Application Note

Food & Beverage Testing

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Analysis of Pesticides in Broccoli Using Universal dSPE with Carbon S and GC/TQ

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Abstract

This application note presents a simple, quick, and dependable GC/TQ method for the extraction of pesticides from broccoli using the Agilent Bond Elut QuEChERS EN 15662 extraction kit, followed by cleanup with the Agilent Bond Elut QuEChERS dSPE universal kit with Carbon S. The Agilent Carbon S sorbent provides efficient and selective matrix pigment removal and increases the recovery of select planar pesticides compared to the traditional graphitized carbon black (GCB) sorbent widely used in food sample extractions.

Introduction

Broccoli has natural, highly abundant pigments, such as chlorophyll and lutein, which cause numerous matrix effects and interfere with the analysis of pesticides on an Agilent 7000D triple quadrupole GC/MS system (GC/TQ). Effective sample extraction and matrix cleanup is necessary to remove problematic background interferences while maintaining high pesticide recoveries. QuEChERS is a common method for extracting pesticides from food matrices using organic solvent, and often employs dispersive solid phase extraction (dSPE) kits containing GCB to further clean the samples.

GCB is widely used in dSPE kits, as it effectively removes pigments and co-extractive compounds, which minimizes interferences in the analysis of pesticides in food matrices and mitigates the contamination of the GC/TQ flow path. However, the interaction of GCB with planar pesticides, such as hexachlorobenzene, often causes unwanted analyte loss. To address the negative interactions of GCB while maintaining its effective pigment removal properties, Agilent developed a new sorbent, Carbon S. Carbon S is a hybrid carbon material with optimized carbon content and pore structure designed to be an alternative to GCB.

Carbon S provides equivalent or better pigment removal from various sample matrices compared to GCB, while improving recovery of GCB-selective compounds (i.e., planar pesticides). As a result, Carbon S sorbent offers an improved balance between analyte recovery and matrix pigment-removal efficiency than traditional GCB sorbent. This application note investigates sample preparation using the QuEChERS dSPE universal kit with Carbon S for the analysis of select pesticides in broccoli by GC/TQ.

Experimental

Consumables and supplies

To assist laboratories in preparing to run the method, a list of Agilent consumables used in this application note is provided in the appendix.

Standard preparation

A combined pesticide standard and internal standard (ISTD) spiking solution was prepared in acetonitrile using mixed solutions from Agilent Technologies (part number 51900551) and individual standard solutions from Sigma-Aldrich (St. Louis, MO, U.S.A.). Matrix-matched calibration standards were prepared at 1, 10, 50, 100, and 200 ppb. Five replicate matrix matched quality control (QC) samples were prepared at 5 ppb. ISTD compounds included alpha-BHC-d6, parathion-d10, and triphenyl phosphate.

Sample preparation

A 10 g sample of homogenized organic broccoli was weighed into a 50 mL centrifuge tube and spiked with appropriate level spiking solution. The sample was vortexed for 30 seconds before adding two ceramic homogenizers and 10 mL of acetonitrile, and vortexing again for 1 minute. An Agilent QuEChERS extraction salt packet for EN method (part number 5982-5650CH) was added to the tube and briefly shaken vigorously by hand. A Geno/Grinder from SPEX SamplePrep was used to shake the sample for 5 minutes at 1,500 rpm. Sample was centrifuged for 5 minutes at 5,000 rpm before transferring 6 mL of extract to an Agilent QuEChERS dSPE universal kit 15 mL tube (Agilent QuEChERS dSPE universal kits with Carbon S (part number 5610-2060) and with GCB (part number 5982-0029) were compared). The dSPE tube was shaken using a Geno/Grinder for 3 minutes at 1,500, then centrifuged for 5 minutes at 5,000 rpm. The final sample extract was transferred to labeled vials for storage and analysis. An outline of this sample preparation procedure is shown in Figure 1. A dMRM chromatogram of planar pesticides extracted from a 10 ppb spiked broccoli sample cleaned using the QuEChERS dSPE universal kit with Carbon S is shown in Figure 3.

