AOAC Official Method 2007.01
Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate

Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry

First Action 2007

[Applicable for the following pesticides in grapes, lettuces, and oranges: atrazine, azoxystrobin, bifenthrin, carbaryl, chlorothalonil, chlorpyrifos, chlorpyrifos-methyl, -cyhalothrin (incurred in lettuces), cyprodinil, *o,p* -DDD, dichlorvos, endosulfan sulfate, ethion (incurred in oranges), imazalil, imidacloprid, kresoxim-methyl (incurred in grapes), linuron, methamidophos, methomyl, permethrins (incurred in lettuces) procymidone, pymetrozine, tebuconazole, thiabendazole (incurred in oranges), tolylfluanid (degraded in lettuces), and trifluralin. These were representative pesticide analytes chosen in representative matrixes, and the method is expected to be applicable to many other similar pesticides and matrixes. Limits of quantitation were demonstrated to be <10 ng/g.]

See Tables **2007.01A–E** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method uses a single-step buffered acetonitrile (MeCN) extraction and salting out liquid–liquid partitioning from the water in the sample with $MgSO_4$. Dispersive-solid-phase extraction (dispersive-SPE) cleanup is done to remove organic acids, excess water, and other components with a combination of primary secondary amine (PSA) sorbent and $MgSO_4$; then the extracts are analyzed by mass spectrometry (MS) techniques after a chromatographic analytical separation. Figure **2007.01** outlines the protocol in a box format. In brief, a well-chopped food sample along with 1 mL of 1% acetic acid (HOAc) in MeCN and 0.5 g anhydrous $MgSO_4$/NaOAc (4/1, w/w) per g sample are added to a centrifuge tube or bottle, which is shaken and centrifuged. A portion of the MeCN extract (upper layer) is added to anhydrous $MgSO_4$/PSA sorbent (3/1, w/w; 200 mg per 1 mL extract), mixed, and centrifuged. This final extract is transferred to autosampler vials for analysis by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) to identify and determine a wide range of pesticide residues. To achieve <10 ng/g detection limits in modern GC/MS, large volume injection (LVI) of 8 L is typically needed, or the final extract can be concentrated and solvent exchanged to toluene (4 g/mL), in which case 2 L splitless injection is used.

Both GC/MS and LC/MS/MS techniques are prone to matrix effects in pesticide residue analysis, albeit for different reasons [Erney, D.R., Gillespie, A.M., Gilvydis, D.M., & Poole, C.F. (1993) *J. Chromatogr.* **638**, 57–63; Hajslova, J., & Zrostlikova, J. (2003) *J. Chromatogr. A* **1000**, 181–197; Alder, L., Luderitz, S., Lindtner, K., & Stan, H.J. (2004) *J. Chromatogr. A* **1058**, 67–79]. To account for these effects, matrix-matched calibration was conducted (calibration standards in solvent solution may also be used if matrix effects are shown not to occur). Due to the situation that some laboratories had LVI capability and others did not, the necessary amounts of matrix blank(s) and final extract volume was different for some laboratories than others. Depending on the water content of the matrix, a 15 g sample typically yields 11–14 mL of initial MeCN extract after centrifugation. In dispersive-SPE, roughly half of the extract is lost to the powders, thus about 6–7 mL of final extract can be expected for a 15 g sample. Two options were provided in the protocol to account for the different situations among the laboratories.

Table 2007.01A. Interlaboratory study results for incurred pesticides (and chlorpyrifos-methyl)

Analyte	Matrix	Avg. concn	s_r[a]	RSD_r[b], %	S_R[c], ng/g	Rec., %	RSD_R[d], %	HorRat	No. of labs	Outlier labs[e]
Chlorpyrifos-methyl	Grapes	165	14	8.5	35	83	21	1.00	11	6-C, 4-C
	Lettuces	178	20	11	30	89	17	0.81	10	11-SG
	Oranges	174	25	14	36	87	20	0.98	12	
Kresoxim-methyl	Grapes	9.2	1.9	21[f]	3.2	NA	35[f]	1.09	12	
Cyprodinil	Grapes	112	NA[g]	NA	18	NA	16	0.73	13	
-Cyhalothrin	Lettuces	58	6.1	11	11	NA	20	0.80	9	11-C
Permethrins	Lettuces	112	9.8	8.7	41	NA	36[f]	1.63	9	6-C, 1-C
Imidacloprid	Lettuces	12	NA	NA	1.6	NA	14	0.44	11	
Ethion	Oranges	198	23	12	36	NA	18	0.89	11	11-C
Thiabendazole	Oranges	53	3.8	7.2	7.6	NA	14	0.58	12	
Imazalil	Oranges	13	NA	NA	4.7	NA	35[f]	1.15	8	7-SG

[a] s_r = Standard deviation for repeatability (within laboratory).

[b] RSD_r = Relative standard deviation for repeatability.

[c] s_R = Standard deviation for reproducibility (among laboratories).

[d] RSD_R = Relative standard deviation for reproducibility.

[e] C = Cochran outlier; SG = single Grubbs outlier.

[f] RSD_r >15%; 120% < Rec. < 70%; RSD_R >25%; HorRat >1.2; and fewer than 8 laboratories in an assessment.

[g] NA = Not applicable.

