

Minimizing the Impact of the Sample Matrix During Routine Pesticide Residue Analysis in Food

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Abstract

This application note describes the application of ultra sensitive detection to minimize the impact of matrix effects during analysis of 81 pesticide residues in a range of food products and also the use of a novel MRM acquisition mode that allows direct monitoring of the matrix background in each sample injected.

Benefits

- Detection of pesticides in complex food matrices using large multi-residue methods to below the required regulatory concentrations.
- Ability to monitor changes in the sample matrix between samples and batches.
- Reduction of matrix concentration to minimize matrix effects while maintaining detection.

Introduction

One of the biggest challenges in ensuring the safety of our food supplies is the measurement of hazardous ultra trace level components in the presence of a highly complex sample matrix. For the analysis of pesticides in food matrices, increased use of liquid chromatography systems, coupled with tandem quadrupole mass spectrometers has allowed progress in reducing the problems caused by the sample matrix. However, difficulties remain when trying to discriminate against matrix components that exhibit similar physiochemical properties. Unawareness of these difficulties in each unique sample can lead to poor quality results, and can impact a laboratory's performance and reputation.

Understanding the matrix challenge of each injected sample is clearly beneficial as is the ability to monitor changes in the sample matrix between samples and batches. This capability can lead to the continuous improvement of analytical quality in the laboratory. Conventional LC tandem quadrupole systems do not allow the direct monitoring of the sample matrix during high sensitivity MRM quantitation and it is only recently with the newest generation of instruments that this has become possible.

Problems caused by the sample matrix can include disruption to chromatography, increased chemical noise, and most notably, ionization suppression.¹⁻⁴ In highly complex matrices such as herbs and spices, these problems can be found in combination to make determination of pesticide residue concentration very difficult.

In addition to problems caused by the sample matrix, there are also pesticides that, by nature, are more difficult to analyze using LC-MS/MS due to a poor (relative) response factor. Successful analysis of these compounds to the regulatory concentration limits is difficult when considering the practicality of increasing sample amount and the balance of extracted matrix concentration. A much more practical solution is to use increased instrument sensitivity to maximize performance at these required concentrations. Also, if enough sensitivity is available, then the reduction of matrix concentration injected onto the system becomes possible.

Described here is the application of ultra sensitive detection to minimize the impact of matrix effects during analysis of 81 pesticide residues in a range of food products. Also described is the use of a novel MRM acquisition mode that allows direct monitoring of the matrix background in each sample injected.

Mass spectrometer acquisition

Quanpedia generated MRM parameters (a full MRM list can be found in Appendix 1) were used as the basis of RADAR-enabled mass spectrometer acquisition method. RADAR is an information-rich acquisition approach that allows measurement of target analytes with precision in MRM mode, while simultaneously scanning the background for all other components.

Figure 1 shows a RADAR-enabled mass spectrometer acquisition method with time scheduled MRMs for target pesticides and a simultaneous full scan (MS2) acquisition.



Figure 1. Mass spectrometer experiment showing RADAR acquisition mode.

Experimental

Waters DisQuE (EN 15662:2008) Extraction Kit (QuEChERS) was used to prepare spiked extracts of grape, avocado, marjoram, and ginger. Sample matrix concentrations were 1g/mL for grape and avocado and 0.1 g/mL for marjoram and ginger. The final acetonitrile extracts from QuEChERS were diluted 10x into mobile phase and 10 μ L were injected onto the analytical system (referred to as original sample). Subsequent

dilutions of this were then made to reduce matrix effects.