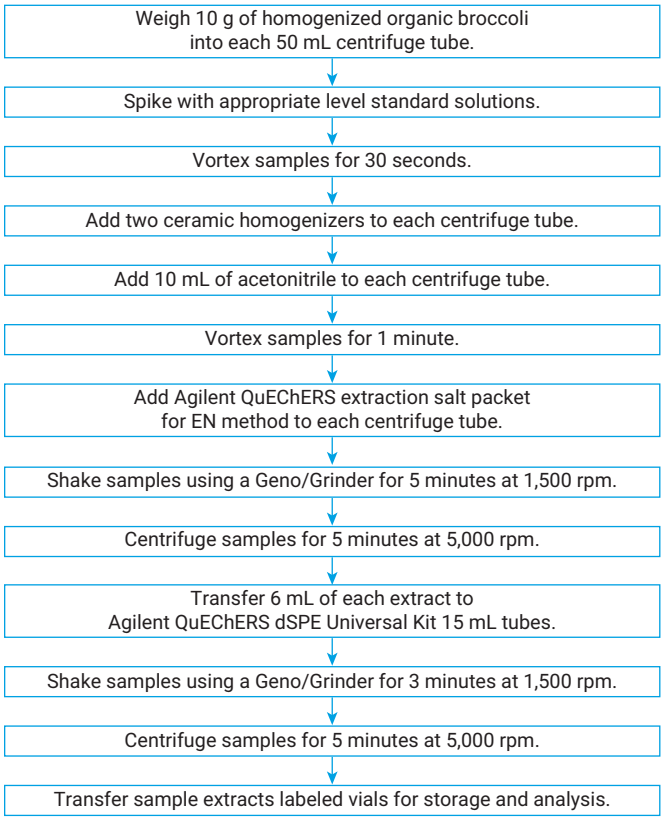


Figure 1. Sample preparation procedure for broccoli samples by Agilent QuEChERS EN method extraction followed by Agilent QuEChERS dSPE universal kit cleanup.

Instrumentation

The analytical system used for this study was an Agilent 8890 GC with a 7000D triple quadrupole GC/MS that was configured to obtain the best performance over a wide calibration range (Figure 2). The GC system was equipped with an Agilent 7693A automatic liquid sampler (ALS) tower and tray, a multimode inlet (MMI), an electronic pneumatic control (EPC), and an Agilent purged Ultimate union (PUU) for backflush system. Agilent MassHunter Workstation software was used for data acquisition and analysis. Dynamic MRM (dMRM) mode was used to accurately quantitate narrow peaks using an efficient dwell-time distribution. The GC/TQ instrument conditions were based on prior published methodology using equivalent instruments, and are listed in Table 1. Target and ISTD dMRM parameters are listed in the appendix.

Table 1. Agilent 8890 GC and Agilent 7000D GC/MS method parameters.

Columns	Agilent J&W HP-5ms UI, 15 m × 250 µm, 0.25 µm (Quantity: 2) (p/n 19091S-431UI)
Carrier Gas	Helium
Column 1 Flow	0.8267 mL/min
Column 2 Flow	1.4 mL/min
Injection Volume	0.5 µL
Inlet Liner	Splitless, single taper, glass frit (p/n 5190-5105)
MMI Temperature Program	75 °C for 0.02 min, 750 °C/min to 350 °C
Inlet Mode	Pulsed splitless Pulse pressure 25 psi until 1 min Purge flow to split vent 30 mL/min at 0.75 min
Oven Temperature Program	60 °C for 1 min, 40 °C/min to 170 °C, 10 °C/min to 310 °C, Hold 3 min
Run Time	20.75 min
Backflush Conditions	1.5 min postrun 310 °C oven temperature Postrun total flow 25 mL/min
Transfer Line Temperature	280 °C
Source	Extractor source with a 3 mm lens (G7000-20444)
Quadrupole Temperature	150 °C
Source Temperature	300 °C
Data Monitoring	dMRM
EM Voltage Gain Factor	10
Solvent Delay	2 min