Table 2007.01B. Interlaboratory study results for fortified pesticides in grapes

Analyte	Avg. C, ng/g	s_r, ng/g	RSD_r, %	s_R, ng/g	Rec., %	RSD_R, %	HorRat	No. of labs	Outlier labs[a]
Atrazine	9.3	0.6	6.9	2.0	93	21	0.65	13	
	45	3.2	7.1	5.7	90	13	0.49	13	
	365	23	6.2	71	91	19	1.04	13	
Azoxystrobin	9.4	0.6	6.6	2.0	94	21	0.64	13	
	92	8.7	9.4	11	92	12	0.51	12	8-SG
	182	17	9.2	26	91	14	0.70	12	8-SG
Bifenthrin	7.8	0.8	11	2.3	78	30[b]	0.89	11	2-C, 10-C
	86	5.9	6.9	14	86	17	0.73	12	6-C
	923	71	7.7	136	92	15	0.91	13	
Carbaryl	12	1.2	11	2.8	104	27[b]	0.85	12	5-SG
	50	6.4	13	11	100	22	0.87	13	
	1003	70	7.0	189	100	19	1.18	12	5-C
Chlorothalonil	6.3	0.9	14	2.1	63[b]	33[b]	0.97	8	10-C
	59	8.3	14	13	79	23	0.93	10	
	140	19	13	38	70	27[b]	1.27[b]	10	
Chloropyrifos	8.1	1.5	19[b]	3.0	81	37[b]	1.12	12	
	68	8.3	12	14	84	20	0.84	13	
	396	25	6.4	50	79	12	0.68	12	11-SG
Cyprodinil[c]	123	13	10	26	101	21	0.95	13	
	240	20	8.3	63	92	26[b]	1.32[b]	13	
	581	42	7.3	110	95	19	1.09	13	
o,p -DDD	8.9	1.4	16[b]	3.2	89	36[b]	1.09	12	
	42	3.1	7.3	7.0	84	17	0.65	12	
	445	32	7.1	47	89	10	0.58	11	6-C
Dichlorvos	7.2	1.0	14	1.3	72	18	0.53	11	8-SG
	85	7.4	8.7	15	85	18	0.77	11	4-C
	294	25	8.5	62	98	21	1.10	12	
Endosulfan sulfate	8.6	0.9	10	1.5	86	17	0.52	7[b]	10-C
	115	14	12	21	77	18	0.81	11	
	415	56	14	111	83	27[b]	1.47[b]	11	
Imazalil	7.6	0.8	9.8	3.1	76	41[b]	1.22[b]	11	
	50	2.5	4.9	15	67[b]	30[b]	1.19	10	8-C
	432	53	12	161	78	37[b]	2.06[b]	11	
Imidacloprid	8.8	0.8	8.9	3.0	88	34[b]	1.04	13	
	45	3.5	7.7	8.9	99	20	0.78	13	
	218	18	8.2	24	97	11	0.56	12	8-SG
Linuron	9.9	1.7	17[b]	2.9	99	29[b]	0.90	11	
	99	7.4	7.4	15	99	15	0.67	12	
	971	65	6.7	191	97	20	1.23[b]	12	
Methamidophos	10	2.9	29[b]	3.0	101	30[b]	0.95	9	5-SG
	80	8.0	10	14	80	18	0.77	12	
	852	72	8.4	119	85	14	0.85	11	8-SG
Methomyl	9.3	1.2	12	2.9	93	32[b]	0.98	12	
	50	3.3	6.7	9.3	100	19	0.74	13	
	204	10	4.9	26	102	13	0.63	13	
Procymidone	8.2	0.7	8.2	2.0	82	24	0.74	11	5-SG
	64	6.0	9.4	16	85	24	1.01	13	
	428	16	3.8	70	86	16	0.90	12	9-C
Pymetrozine	6.2	1.2	20[b]	1.6	62[b]	27[b]	0.77	11	
	47	3.1	6.7	9.6	62[b]	20	0.81	11	
	341	20	5.8	59	68[b]	17	0.92	11	
Tebuconazole	9.2	1.1	12	1.2	92	13	0.41	12	3&4-DG
	63	5.5	8.7	8.8	84	14	0.58	13	
	439	29	6.7	84	88	19	1.06	13	
Tolylfluanid	7.9	1.0	12	3.1	79	39[b]	1.19	13	
	34	4.3	13	13	67[b]	37[b]	1.41[b]	13	
	144	13	8.8	42	72	29[b]	1.37[b]	13	
Trifluralin	7.8	0.7	8.5	1.8	78	23	0.68	12	10-C
	58	3.7	6.4	14	77	25	1.02	13	
	379	19	5.1	48	76	13	0.69	10	6-C, 4-C, 11-SG

[a] C = Cochran outlier; SG = single Grubbs outlier; DG = double Grubbs outliers.

[b] RSD_r >15%; 120% < Rec. < 70%; RSD_R >25%; HorRat >1.2; or fewer than 8 laboratories in an assessment.

[c] Cyprodinil was incurred in the grapes and affected quantitation.

Table 2007.01C. Interlaboratory study results for fortified pesticides in lettuces

Analyte	Avg. C, ng/g	s_r, ng/g	RSD_r, %	s_R, ng/g	Rec., %	RSD_R, %	HorRat	No. of labs	Outlier labs[a]
Atrazine	9.9	1.5	15	1.8	99	18	0.56	11	
	70	7.9	11	15	93	21	0.88	12	
	930	50	5.4	166	93	18	1.11	11	5-C
Azoxystrobin	10	0.8	7.6	1.8	102	18	0.56	12	
	47	2.4	5.2	6.5	93	14	0.55	12	
	531	32	6.1	88	106	17	0.94	12	
Bifenthrin	9.1	0.8	9.1	1.4	91	16	0.48	11	
	66	8.0	12	9.4	88	14	0.59	11	
	217	27	12	33	87	15	0.77	11	
Carbaryl	9.4	1.1	12	2.0	94	22	0.67	12	
	92	6.1	6.7	9.0	92	9.8	0.43	11	8-SG
	589	38	6.4	127	98	22	1.24[b]	12	
Chlorothalonil	6.2	0.8	14	2.0	62[b]	32[b]	0.93	6[b]	
	28	10	37[b]	14	70	48[b]	1.77[b]	7[b]	
	684	134	20[b]	205	68[b]	30[b]	1.77[b]	6[b]	
Chloropyrifos	9.0	2.1	24[b]	2.3	90	26[b]	0.79	9	12-SG, 10&11-DG
	86	9.4	11	20	86	23	1.01	11	11-SG
	179	18	10	30	90	17	0.82	11	11-SG
Cyprodinil	9.7	1.0	10	1.4	97	14	0.44	11	11-SG
	44	2.7	6.1	8.9	89	20	0.79	11	11-SG
	848	61	7.2	117	85	14	0.84	10	8-SG
o,p -DDD	8.9	0.6	7.0	1.9	89	21	0.66	8	
	81	4.8	5.9	12	81	15	0.63	9	11-C
	214	19	8.7	27	86	13	0.62	10	
Dichlorvos	5.2	1.0	20[b]	2.4	52[b]	45[b]	1.29[b]	12	
	58	6.6	11	12	77	20	0.81	12	
	838	50	6.0	224	84	27	1.63[b]	11	
Endosulfan sulfate	5.6	3.3	59[b]	2.5	56[b]	45[b]	1.28[b]	2[b]	
	38	9.6	25[b]	15	75	39[b]	1.48[b]	7[b]	
	769	330	43[b]	312	77	40[b]	2.44[b]	7[b]	
Imazalil	7.6	0.3	3.5	3.5	76	39[b]	1.18	8	2-C
	72	3.7	5.2	24	57[b]	33[b]	1.39[b]	11	
	589	47	7.9	229	59[b]	39[b]	2.25[b]	11	
Imidacloprid[c]	22	1.3	6.2	1.7	100	7.9	0.28	11	8-SG
	84	6.2	7.4	8.1	97	9.6	0.41	12	
	515	21	4.2	53	101	10	0.58	11	5-C
Linuron	8.6	1.1	12	1.5	86	17	0.53	11	
	46	2.2	4.9	7.4	91	16	0.63	10	2-C
	234	14	5.8	25	94	10	0.53	11	
Methamidophos	8.8	0.8	8.5	1.3	88	15	0.46	8	6-C
	66	4.5	6.9	12	82	18	0.72	11	
	538	37	6.8	63	84	12	0.67	9	5-C, 8-SG
Methomyl	9.7	0.8	8.6	1.0	96	10	0.32	10	
	99	8.0	8.1	6.4	99	6.5	0.29	10	2-SG
	997	24	2.4	168	100	17	1.05	11	
Procymidone	10	0.6	6.2	2.2	101	22	0.68	8	2-C
	92	8.5	9.2	15	92	17	0.73	11	
	967	118	12	129	97	13	0.83	11	
Pymetrozine	6.9	0.4	6.1	1.4	69[b]	20	0.59	10	
	33	1.6	4.7	4.6	67[b]	14	0.51	9	11-C
	127	8.5	6.7	17	63[b]	13	0.61	10	
Tebuconazole	9.7	0.7	6.9	1.2	97	13	0.40	11	4-C
	89	6.8	7.7	11	89	12	0.52	12	
	948	42	4.4	226	95	24	1.48[b]	11	4-C
Tolylfluanid	3.7	1.1	30[b]	2.2	37[b]	59[b]	1.59[b]	4[b]	
	9.3	3.7	40[b]	4.1	9.3[b]	44[b]	1.37[b]	8	3-SG, 8-SG
	142	22	15	86	14[b]	61[b]	2.84[b]	8	12-C, 3&8-DG
Trifluralin	10	1.4	13	1.7	103	17	0.54	11	
	42	4.5	11	9.0	84	22	0.83	11	
	169	25	15	30	84	18	0.84	11	