LC conditions

LC system:	ACQUITY UPLC
Column:	ACQUITY BEH C ₁₈ 100 mm x 2.1 mm, 1.7 µm
Mobile phase A:	0.1% HCOOH in H ₂ O
Mobile phase B:	0.1% HCOOH in MeOH
Run time:	10.00 min

UPLC gradient:

Time (min)	Flow (mL/Min)	%A	%B
-	0.5	90	10
0.25	0.5	90	10
7.75	0.5	2	98
8.5	0.5	2	98
8.51	0.5	90	10

MS conditions

MS system:	Xevo TQ-S
Ionization mode:	ES positive

Capillary voltage:	0.60 kV
Source temp:	130 °C
Desolvation temp:	650 °C
Cone gas flow:	150 L/hr
Desolvation gas flow:	1200 L/hr

Results and Discussion

Detection to below regulatory limits

European Union (EU) regulations to control pesticide exposure from food consumption are among the toughest in the world. In order to import food and food commodities into Europe, the level of pesticide contamination must be below the stated maximum residue limits (MRLs) for that product.⁵ Confirmation of positive results requires good quantitative performance well below these concentrations, which can be very challenging in more complex matrices.

Figure 2 shows a selection of extracted MRM chromatograms for pesticides spiked into avocado at 0.005 mg/kg. Quantitative and confirmatory transitions are both detected at this level, which is 10x below the European MRL (except zoxamide, which is 4x below). This includes parathion, which has a relatively poor response factor when analyzed using electrospray ionization. Comfortable quantitation of pesticides at these low concentrations allows high confidence when reporting results around maximum residue limits.