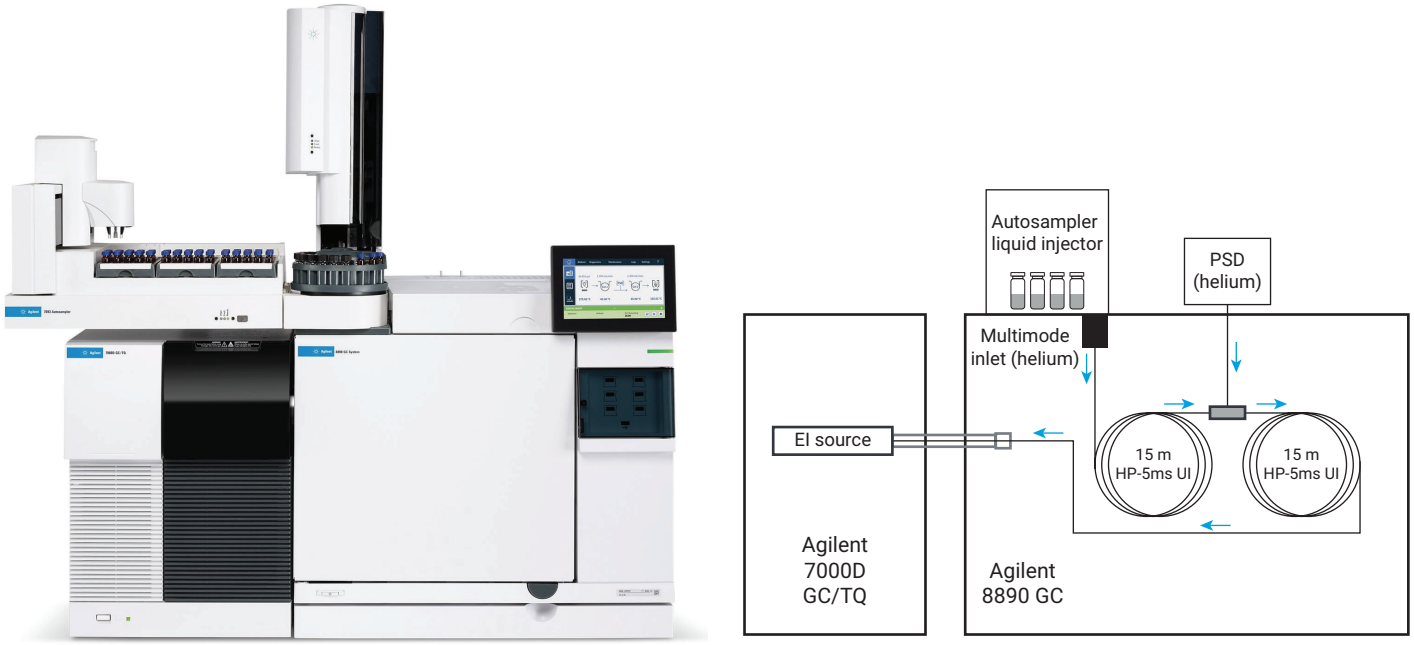
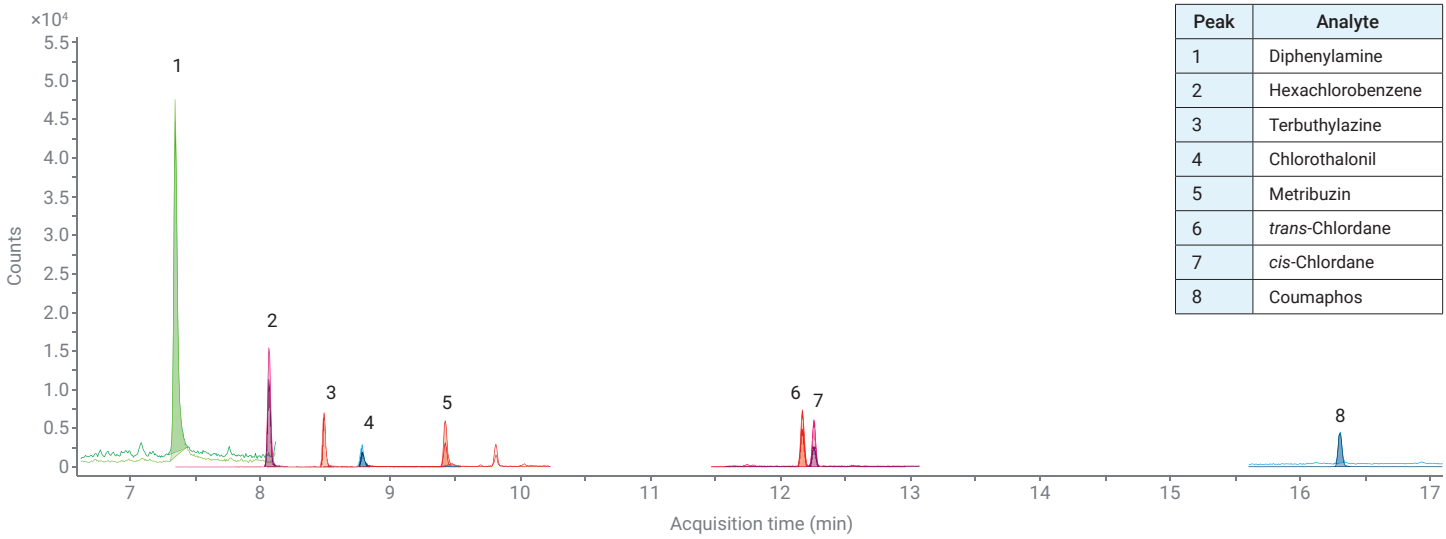