[a] C = Cochran outlier; SG = single Grubbs outlier; DG = double Grubbs outliers.

[b] RSD_r >15%; 120% < Rec. < 70%; RSD_R >25%; HorRat >1.2; or fewer than 8 laboratories in an assessment.

[c] Imidacloprid was incurred in the lettuces unbeknownst to the SD.

Table 2007.01D. Interlaboratory study results for fortified pesticides in oranges

Analyte	Avg. C, ng/g	s_r, ng/g	RSD_r, %	s_R, ng/g	Rec., %	RSD_R, %	HorRat	No. of labs	Outlier labs[a]
Atrazine	8.9	1.0	11	1.9	89	21	0.65	11	2-C
	90	8.2	9.1	12	90	13	0.57	12	
	187	19	10	27	93	14	0.69	12	
Azoxystrobin	8.4	1.3	16[b]	1.8	84	21	0.65	11	
	65	5.2	8.0	8.1	86	12	0.52	12	
	853	35	4.1	82	85	9.6	0.59	11	11-C
Bifenthrin	9.7	2.3	24[b]	2.3	97	24	0.75	10	9-SG
	45	2.5	5.6	6.8	91	15	0.59	10	2-C
	488	51	10	76	98	16	0.87	12	
Carbaryl	8.4	0.6	7.3	2.1	84	25	0.77	10	
	66	5.0	7.5	14	88	21	0.88	11	
	172	8.8	5.1	34	86	20	0.95	12	
Chlorothalonil	4.8	0.8	16[b]	2.7	48[b]	56[b]	1.57[b]	3[b]	
	70	14	20[b]	29	70	42[b]	1.74[b]	6[b]	
	330	137	42[b]	131	66[b]	40[b]	2.09[b]	7[b]	
Chloropyrifos	11	1.6	14	5.0	111	45[b]	1.58[b]	9	2-C
	82	4.5	5.6	12	82	15	0.64	10	9-C
	953	97	10	284	95	30[b]	1.85[b]	12	11-C
Cyprodinil	8.7	0.9	10	2.0	87	23	0.72	12	
	56	4.5	8.0	9.0	75	16	0.65	12	
	199	12	6.2	35	80	18	0.86	12	
o,p -DDD	9.1	0.6	7.2	1.8	91	20	0.60	9	9-C
	74	5.1	6.9	9.8	99	13	0.56	10	10-C
	967	81	8.4	191	97	20	1.22[b]	11	
Dichlorvos	9.3	0.8	8.1	1.0	93	11	0.35	7[b]	12-C
	43	2.2	5.2	8.0	85	19	0.73	8	12-SG
	446	22	5.0	54	89	12	0.68	10	
Endosulfan sulfate	12	5.4	44[b]	5.4	124[b]	43[b]	1.40[b]	4[b]	3-SG
	83	19	23[b]	19	83	23	1.01	10	
	240	35	15	61	80	25	1.28[b]	10	
Imazalil[c]	22	1.7	7.7	6.2	96	28[b]	0.98	8	7-C
	58	4.3	7.4	13	92	22	0.91	9	
	186	9.7	5.2	41	87	22	1.06	10	
Imidacloprid	10	1.1	10	2.8	104	27[b]	0.86	11	
	93	6.5	7.0	12	93	13	0.57	11	
	989	64	6.5	124	99	13	0.78	11	
Linuron	7.8	1.3	17[b]	2.7	78	35[b]	1.04	11	
	60	3.0	5.0	13	86	21	0.86	11	
	387	26	6.6	42	79	11	0.59	9	11,1-DG
Methamidophos	9.2	1.1	12	1.5	92	16	0.49	8	9-C
	42	3.5	8.2	5.6	85	13	0.52	8	4-C
	211	12	5.5	31	85	15	0.73	9	4&9-DG
Methomyl	8.5	0.8	8.9	2.8	85	33[b]	0.99	9	7-C
	68	4.8	7.0	8.7	91	13	0.54	12	
	492	19	3.9	60	98	12	0.69	12	
Procymidone	11	0.9	8.1	3.9	108	36[b]	1.15	8	12-C
	43	3.5	8.0	5.8	86	14	0.53	10	10-C
	170	16	9.7	25	85	15	0.71	11	
Pymetrozine	7.5	1.3	18[b]	2.1	75	28[b]	0.82	10	
	77	5.9	7.7	10	77	14	0.57	10	
	789	38	4.8	117	79	15	0.89	9	12-C
Tebuconazole	8.7	0.7	8.0	1.2	87	14	0.42	11	
	41	2.2	5.4	6.2	82	15	0.58	12	
	177	14	7.9	28	88	16	0.76	12	
Tolylfluanid	5.8	1.2	20[b]	1.4	58[b]	24	0.69	9	11-SG
	46	7.5	16[b]	14	61[b]	31[b]	1.21[b]	11	9-C
	356	54	15	134	71	38[b]	2.02[b]	12	
Trifluralin	8.6	0.4	4.5	2.4	86	28[b]	0.87	9	9-C
	92	8.6	9.4	11	92	12	0.54	12	
	915	60	6.5	194	92	21	1.31[b]	11	6-C

[a] C = Cochran outlier; SG = single Grubbs outlier; DG = double Grubbs outliers.