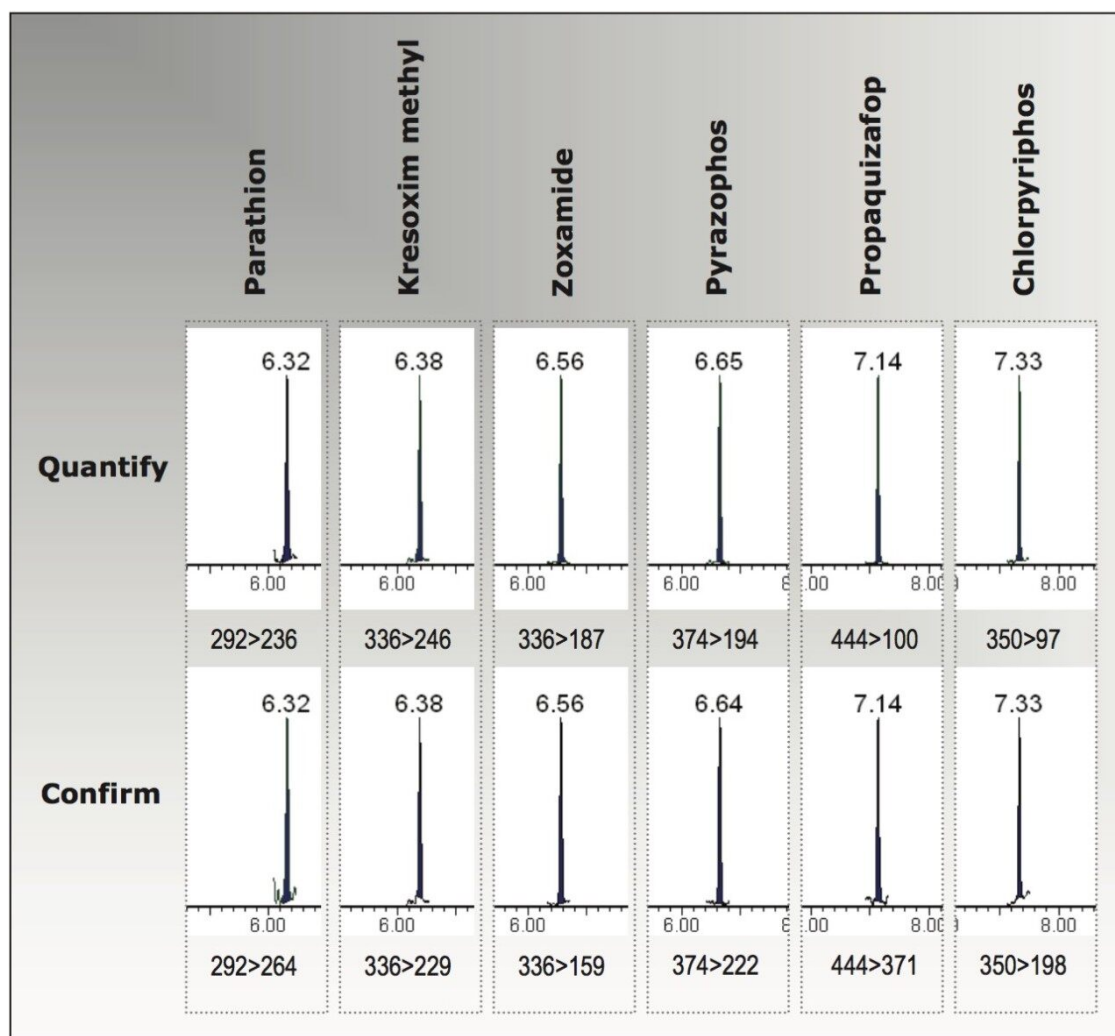


Figure 2. Quantitative and confirmatory MRM transitions for pesticides spiked into avocado at 0.005 mg/kg.

Monitoring matrix complexity

Each sample analyzed had full scan data available along with the MS/MS data. This was due to the RADAR functionality of the Xevo TQ-S being enabled. These data were used to monitor the complexity of the sample matrix background in each sample.

Differences in the co-extracted background for grape, avocado, marjoram, and ginger were observed by plotting the base peak intensity (BPI) chromatogram. For ginger and marjoram, 10x less sample was extracted using QuEChERS to give a 0.1 g/mL matrix, as opposed to the usual 1 g/mL matrix for grape and avocado. This is due to the extremely high complexity of the sample matrix, as well as to aid extraction of these drier samples. Figure 3 shows base peak intensity (BPI) chromatograms overlaid with MRM

chromatograms for pesticides spiked at 1.0×10^5 g/kg for each matrix.