Figure 2. The Agilent 7000D triple quadrupole GC/MS (GC/TQ) coupled with an Agilent 8890 GC.



Peak	Analyte
1	Diphenylamine
2	Hexachlorobenzene
3	Terbuthylazine
4	Chlorothalonil
5	Metribuzin
6	*trans*-Chlordane
7	*cis*-Chlordane
8	Coumaphos

Figure 3. A dMRM chromatogram of planar pesticides extracted from a 10 ppb spiked broccoli sample cleaned using an Agilent QuEChERS dSPE universal kit with Agilent Carbon S.

Results and discussion

The QuEChERS sample preparation method was evaluated for broccoli using the existing recommended dSPE cleanup containing GCB alongside the QuEChERS dSPE universal kit with Carbon S. Matrix matched calibration curves were prepared over a range of 1 to 200 parts per billion (ppb). QC samples were prepared at 5 ppb. Calibration curves had linearities with R^2 >0.990 for all targeted pesticides using both cleanup methods. Figure 4 illustrates a comparison of planar pesticide recoveries between Carbon S and GCB methodologies from the 5 ppb QC sample, and Table 2 shows recovery comparison for all targeted pesticides.

Quantitation via matrix matched calibration showed that 95% of targeted analytes had recoveries between 90% and 110% at 5 ppb in broccoli when cleaned up using the universal kit with Carbon S, whereas GCB cleanup yielded only 75% of analytes with recoveries between 90% and 110%. Carbon S improved planar pesticide recoveries compared to GCB, nearly doubling the recovery of hexachlorobenzene (69% versus 34%) and chlorothalonil (93% versus 52%). Replicate samples (n = 5) were tested to determine accuracy and precision data. Both Carbon S and GCB cleanup methods had relative standard deviations (RSDs) <20%.

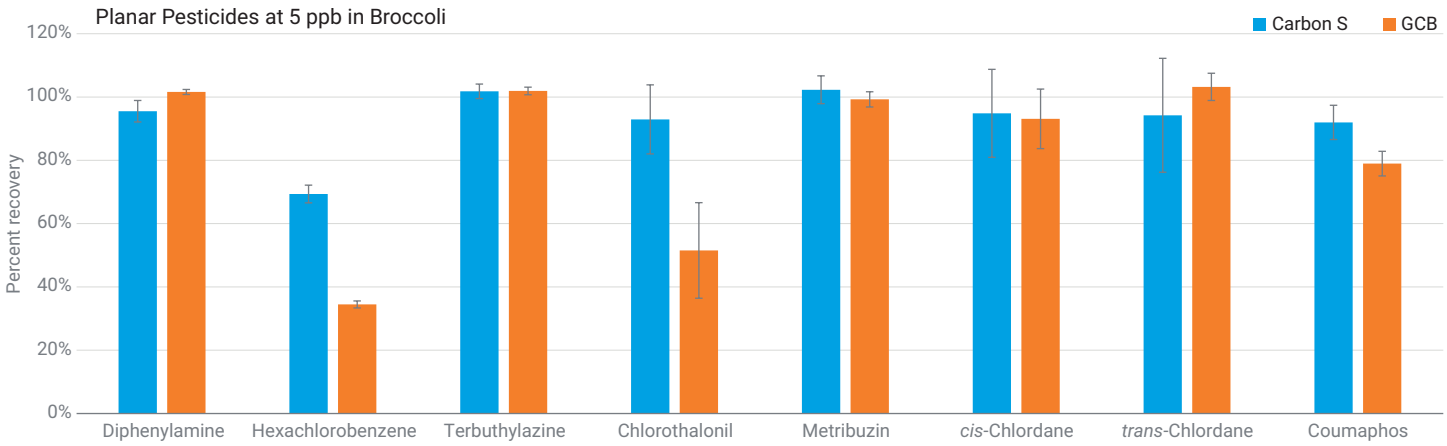


Figure 4. Recoveries of 5 ppb planar pesticides in broccoli using QuEChERS EN method extraction followed by Agilent QuEChERS dSPE universal kit cleanup.