[b] RSD_r >15%; 120% < Rec. < 70%; RSD_R >25%; HorRat >1.2; or fewer than 8 laboratories in an assessment.

[c] Imazalil was incurred in the oranges unbeknownst to the SD.

Table 2007.01E. Averaged interlaboratory study results for the fortified and incurred pesticides[a]

Matrix	Recovery, %	RSD_r, %	RSD_R, %	HorRat	No. of labs (*n*)
Grapes	86 ± 11	10 ± 4	22 ± 8	0.90 ± 0.29	12 ± 1
Lettuces	87 ± 12	10 ± 7	20 ± 9	0.83 ± 0.45	10 ± 1
Oranges	87 ± 15	10 ± 6	20 ± 8	0.84 ± 0.37	10 ± 2
Overall	87 ± 11	10 ± 6	21 ± 8	0.86 ± 0.37	11 ± 2
Incurred	NA[b]	12 ± 4	22 ± 8	0.92 ± 0.30	11 ± 2

[a] Data from fewer than 7 laboratories in an assessment were excluded.

[b] NA = Not applicable.

In Option A, if the laboratory had LVI capability, then 1 or 2 mL extracts were taken for dispersive-SPE (the volume depended on the analyst preference and the type of centrifuge and tubes available in the laboratory). The final extract volume was 0.5 mL if 1 mL was taken for dispersive-SPE, and 1 mL if 2 mL underwent the cleanup step. In either case, two 15 g blank samples were used for the matrix blank (0-standard) and 6 matrix-matched calibration standards (5, 10, 50, 100, 250, and 1000 ng/g equivalent concentrations). For dispersive-SPE of the matrix blanks, either 7 separate tubes using the same 1–2 mL extract volumes as the test samples could have been used, or 1–2 dispersive-SPE tube(s) with 7-fold greater extract volume(s).

In Option B, if LVI is not available for GC/MS, then 30 mL of matrix blank extract was needed after dispersive-SPE cleanup to prepare the matrix-matched calibration standards (or ≥60 mL initial extract). In this case, 6 matrix blanks of 15 g each were extracted along with the test samples to provide enough blank extract volume, which were combined, and seven 8 mL aliquots were distributed to 7 dispersive-SPE tubes containing 0.4 g PSA + 1.2 g anhydrous $MgSO_4$.

B. Apparatus and Conditions

Note: Tables 4 and 5 of the collaborative study [J. AOAC Int. 90, 485(2007)] list the analytical instrumentation and sources of sample preparation materials used by each laboratory in the study. Further information appears in the full report. Since the time of the collaborative study, at least 3 vendors, United Chemical Technologies (Bristol, PA, USA), Restek (Bellefonte, PA, USA) and Supelco (Bellefonte, PA, USA) have introduced commercial dispersive-SPE products for QuEChERS and other applications. *See* Table 4 [J. AOAC Int. 90, 485(2007)] for sources of analytical instruments.

(**a**) *Gas chromatograph/mass spectrometer.*—An ion trap, quadrupole, time-of-flight (TOF), or other GC/MS instrument may be used with electron impact (EI) ionization, an autosampler (AS), and computerized instrument control/data collection. Either LVI of 8 L for a 1 g/mL MeCN extract (e.g., 75 C ramped to 275 C at 200 C/min) or 2 L splitless injection of 4 g/mL extracts in toluene at 250 C may be used. A 3–5 m, 0.25 mm id, phenylmethyl-deactivated guard column must be used as a retention gap in either case. The analytical column is a 30 m, 0.25 mm id, 0.25 m film thickness (5%phenyl)-methylpolysiloxane (low bleed) analytical column (DB-5ms or equivalent). Set He head pressure on the column to be 10 psi or constant flow to be 1.0 mL/min with systems capable of electronic pressure/flow control. After an appropriate time for solvent delay, use an appropriate oven temperature program, for example, starting at 75 C for MeCN extracts or 100 C for toluene ramped to 150 C at 25 C/min, then to 280 C at 10 C/min, and hold for 10 min. All collaborators had much experience in pesticide residue analysis and were free to use their own analytical conditions provided that peak shapes were Gaussian, peak widths at half heights were <5 s, and signal-to-noise ratio (S/N) of the quantitative ion for the pesticides at 10 ng/g equivalent concentrations in the sample were >10. For qualitative purposes (which were not the focus of this study), at least 3 ions yielding relative abundances that reasonably match a contemporaneously analyzed reference standard are typically needed to make an analyte identification.

(**b**) *Liquid chromatograph/tandem mass spectrometer.*— A triple quadrupole, ion trap, or other LC/MS/MS instrument may be used provided it is capable of electrospray ionization (ESI) in the positive mode with computerized instrument control/data collection and has an AS. An injection volume (5–100 L) will be determined for each instrument to achieve S/N > 10 for the quantitation ion for a 10 ng/g equivalent sample concentration. As in GC/MS, the collaborators had much experience in the analysis of pesticides and were free to use their own conditions. Suggested LC conditions, however, include a 15 cm long, 3.0 mm id, 3 m particle size C_{18} column, flow rate of 0.3 mL/min, and gradient elution with an initial condition of 25% MeOH in 5 mM formic acid solution taken linearly in 15 min to 90% MeOH in 5 mM formic acid solution and held for 15 min. A short C_{18} guard column must be used to protect the analytical column, and a bypass valve must be used before the MS instrument to avoid introduction of the early and late eluting nonanalyte components into the detector. The MS/MS conditions were optimized in each laboratory using direct infusion into the ESI source to provide highest S/N for the quantitation ion of each LC-type analyte from a single MS/MS transition. A second transition with reasonably matching relative abundance ratios vs a contemporaneously analyzed reference standard is typically needed for qualitative purposes.

(**c**) *Centrifuge(s).*—Capable of holding the 50 mL centrifuge tubes or bottles used for extraction and 10–15 mL graduated centrifuge tubes or 2 mL mini-tubes used in dispersive-SPE. Determine the rpm settings that yield a given relative centrifugal force (RCF), and ensure that maximum ratings of the centrifuge, tube/bottles, and rotors for the instrument are not exceeded.

(**d**) *Balance(s).*—Capable of accurately measuring weights from 0.05 to 100 g within ±0.01 g.

(**e**) *Freezer.*—Capable of continuous operation <–20 C.

(**f**) *Furnace/oven.*—Capable of 500 C operation.