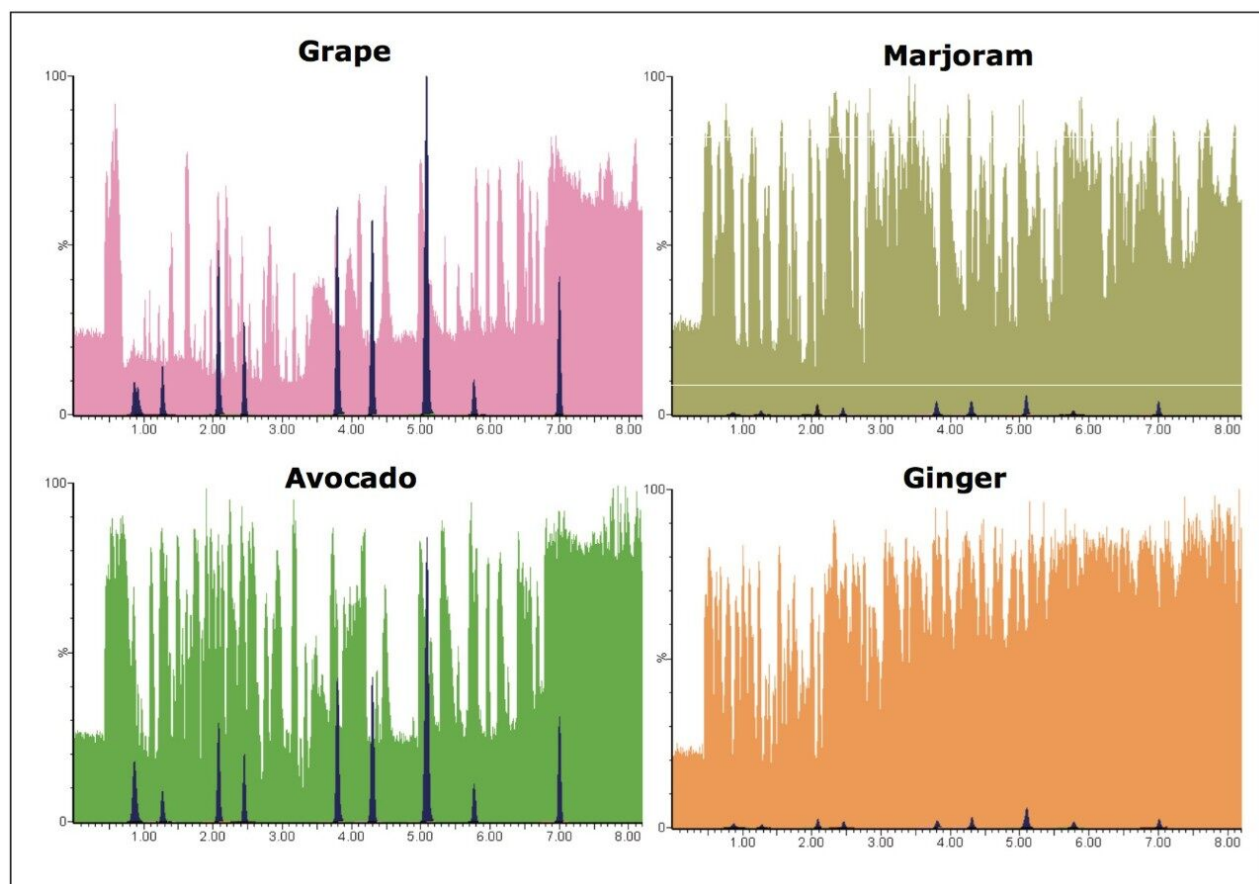


Figure 3. BPI chromatograms overlaid with MRM chromatograms for pesticides spiked at 0.01 mg/kg into grape (1.0 g/mL matrix), avocado (1.0 g/mL), marjoram (0.1 g/mL), and ginger (0.1 g/mL).

Despite the reduction in matrix concentration, the ionizable background is high in marjoram and ginger samples, compared with grape and avocado; as a consequence, the likelihood for analyte ion suppression (and enhancement) may be higher for these types of samples.

With simultaneous full scan it is also possible to observe specific components that co-elute with target analytes. Figure 4 shows BPI and MRM mass chromatograms for a grape sample spiked with dimethoate at 0.01 mg/kg. Full scan spectra from the elution region of dimethoate were combined and the most intense ion from the mass spectrum extracted into another chromatogram (XIC), revealing a discrete peak that co-elutes with dimethoate, as shown in Figure 4.

