.4	3.5	97.6	4.5	97.1	6.6
Monocrotophos	11.75	81.6	5.9	96.8	4.5	105.5	3.9
BHC alpha isomer	12.08	103.4	6.5	94.7	9.3	112.0	3.3
Hexachlorobenzene	12.37	100.5	2.1	99.3	3.4	92.3	2.0
Dimethoate	12.68	83.4	6.1	92.7	5.0	101.9	3.2
BHC beta isomer	13.21	95.0	6.0	100.9	7.2	87.5	6.2
Lindane	13.45	102.4	7.3	110.8	7.1	93.5	7.0
Pentachloronitrobenzene	13.68	86.5	3.1	93.1	2.9	101.9	2.4
Diazinon	14.48	92.4	3.3	96.0	3.4	104.9	2.3
BHC Delta isomer	14.55	89.6	4.0	105.5	4.3	99.4	3.8
Propanil	16.12	102.9	4.5	103.8	4.9	103.6	5.3
Methyl parathion	16.59	87.5	7.7	102.8	7.4	103.2	3.2
Chlorpyrifos methyl	16.60	105.3	3.0	103.9	3.3	104.8	2.4
Vinclozolin	16.63	116.4	4.8	115.5	4.2	115.1	4.4
Heptachlor	16.79	102.8	1.6	96.6	3.9	96.9	3.2
Metalaxyl	17.35	105.8	3.6	104.5	5.4	108.7	5.0
Fenitrothion	18.08	81.9	2.7	92.7	6.7	106.9	4.2
Pirimiphos-methyl	18.31	108.9	2.7	107.3	3.2	107.8	3.4
Aldrin	18.51	106.0	2.3	105.0	3.5	99.9	1.4
Malathion	18.81	97.6	5.0	95.1	7.4	111.8	3.3
Fenthion	19.12	105.2	2.5	102.8	4.1	104.4	2.2
Chlorpyrifos	19.24	105.3	2.5	104.7	3.1	102.4	4.0
Parathion	19.27	72.8	4.6	77.6	7.3	112.9	3.3
Triadimefon	19.40	99.9	6.2	93.3	7.0	102.5	4.0
Heptachlor exo-epoxide	20.71	106.6	4.7	97.7	4.0	100.3	2.1
Pendimethalin	20.99	117.8	4.6	92.0	4.6	102.5	2.6
Isofenphos	21.60	109.2	5.8	102.2	8.2	106.9	3.9
Quinalphos	21.64	111.0	6.0	110.3	7.8	105.7	4.1
Triadimenol	21.71	96.6	6.2	107.3	6.4	92.0	9.4
Methidathion	22.29	94.9	8.9	95.3	9.5	111.9	4.2
Butachlor	23.23	117.6	5.8	110.2	8.5	102.9	3.6
Dieldrin	23.85	112.3	5.0	112.4	6.7	105.5	4.7
Isoprothiolane	23.88	123.7	3.9	100.5	6.6	108.5	2.4
p,p'-DDE	24.02	100.7	4.4	100.8	5.3	104.5	2.8
Myclobutanil	24.44	100.5	5.1	89.8	7.9	106.2	3.7
Endrin	24.74	108.6	5.2	97.2	5.9	107.4	5.6
p,p'-DDD	25.67	98.2	5.9	99.6	6.6	102.1	5.9

o,p'-DDT	25.76	90.9	5.2	90.6	6.3	108.9	3.0
Ethion	25.99	89.9	5.4	86.9	8.3	111.6	5.5
p,p'-DDT	26.98	87.0	5.8	88.6	7.4	106.0	3.4
Iprodione	28.39	99.6	5.5	108.6	5.3	98.9	6.2
Phosmet	28.49	91.7	6.0	96.0	8.8	99.6	5.5
Bifenthrin	28.84	104.8	5.0	104.2	7.9	108.5	7.1
Fenpropathrin	28.98	106.2	6.0	109.0	8.1	104.7	4.6
Tetradifon	29.36	114.3	5.1	107.5	7.1	109.2	6.7
Phosalone	29.66	97.2	6.7	91.4	8.7	105.2	4.0
Cyhalothrin (lambda)	30.37	84.0	6.5	91.0	8.1	105.9	5.3
Permethrin I	31.37	98.2	8.1	97.6	7.6	103.3	4.3
Permethrin II	31.55	100.1	5.7	96.7	8.1	101.0	5.2
Cypermethrin I	32.70	89.3	7.7	102.9	9.3	104.4	5.2
Fenvalerate	34.30	75.0	9.1	93.3	9.0	107.5	5.3
Cypermethrin II	34.71	86.4	8.5	97.2	8.6	100.1	7.7
Deltamethrin	35.88	67.3	8.4	87.6	8.6	95.4	4.9

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