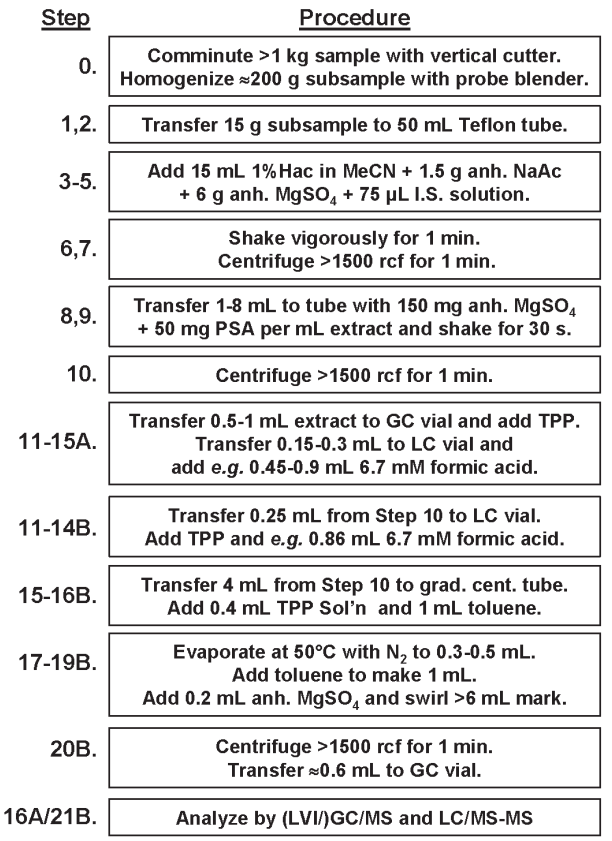

Figure 2007.01. Outline of the QuEChERS protocol used in the collaborative study.

(**g**) *Food chopper and/or blender.*—Preferably an s-blade vertical cutter (e.g. Stephan, Robotcoupe) and and probe blender (e.g. Ultra-Turrax, Propsep).

(**h**) *Solvent evaporator* (*optional*).—For the evaporation of MeCN extracts, if LVI is not used in GC/MS.

C. Reagents

[*See* Table 5 [J. AOAC Int. 90, 485(2007)] for sources of chemicals.]

(**a**) *Anhydrous magnesium sulfate ($MgSO_4$).*—Powder form; purity >98%; heated in bulk to 500 C for >5 h to remove phthalates and residual water.

(**b**) *Acetonitrile (MeCN).*—Quality of sufficient purity that is free of interfering compounds.

(**c**) *Acetic acid (HOAc).*—Glacial; quality of sufficient purity that is free of interfering compounds.

(**d**) *1% HOAc in MeCN.*—Prepared on a v/v basis (e.g., 10 mL glacial HOAc in a 1 L MeCN solution).

(**e**) *Anhydrous sodium acetate (NaOAc).*—Powder form ($NaOAc{\cdot}3H_2O$ may be substituted, but 0.17 g per g sample must be used rather than 0.1 g anhydrous NaOAc per g sample).

(**f**) *Primary secondary amine (PSA) sorbent.*—40 m particle size (Varian Part No. 12213024 or equivalent). (*Note*: Premade dispersive-SPE tubes are now available from at least 3 vendors.)

(**g**) *C_{18} sorbent* (*optional*).—40 m particle size, if samples contain >1% fat.

(**h**) *Graphitized carbon black (GCB) sorbent* (*optional*).—120/400 mesh size, if no structurally planar pesticides are included among the analytes.

(**i**) *Helium.*—Purity that has been demonstrated to be free of interfering compounds in GC/MS.

(**j**) *Toluene* (*optional*).—Quality of sufficient purity that is free of interfering compounds; only needed if LVI is not used in GC/MS.

(**k**) *Methanol (MeOH).*—Quality of sufficient purity that is free of interfering compounds in LC/MS/MS prepared in mobile phase solution.

(**l**) *Water.*—Quality of sufficient purity that is free of interfering compounds in LC/MS/MS.

(**m**) *Formic acid.*—Quality of sufficient purity that is free of interfering compounds in LC/MS/MS prepared in mobile phase solution.

(**n**) *Pesticide standards.*—High purity reference standards of the pesticide analytes, and quality control (QC) and internal standards (ISs) prepared at highly concentrated stock solutions (e.g., 2000 ng/ L) in MeCN with 0.1% HOAc. Stored in dark vials in the freezer. Check annually for stability.

(**o**) *Standard solutions.*—Prepared in MeCN for all collaborators: IS solution = 40 ng/ L of both d_{10}-parathion and d_6- -HCH in MeCN; triphenylphosphate (TPP) solution = 2 ng/ L TPP in 1% HOAc in MeCN solution; QC-spike solution = 40 ng/ L of the 27 pesticide analytes in 0.1% HOAc in MeCN; and individual test solutions = 10 ng/ L of each of the 30 compounds to be detected (except 40 ng/ L TPP) in 0.1% HOAc in MeCN solution. Collaborators prepared a test mix and calibration standard spike solutions from those provided as described in **E**.

(**p**) *Blank sample.*—Verified to be free of analytes above the detection limit.

(**q**) *Other reagents.*—Certain instruments may require nitrogen or other materials/devices for their operation.

D. Materials

(**a**) *Fluorinated ethylene propylene (FEP) centrifuge tubes.*—50 mL; e.g., Nalgene Part No. 3114-0050 or equivalent for <16 g sample (or 250 mL FEP centrifuge bottles for 16–75 g sample size).

(**b**) *Spatula/spoon and funnel.*—For transferring sample into centrifuge tubes.

(**c**) *Solvent dispenser and 1–4 L solvent bottle.*—For transferring 15 mL 1% HOAc in MeCN per 15 g sample in FEP centrifuge tubes or bottles.

(**d**) *Centrifuge tubes (optional).*—10–15 mL graduated. For evaporation and/or dispersive-SPE.

(**e**) *Mini-centrifuge tubes (optional).*—2 mL. For dispersive-SPE (use tubes with o-ring-sealed caps to avoid leaks).

(**f**) *Syringes/pipets.*—Capable of accurate sample introduction of 2 or 8 L volume into GC/MS and appropriate volumes of matrix spike, IS, and calibration standard solutions (12.5–300 L).

(**g**) *Repeating or volumetric pipets.*—Capable of accurately transferring 0.5–8 mL solvent.

(**h**) *Containers.*—Graduated cylinders, volumetric flasks, weigh boats, vials, and/or other general containers in which to contain samples, extracts, solutions, standards, and reagents.

E. Preparation of Reagent Materials and Comminuted Sample

(*1*) Prepare the necessary number of sealable vials/cups containing 6.0 0.3 g anhydrous $MgSO_4$ + 1.5 0.1 g anhydrous NaOAc (or 2.5

0.2 g $NaOAc \cdot 3H_2O$) per 15 g sample. Scoops of appropriate volume can be used to speed the process, but weighing should still be done to check consistency. The containers should be sealed during storage and can be refilled and re-used without cleaning in between usages.