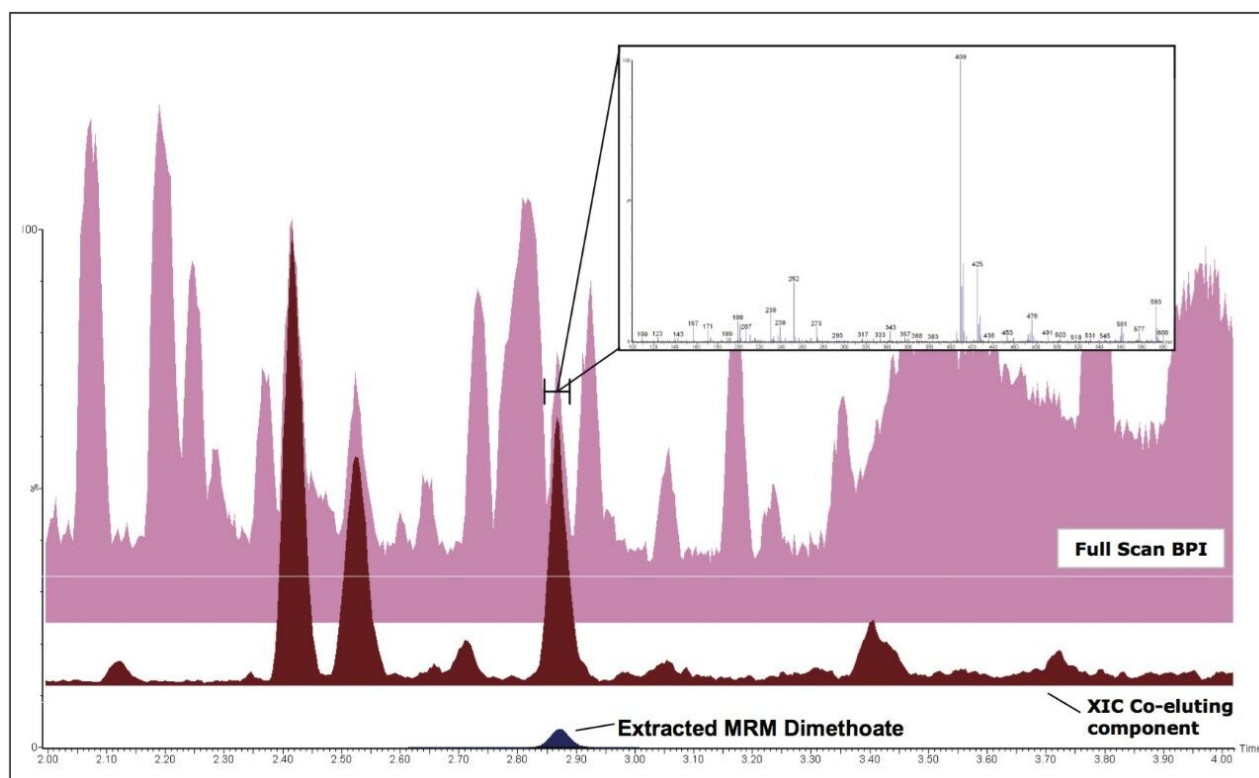


Figure 4. RADAR full scan BPI and MRM mass chromatograms for a grape sample spiked with dimethoate at 0.01 mg/kg. Also shown is the extracted ion chromatogram (XIC) of the co-eluting component with the subtracted mass spectrum inset.

If significant problems are observed with this or any other components in the matrix, the ability to observe them allows for further investigation and necessary remedial action to be carried out. Also, this acquisition mode can help to track the clean-up efficiency of the methodology employed.

Reduction of matrix effects

Minimizing matrix effects allows higher confidence in the quality of analytical data obtained. Reducing matrix concentration injected onto the analytical system is a simple and effective means to do this. When using a standard flow ESI source this can be achieved by reducing the amount of sample to be extracted, reducing the number of sample enrichment steps, or diluting final extracts. In any case, this is only a possibility if enough sensitivity is available to maintain detection at the required concentrations.

Ginger samples showed the highest ionizable background when compared to all other samples, despite having a relatively low matrix concentration (0.1 g/mL), as shown in Figure 3. Matrix effects were observed in the ginger samples with ion suppression and chromatography problems most apparent.

Diluting the ginger extracts 10x allowed recovery of distorted peak shape for cyromazine and reduction in matrix suppression for a number of pesticides, as shown in Figure 5. Table 1 shows reduction of ion suppression with a 10x dilution of sample. This reduction in suppression is clear when comparing peak area of pesticides in ginger to standards with no matrix present. As the matrix concentration is reduced the peak area response begins to correlate closely with standard peak areas.