Table 2. Comparison of Agilent QuEChERS dSPE universal kits with and without Carbon S using 5 ppb of pesticides in broccoli. Analytes with significantly better recoveries when using Carbon S rather than GCB are highlighted.

Analyte	Description	Carbon S Recovery	GCB Recovery
Dichlobenil	Nonplanar	100%	102%
Methacrifos	Nonplanar	104%	105%
Diphenylamine	Planar	96%	102%
Ethalfluralin	Nonplanar	107%	103%
Sulfotep	Nonplanar	102%	104%
Hexachlorobenzene	*Planar*	*69%*	*34%*
Demeton-S	Nonplanar	101%	104%
Simazine	Nonplanar	100%	98%
Terbuthylazine	Planar	102%	102%
Lindane	Nonplanar	104%	103%
Chlorothalonil	*Planar*	*93%*	*52%*
Chlorpyrifos-methyl	Nonplanar	102%	102%
Metribuzin	Planar	102%	99%
Fenitrothion	Nonplanar	104%	108%
Aldrin	Nonplanar	104%	103%
Pendimethalin	Nonplanar	105%	101%
cis-Chlordane	Planar	95%	93%
trans-Chlordane	Planar	94%	103%
Bupirimate	Nonplanar	102%	101%
Dieldrin	Nonplanar	102%	102%
Triazophos	Nonplanar	102%	105%
EPN	Nonplanar	101%	104%
Phosalone	Nonplanar	99%	102%
Mirex	Nonplanar	102%	103%
Coumaphos	*Planar*	*92%*	*79%*
Pyraclostrobin	Nonplanar	103%	88%
Deltamethrin	Nonplanar	99%	118%

Conclusion

A simple, quick, and dependable GC/TQ method was validated for the extraction of pesticides from broccoli using the Agilent Bond Elut QuEChERS EN extraction kit, followed by cleanup with the Agilent Bond Elut QuEChERS dSPE universal kit with Carbon S. Method performance was evaluated based on analyte recovery comparison in broccoli, particularly planar pesticides, using the dSPE universal kit with Carbon S compared to traditional GCB. The dSPE universal kit with Carbon S was proven to provide equivalent or better performance on planar pesticides analysis in broccoli than the current GCB recommendation.

References

1. González-Curbelo, M. Á. *et al.* Evolution and Applications of the QuEChERS Method. TrAC **2015**, 71, 169–185.
2. Andrianova, A.; Zhao, L. Five Keys to Unlock Maximum Performance in the Analysis of Over 200 Pesticides in Challenging Food Matrices by GC/MS/MS. *Agilent Technologies application note*, publication number 5994-4965EN, **2022**.
3. Andrianova, A. *et al.* Quantitation of Pesticides in Strawberries at Tolerance Levels Established by the US EPA. *Agilent Technologies application note*, publication number 5994-0799EN, **2019**.
4. Westland, J. Analysis of Pesticides in Tomato and Kale on GC/MS/MS with Agilent Bond Elut QuEChERS High Pigment dSPE with Carbon S. *Agilent Technologies application note*, publication number 5994-5050EN, **2022**.
5. Zhao, L.; Andrianova, A. Determination of Over 3000 Pesticides in Cayenne Pepper. *Agilent Technologies application note*, publication number 5994-5630EN, **2023**.

Appendix

Table A1. Agilent consumables and supplies used in this application note.