(*2*) Prepare the necessary number of appropriate centrifuge tubes (2 mL mini-centrifuge tubes or 10–15 mL centrifuge tubes) containing 0.05 0.01 g PSA sorbent + 0.15 0.03 g anhydrous $MgSO_4$ per 1 mL extract taken for dispersive-SPE cleanup. (*Note*: At least United Chemical Technologies, Restek, and Supelco now provide dispersive-SPE products commercially to replace this step.) If LVI is not available for GC/MS, then evaporation of the extracts will be needed, and 8 mL extract will be transferred to 10–15 mL sealable centrifuge tubes containing 0.40 0.08 g PSA sorbent + 1.20 0.24 g anhydrous $MgSO_4$. For matrixes that contain >1% fat, add an additional 0.05 0.01 g C_{18} sorbent per mL extract to the container. If no planar pesticides are among the analytes (e.g., thiabendazole, terbufos, quintozene, and hexachlorobenzene), then 0.05 0.01 g GCB sorbent per mL extract can also be added to the tube. (*Note*: Final extract volume may have to be reduced to 0.4 mL per 1 mL aliquot in dispersive-SPE if all 4 powders are used.)

(*3*) Prepare 1% HOAc in MeCN in dispenser bottle by adding 10 mL HOAc to 990 mL volume of MeCN or different desired amount in the same ratio.

(*4*) Label all vials and tubes appropriately that will be used in the method.

(*5*) *Note*: Step 5 was conducted by the Study Director (SD) when preparing the test samples. An appropriate chopper must be used to comminute large, representative sample portions. An uncommon or deuterated pesticide standard may be spiked into the sample during homogenization to determine the effectiveness of the procedure. Blend the sample until it gives a consistent texture. Transfer ≈200 g to a sealable container for freezer storage after further homogenization with a probe blender. Blend this subsample with the mixer until it is homogeneous. The test portion (e.g., 15 g) is taken for extraction immediately, and the container is then sealed and stored in the freezer in case re-analysis is necessary. The advantages of this approach are that the 15 g portion is highly representative of the original sample, the sample is well-comminuted to improve extraction by shaking, less time is spent on the overall homogenization process than trying to provide equivalent homogenization of the large initial sample with the chopper, and a frozen subsample is available for re-analysis if needed.

To provide the most homogeneous comminuted samples, frozen conditions, sufficient chopping time, and appropriate sample size to chopper volume ratio should be used. Use of frozen samples also minimizes degradative and volatilization losses of certain pesticides. In this case, cut the sample into 2–5 cm^3 portions with a knife and store the sample in the freezer prior to processing. Cryogenic blending devices, liquid nitrogen, or dry ice may also be used (but make sure all dry ice has sublimed before weighing samples and ensure that water condensation is minimal, especially in a humid environment).

(*6*) For laboratories with LVI in GC/MS, prepare a test mix of the pesticides in MeCN + 0.1% HOAc to determine the retention times (t_R) and MS quantitation/diagnostic ions at the particular GC/MS conditions to be used in the analysis [*see* Table 2 of the collaborative study (J. AOAC Int. 90, 485(2007))].

The preparation of the test mix and calibration spiking standards are described as follows:

(*1*) *Test mix in MeCN + 0.1% HOAc*.—4 ng/ L in 10 mL of all 30 compounds to be analyzed. Add 1 mL each of QC-spike solution + IS solution + TPP test solution + 1% HOAc in MeCN and fill to 10 mL with MeCN. Calibration spike standards in MeCN for 27 pesticide analytes (make 10 mL each in volumetric flasks, then transfer to 15 mL dark glass vials and store in freezer).

(*2*) *Cal-standard-1000*.—20 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 5 mL QC-spike solution + 1 mL IS solution + 1 mL 1% HOAc in MeCN and fill to the mark with MeCN.

(*3*) *Cal-standard-250*.—5 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 1.25 mL QC-spike solution + 1 mL IS solution + 1 mL 1% HOAc in MeCN and fill to the mark with MeCN.

(*4*) *Cal-standard-100*.—2 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 500 L QC-spike solution + 1 mL IS solution + 1 mL 1% HOAc in MeCN and fill to the mark with MeCN.

(*5*) *Cal-standard-50*.—1 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 250 L QC-spike solution + 1 mL IS solution + 1 mL 1% HOAc in MeCN and fill to the mark with MeCN.

(*6*) *Cal-standard-10*.—0.2 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 50 L QC-spike solution + 1 mL IS solution + 1 mL 1% HOAc in MeCN and fill to the mark with MeCN.

(*7*) *Cal-standard-5*.—0.1 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 25 L QC-spike solution + 1 mL IS solution + 1 mL 1% HOAc in MeCN and fill to the mark with MeCN.

For laboratories without LVI in GC/MS, the preparation of the test mix and the calibration spiking standards are described below:

(*1a*) *Test mix for GC in toluene*.—4 ng/ L in 10 mL of all 30 compounds to be analyzed. Add 1 mL QC-spike solution + 1 mL IS solution + 1 mL TPP test solution and fill to 10 mL with toluene. Calibration spike standards in MeCN for LC/MS/MS (in dark glass AS vials stored in freezer).

(*2a*) *Cal-standard-1000*.—20 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 500 L QC-spike solution + 100 L IS solution + 100 L 1% HOAc in MeCN + 320 L MeCN.

(*3a*) *Cal-standard-250*.—5 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 125 L QC-spike solution + 100 L IS solution + 100 L 1% HOAc in MeCN + 695 L MeCN.

(*4a*) *Cal-standard-100*.—2 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 50 L QC-spike solution + 100 L IS solution + 100 L 1% HOAc in MeCN + 770 L MeCN.

Dilute QC-spike solution.—4 ng/ L. Transfer 100 L QC-spike solution to AS vial and add 900 L MeCN.

(*5a*) *Cal-standard-50*.—1 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 250 L dilute QC-spike solution + 100 L IS solution + 100 L 1% HOAc + 570 L MeCN.

(*6a*) *Cal-standard-10*.—0.2 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 50 L dilute QC-spike solution + 100 L IS solution + 100 L 1% HOAc and 770 L MeCN.

(*7a*) *Cal-standard-5*.—0.1 ng/ L of each pesticide + 4 ng/ L IS in MeCN + 0.1% HOAc. Add 25 L dilute QC-spike solution + 100 L IS solution + 100 L 1% HOAc + 795 L MeCN.

Calibration spike standards in toluene.—Make 10 mL each in volumetric flasks, then transfer to 15 mL dark glass vials and store in freezer.

(*8a*) *Cal-standard-1000-tol*.—20 ng/ L of each pesticide + 4 ng/ L IS in toluene. Add 5 mL QC-spike solution + 1 mL IS solution and fill to the mark with toluene.