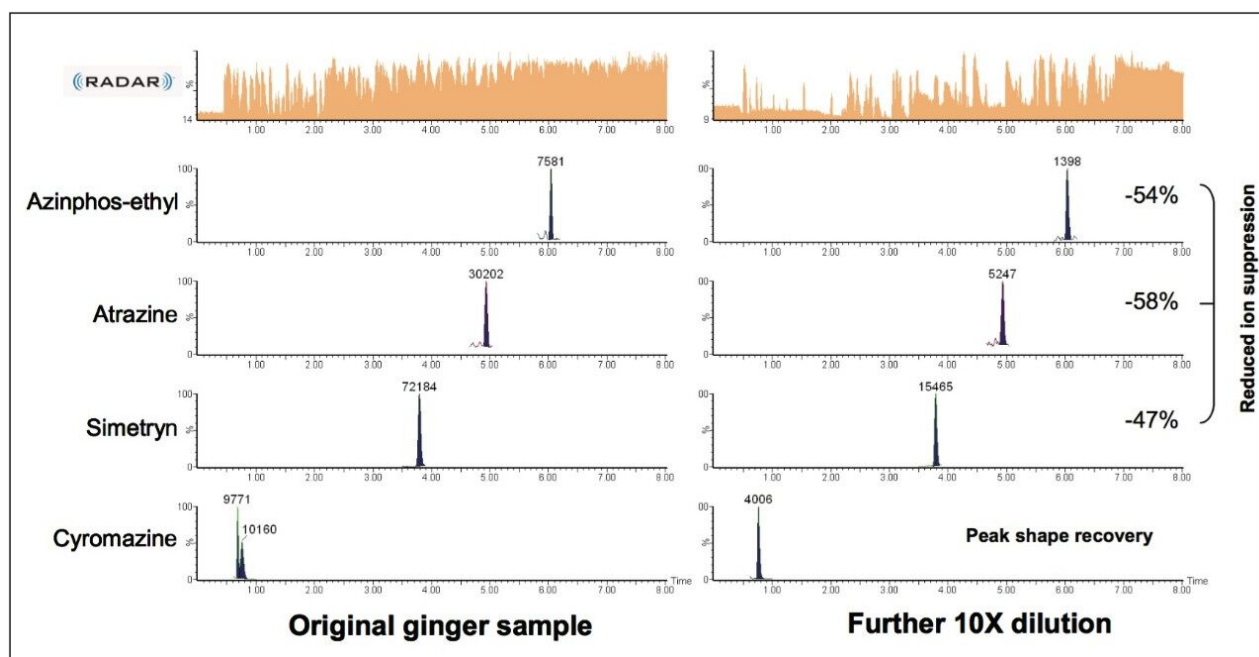


Figure 5. Effects of reducing sample matrix concentration by dilution for ginger. The full scan RADAR background is shown in the top chromatogram with MRM chromatograms for a selection of pesticides below.

	% Peak area recovery to standard	
	Original Extract	Diluted Extract
Thiabendazole	89.2	105.2
Atrazine-desisopropyl	71.6	100.8
Aldicarb	36.4	91.2
Desmetryn	49.2	97.0
Prometon	85.8	109.2
Simazine	63.1	103.4
Hexazinone	80.0	98.7
Demeton S Methyl	69.7	117.0
Tebuthiuron	79.1	96.3
Ametryn	66.7	103.4
Terbutryn	81.7	102.8
Azinphos Methyl	58.1	91.8
Trietazine	46.8	91.6
Azinphos Ethyl	60.5	86.1

Table 1. Reduction of ion suppression for a ginger extract upon 10x dilution of original samples. Calculated as percent peak area recovery to a standard injection with no matrix present.

Conclusion

- Xevo TQ-S allows detection of pesticides in complex food matrices using large multi-residue methods to below the required regulatory concentrations. This includes compounds with poor relative response factors.
- The RADAR mode of acquisition enables the collection of spectral information on background components in the sample matrix while simultaneously collecting MRM data. This can help identify areas of potential ion suppression, observe untargeted contaminants, and aid in the development of matrix reduction strategies.
- Where matrix effects are observed, the high sensitivity offered by Xevo TQ-S allows matrix concentration in samples to be reduced to counteract these effects. This is possible while maintaining detection at regulatory concentrations and allows higher confidence in reported data.

References

1. J M Marín *et al.* *Journal of Chromatography A*. 1216: 9, 1410-1420. 27 February 2009.
2. Hajšlová & Zrostlíková. *Journal of Chromatography A*. 1000:1-2, 181-197. 6 June 2003.
3. Gosetti *et al.* *Journal of Chromatography A*. 1217: 25, 3929-3937, 18 June 2010.
4. Kruve *et al.* *Journal of Chromatography A*. 1187: 1-2, 58-66, 11 April 2008.
5. website: http://ec.europa.eu/sanco_pesticides/public/index.cfm