Description	Specifics	Part Number
Analytical Column	J&W HP-5ms UI, 15 m × 250 µm, 0.25 µm	19091S-431UI
GC Inlet Liner	Ultra Inert, universal, mid-frit, 870 µL, 4 mm	5190-5105
Purged Ultimate Union	Ultimate union kit, deactivated	G3182-61580
QuEChERS Kit	QuEChERS dSPE universal kit with Carbon S, 15 mL	5610-2060
QuEChERS Kit	QuEChERS dSPE universal kit with GCB, 15 mL	5982-0029
Autosampler Vials	Certified A-Line 2 mL, amber, screw top, write on spot, 100/pk	5190-9590
Autosampler Caps	9 mm green screw cap, PTFE/RS, 500/pk	5185-5829
Vial Inserts	250 µL vial inserts with polymer feet, 100/pk	5181-1270
Centrifuge Tubes	Centrifuge tubes and caps, 15 mL, 50/pk	5610-2039
Centrifuge Tubes	Centrifuge tubes and caps, 50 mL, 50/pk	5610-2049

Table A2. dMRM transitions for pesticides on the Agilent 8890 GC with an Agilent 7000D triple quadrupole GC/MS.

Compound Name	Target/ISTD	Precursor Ion	Product Ion	RT (min)	CE
Dichlobenil	Target	171	136.1	5.44	15
Dichlobenil	Target	171	100	5.44	25
Methacrifos	Target	207.9	180.1	6.31	5
Methacrifos	Target	124.9	47.1	6.31	10
Diphenylamine	Target	169	168.2	7.36	15
Diphenylamine	Target	168	167.2	7.36	15
Ethalfluralin	Target	315.9	275.9	7.41	10
Ethalfluralin	Target	275.9	202.1	7.41	15
Sulfotep	Target	237.8	145.9	7.64	10
Sulfotep	Target	201.8	145.9	7.64	10
alpha-BHC-d6	ISTD	224	187	7.78	15
alpha-BHC-d6	ISTD	224	150	7.78	15
Hexachlorobenzene	Target	283.8	248.8	8.1	15
Hexachlorobenzene	Target	283.8	213.9	8.1	30
Demeton-S	Target	126	65	8.12	10
Demeton-S	Target	88	60	8.12	5
Simazine	Target	201.1	173.1	8.24	5
Simazine	Target	173	172.1	8.24	5
Terbuthylazine	Target	228.9	173.1	8.54	5
Terbuthylazine	Target	172.9	172	8.54	5
Lindane	Target	216.9	181	8.56	5
Lindane	Target	181	145	8.56	15
Chlorothalonil	Target	263.8	229	8.8	20
Chlorothalonil	Target	263.8	168	8.8	25
Parathion-d10	ISTD	301	115	9.75	15
Parathion-d10	ISTD	301	83	9.75	35
Metribuzin	Target	198	82	9.48	15
Metribuzin	Target	198	55	9.48	30
Chlorpyrifos-methyl	Target	124.9	78.9	9.5	5
Chlorpyrifos-methyl	Target	124.9	47	9.5	15
Progargite	Target	149.9	135.1	9.94	5
Progargite	Target	135	107.1	9.94	10
Fenitrothion	Target	125.1	79	10.03	5
Fenitrothion	Target	125.1	47	10.03	15
Aldrin	Target	262.9	192.9	10.49	335
Aldrin	Target	254.9	220	10.49	20
Pendimethalin	Target	251.8	162.2	10.91	10
Pendimethalin	Target	251.8	161.1	10.91	15
trans-Chlordane	Target	374.9	265.9	11.58	20
trans-Chlordane	Target	372.8	265.8	11.58	15
cis-Chlordane	Target	374.9	265.9	11.81	15
cis-Chlordane	Target	372.9	265.8	11.81	20
Bupirimate	Target	272.9	193.1	12.22	5
Bupirimate	Target	272.9	108	12.22	15
Dieldrin	Target	277	241	12.32	5
Dieldrin	Target	262.9	193	12.32	35
Triazophos	Target	161.2	134.2	13.15	5
Triazophos	Target	161.2	106.1	13.15	10
Triphenyl phosphate	ISTD	326	325	14.01	5
Triphenyl phosphate	ISTD	232.9	215.1	14.01	10
EPN	Target	169	141.1	14.49	5
EPN	Target	169	77	14.49	25
Phosalone	Target	182	111	15.1	15
Phosalone	Target	182	102.1	15.1	15
Mirex	Target	273.8	238.8	15.64	15
Mirex	Target	271.8	236.8	15.64	15
Coumaphos	Target	361.9	109	16.35	15
Coumaphos	Target	210	182	16.35	10
Pyraclostrobin	Target	164	132.1	18.07	10
Pyraclostrobin	Target	164	77.1	18.07	35
Deltamethrin	Target	252.9	93	18.79	15
Deltamethrin	Target	181	152.1	18.79	25

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