(*9a*) *Cal-standard-250-tol*.—5 ng/ L of each pesticide + 4 ng/ L IS in toluene. Add 1.25 mL QC-spike solution + 1 mL IS solution and fill to the mark with toluene.

(*10a*) *Cal-standard-100-tol*.—2 ng/ L of each pesticide + 4 ng/ L IS in toluene. Add 500 L QC-spike solution + 1 mL IS solution and fill to the mark with toluene.

(*11a*) *Cal-standard-50-tol*.—1 ng/ L of each pesticide + 4 ng/ L IS in toluene. Add 250 L QC-spike solution + 1 mL IS solution and fill to the mark with toluene.

(*12a*) *Cal-standard-10-tol*.—0.2 ng/ L of each pesticide + 4 ng/ L IS in toluene. Add 50 L QC-spike solution + 1 mL IS solution and fill to the mark with toluene.

(*13a*) *Cal-standard-5-tol*.—0.1 ng/ L of each pesticide + 4 ng/ L IS in toluene. Add 25 L QC-spike solution + 1 mL IS solution and fill to the mark with toluene.

F. 10-Step Streamlined Extraction and Cleanup Procedure

The method may be scaled appropriately to any subsample size shown to be adequately representative of the original sample. If LVI is not used for GC/MS, then 12 g must be extracted. These instructions will be given for 15 g samples extracted in 50 mL FEP centrifuge tubes. [*Note*: Sample size may have to be reduced to 10–12 g for less dense matrixes (e.g., broccoli)]. *Safety notes:* Dispense solvents in a hood and wear appropriate laboratory safety glasses, coat, and gloves. In centrifugation, do not exceed the tolerance of tube/bottle or rotor, and, if needed, pair the tubes of the most similar weights to best counterbalance the centrifuge.

(*1*) (*Note*: Step 1 was done by the SD.) Weigh 15.0 0.1 g of thoroughly comminuted samples into FEP centrifuge tubes (use 13 mL distilled water for a reagent blank in 1 of the 3 sets of samples).

(*2*) Weigh 15 g blanks (3 or 7) for matrix-matched calibration standards (*see* **A** and **G** for options) and QC spike. To one of the blanks, add 75 L QC-spike solution (40 ng/ L of the 27 pesticide analytes) to make a 200 ng/g QC spike.

(*3*) Add 15 mL 1% HOAc in MeCN per 15 g sample in each tube using the solvent dispenser.

(*4*) Add 75 L IS solution per 15 g sample (this will give 200 ng/g equivalent concentration). Do not add the IS solution to the 2 or 6 matrix blanks to be used for matrix-matched calibration standards.

(*5*) Add 6 g anhydrous $MgSO_4$ + 1.5 g anhydrous NaOAc (or 2.5 g $NaOAc{\cdot}3H_2O$) per 15 g sample to the tubes (the extract will reach 40–45 C) and seal the tubes well (ensure that powder does not get into the screw threads or rim of the tube).

(*6*) Shake the tubes vigorously by hand for 1 min with 3–5 tubes at once in each hand (using the elbows and shoulders more so than the wrists), ensuring that the solvent interacts well with the entire sample and that crystalline agglomerates are broken up sufficiently during shaking (a mechanical shaker may be faster for parallel extraction of larger samples in FEP bottles).

(*7*) Centrifuge the tubes at >1500 rcf (e.g., 3500) for 1 min. The greater the force used, the better for forming a solid sample plug and providing cleaner extracts. Combine the 6 matrix blank extracts if Option B will be followed in **G**.

(*8*) Transfer needed amount (1–2 mL in Option A or 8 mL in Option B) of the MeCN extracts (upper layer) to the dispersive-SPE tubes containing 50 mg PSA sorbent + 150 mg $MgSO_4$ per mL extract (*see* **A** and **G**). For Option A, it is possible to scale up this step 7-fold in 1 or 2 tubes for dispersive-SPE of the matrix blanks.

(*9*) Seal the tubes well and mix by hand (or mix on a Vortex mixer) for 30 s.

(*10*) Centrifuge the dispersive-SPE tubes at >1500 rcf for 1 min. Combine the matrix blank extracts.

G. Options for Handling Extracts for Analysis

Option A.—If 1 mL extracts are taken for dispersive-SPE in Step 8, prepare extracts for concurrent LVI/GC/MS and LC/MS/MS analyses as given (if 2 mL extracts are taken for dispersive-SPE in Step 8, then double all volumes given).

(*11A*) Transfer 500 L final extract from dispersive-SPE tubes to AS vials for LVI/GC/MS.

(*12A*) Add 50 L TPP solution to all vials and 25 L MeCN to test sample extracts, QC spike, 0-standard, and reagent blank.

(*13A*) For the 6 calibration standards, add 25 L each of the respective cal-standard mix to the appropriately labeled vials.

(*14A*) Cap the vials, shake to mix, uncap the vials, and transfer 150 L aliquots to similarly labeled AS vials for LC/MS/MS.

(*15A*) Add formic acid solution in water to achieve the acid concentration and organic solvent content at the initial LC mobile phase conditions (e.g., after transfer of 150 L extract, then add 450 L of 6.7 mM formic acid in water to yield 25% MeCN in 5 mM formic acid aqueous solution).

(*16A*) Cap the vials, and conduct LVI/GC/MS and LC/MS/MS analytical sequences according to **H**. (*Note*: Ensure that the AS needle is set sufficiently low to uptake the relatively small volumes contained in the AS vials.)

Option B.—If LVI is not available, then ≥8 mL of each extract must be taken for dispersive-SPE. Prepare extracts for concurrent GC/MS and LC/MS/MS analysis as follows:

(*11B*) Transfer 250 L MeCN extract from dispersive-SPE tube to AS vial for LC/MS/MS.

(*12B*) Add 25 L TPP solution to all vials and 12.5 L MeCN to test sample extracts, QC spike, 0-standard, and reagent blank.

(*13B*) For the 6 calibration standards, add 12.5 L each of the respective cal-standard mix to the appropriate vials.

(*14B*) Add formic acid solution in water to achieve the acid concentration and organic solvent content at the initial LC mobile phase conditions (e.g., add 860 L of 6.7 mM formic acid in water to yield 25% MeCN in 5 mM formic acid aqueous solution).

(*15B*) For evaporation and solvent exchange to toluene for GC/MS analysis, transfer 4 mL MeCN extracts to 10–15 mL graduated centrifuge tubes.

(*16B*) Add 400 L TPP solution and 1 mL toluene to all tubes.

(*17B*) Evaporate the extract in a Turbovap or N-Evap at 50°C and sufficient N_2 flow until volume is 0.3–0.5 mL.