Appendix 1 Pesticide MRM Parameters

	Precursor ion	Product ion	Collision (V)		Precursor ion	Product ion	Collision (V)
Acephate	206	64	10	Imazapyr	262	69	24
	206	117	12		262	86	24
Acetamiprid	223	56	28	Imazaquin	312	86	26
	223	126	12		312	267	18
Aldicarb	213	89	14	Imidacloprid	256	175	18
	213	116	19		256	209	14
Ametryn	228	68	15	Isoproturon	207	46	15
	228	186	10		207	72	20
Atrazine	216	96	34	Isosaben	333	107	56
	216	174	16		333	165	16
Atrazine-desethyl	188	79	21	Kresoxim Methyl	336	229	15
	188	146	17		336	246	15
Atrazine-desisopropyl	174	79	25	Linuron	249	160	15
	174	96	15		249	182	15
Azamethiphos	325	112	16	Malaaxon	315	99	22
	325	139	16		315	127	11
Azinphos Ethyl	368	132	22	Metaxyl	280	192	16
	368	160	35		280	220	12
Azinphos Methyl	340	132	15	Metamitron	203	104	20
	340	160	10		203	175	15
Azoxystrobin	404	329	15	Methamidophos	142	94	12
	404	372	10		142	125	12
Buturon	237	84	28	Metobromuron	259	148	14
	237	126	14		259	170	18
Cadusafos	271	131	15	Metosulam	418	140	50
	271	159	28		418	175	26
Carbaryl	202	117	20	Mevinphos	225	127	14
	202	145	15		225	193	9
Chlorbromuron	293	182	22	Monolinuron	215	99	32
	293	204	12		215	126	20
Chlorpyrifos	350	97	15	Monuron	199	72	15
	350	198	20		199	126	23
Chlorpyrifos Methyl	322	125	25	Omethoate	214	125	20
	322	290	15		214	183	10
Chlortoluron	213	46	15	Parathion	292	236	12
	213	72	15		292	264	10
Clodinafop-propargyl	350	91	15	Phoxim	299	129	15
	350	266	16		299	153	7
Coumaphos	363	289	30	Pirimiphos-ethyl	334	182	23
	363	307	15		334	198	21
Cyanazine	241	96	22	Pirimiphos-methyl	306	108	30
	241	214	14		306	164	20
Cyromazine	167	60	23	Prometon	226	86	26
	167	108	15		226	184	16
Demeton S Methyl	253	61	17	Propaquizafop	444	100	15
	253	89	17		444	371	15
Demeton S methyl sulfone	263	121	28	Pymetrozine	218	79	28
	263	169	14		218	105	18
Desmetryn	214	82	28	Pyraclostrobin	388	163	23
	214	172	19		388	194	11
Dicrotophos	238	112	10	Pyrazophos	374	194	30
	238	193	10		374	222	20
Difenoxyuron	287	72	18	Quinmerac	222	141	28
	287	123	18		222	204	14
Diflubenzuron	311	141	30	Quizalofop-ethyl	373	91	30
	311	158	15		373	299	16
Dimeturon	339	72	24	Siduron	233	94	23
	339	167	18		233	137	15
Dimethoate	230	125	18	Simazine	202	96	22
	230	199	10		202	124	16
Dimethomorph	388	165	28	Simetryn	214	96	23
	388	301	18		214	124	18
Disulfoton	297	61	32	Spiroxamine	298	100	30
	297	89	12		298	144	19
Diuron	233	46	13	Sulfotep	323	97	30
	233	72	16		323	171	14
Ethoprophos	243	97	29	Tebuthiuron	229	116	24
	243	131	18		229	172	16
Fenuron	165	46	13	Terbutylazine	230	96	26
	165	72	15		230	174	15
Flamprop-methyl	336	77	46	Terbutryn	242	186	15
	336	105	15		242	200	15
Fluazafop-P-butyl	384	282	20	Tetrachlorvinphos	365	127	15
	384	328	15		365	239	18
Flufenacet	364	152	18	Thiabendazole	202	131	26
	364	194	10		202	175	24
Fluomethuron	233	46	16	Trietazine	230	71	28
	233	72	16		230	99	21
Heptenophos	251	125	13	Zoxamide	336	159	36
	251	127	13		336	187	23
Hexazinone	253	71	28				
	253	171	15				

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