(*18B*) For the 6 matrix-matched calibration standards, add 200 L each of the respective cal-standard mix-tol (in toluene) to the appropriate vials.

(*19B*) Add toluene to take the extract up to 1 mL and add anhydrous $MgSO_4$ to reach the 0.2 mL mark on the tube and swirl to rinse above the 6 mL mark.

(*20B*) Centrifuge the tubes at >1500 rcf for 1 min and transfer ≈0.6 mL of the final extracts to the appropriate AS vials for GC/MS analysis.

(*21B*) Cap the vials, and conduct GC/MS and LC/MS/MS analytical sequences according to **H**.

H. LVI/GC/MS and LC/MS/MS Analyses

Conduct proper LVI/GC, LC, and MS maintenance to ensure adequate operation of the instruments. Inject the 10 ng/g matrix standard at the conditions to be used. In GC, ensure that peak shapes of the analytes are Gaussian, widths are <5 s at half height, and S/N >10 is achieved for the pesticides using the quantitation ions chosen at the appropriate t_R. It is anticipated that some analytes will be problematic at 10 ng/g, but LC/MS/MS often provides good results for those difficult compounds in GC. Perform maintenance to correct problems if poor GC quality is observed for those analytes that are not detected by LC/MS/MS. Use alternate or additional quantitation ions if S/N is inadequate and/or matrix interferences occur. In that case, inject the 0-standard to determine if significant interferences are present at the t_R of the analyte(s).

Conduct a similar system suitability assessment of LC/MS/MS for the analytes. Use an injection volume that achieves S/N >10 for the least sensitive analyte in the 10 ng/g standard and provides Gaussian peaks with widths <30 s at half maximum height.

Once the suitability of the instruments has been shown to be acceptable, inject the extract sequences in the following suggested order: (*1*) 0-standard; (*2*) 250 ng/g standard; (*3*) 10 ng/g standard; (*4–7*) test samples 1–4; (*8*) 5 ng/g standard; (*9*) 50 ng/g standard; (*10–12*) test samples 5–7; (*13*) QC spike; (*14*) 100 ng/g standard; (*15*) 1000 ng/g standard; and (*16*) reagent blank. No evidence of carry-over should be present in the reagent blank. Store the extracts at <–20 C if the analyses cannot be conducted immediately after sample preparation, but degradation of certain pesticides in the extracts will likely occur during prolonged storage.

I. Data Analysis

Quantitation is based on linear least squares calibration of analyte peak areas plotted versus analyte concentration. The *y*-intercept should be near zero and correlation coefficient (r^2) of the line should be >0.995. The integrated peak area (or the analyte peak area/IS peak area ratio if the IS is used) becomes the signal, S. Peak heights may be evaluated if peak areas are shown to give a problem. The analyte concentrations in the matrix-matched calibration standards on a per sample basis (ng/g) can be determined by multiplying the volume (L) added to the extract by the analyte concentrations in the cal-standard mix solutions (ng/L) and dividing by the equivalent weight (g) of sample in the final extract (1 g/mL for MeCN extracts and 4 g/mL for those in toluene). The concentrations, C (ng/g), of the pesticide analytes in the test samples and QC spike are determined from the equation:

$$C = (S - y\text{-intercept})/\text{slope}$$

If a well-characterized quadratic relationship occurs, then a best-fitted quadratic curve should be employed for calibration instead.

A spreadsheet was provided to all collaborators that automatically calculated results for each analyte in GC/MS and LC/MS/MS for each matrix. Figure 1 of the collaborative study [J. AOAC Int. 90, 485(2007)] shows a small section of the spreadsheet for Laboratory 1. The collaborators entered the analytical conditions, integrated peak areas, t_R, and quantitation ions used for each analyte into the appropriate cells in the spreadsheets. The spreadsheet then provided calibration plots in the 5–100 and 50–1000 ng/g ranges for determination of C according to the equation above. The "recoveries" of the calibration standards were back-calculated to verify the accuracy of the calibration. In nearly all cases, C < 75 ng/g used the low range plot and C > 75 ng/g used the high range plot, but a few exceptions were made when 1 plot was observed to be considerably better than the other. Results were also noted in bold when the calculated C was >20% higher (typically >1200 ng/g) or lower (typically <4 ng/g) than the highest or lowest calibration standards in the plot used. Independent of QC measures and quantitation issues, all results were still included in the statistical calculations, except when calibration plots were very poor. Figure 2 of the collaborative study [J. AOAC Int. 90, 485(2007)] shows some examples of calibration curves that yielded untrustworthy results. Data transfer errors, interferences, poor system suitability, misintegrations, or mislabeling are the likely causes for those problems when they occurred.

J. Statistical Analysis of the Results

After the data was compiled and organized, the statistical analysis was performed using AOAC guidelines [*Official Methods of Analysis* (2002) *Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*, AOAC INTERNATIONAL, Gaithersburg, MD]. The SD conducted an evaluation of the results using a self-designed spreadsheet template following the examples in the guidelines. A U.S. Department of Agriculture, Agricultural Research Service, statistician and AOAC volunteer, John Phillips, also calculated the results independently using a statistical spreadsheet program designated by AOAC. Comparison of the results was made, and the causes of any discrepancies were corrected until all results were in agreement.

The SD evaluated the data in several different ways entailing the use of an IS or not. The statistician only evaluated the data without use of an IS, and in these cases, outliers were removed for statistical reasons only. Outliers (P = 0.025) were removed based on calculations of repeatability within a laboratory (Cochran outlier test) and reproducibility among laboratories (Grubbs outlier test).

The acceptability of the results was judged predominantly with respect to recoveries, intralaboratory repeatability, interlaboratory reproducibility, and the Horwitz ratio (HorRat), which is calculated from the equation:

$$\text{HorRat} = \text{RSD}_R/2C^{-0.1505}$$

where RSD_R is the overall relative standard deviation of reproducibility for all laboratories in the study and C is the determined concentration (weight analyte/weight sample) of the analyte in the blind duplicate test samples. This relationship provides an easy comparison of the results from this collaborative study with other collaborative studies that were used to calculate the Horwitz equation [Horwitz, W., Kamps, L.R., & Boyer, K.W. (1980) *J. Assoc. Off. Anal. Chem.* **63**, 1344–1354]. The Horwitz equation is an empirically derived relationship between analyte concentration and acceptable variability of results. Essentially, a HorRat value between 0.5–2 indicates that the result meets acceptance criteria for AOAC in collaborative study trials [Horwitz, W., & Albert, R. (2006) *J. AOAC Int.* **89**, 1095–1109]. In pesticide residue analysis, it is desirable to achieve recoveries between 70–120% (or 50–150% depending on the purpose of the analysis), repeatabilities <15% RSD, and reproducibility <25% RSD.

Reference: J. AOAC Int. 90, 485(2007).