RESIDUES AND TRACE ELEMENTS

Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate: Collaborative Study

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A collaborative study was conducted to determine multiple pesticide residues in fruits and vegetables using a quick, simple, inexpensive, and effective sample preparation method followed by concurrent analysis with gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS). For short, the method is known as QuEChERS, which stands for quick, easy, cheap, effective, rugged, and safe. Twenty representative pesticides were fortified in 3 matrixes (grapes, lettuces, and oranges) at 3 duplicate levels unknown to the collaborators ranging from 10 to 1000 ng/g. Additionally, 8 incurred pesticide residues were determined. Thirteen laboratories from 7 countries provided results in the study, and a variety of different instruments were used by collaborators. The QuEChERS procedure simply entails 3 main steps: (*1*) a 15 g homogenized sample is weighed into a 50 mL centrifuge tube to which 15 mL acetonitrile containing 1% HOAc is added along with 6 g $MgSO_4$ and 1.5 g NaOAc, and the tube is shaken and centrifuged; (*2*) a portion of the extract is mixed with 3 + 1 (w/w) $MgSO_4$–primary secondary amine sorbent (200 mg/mL extract) and centrifuged; and (*3*) the final extract is analyzed by GC/MS and LC/MS/MS. To detect residues <10 ng/g in GC/MS, large-volume injection of 8 μL is typically needed, or the extract can be concentrated to 4 g/mL in toluene, in which case 2 μL splitless injection is used. In the study, the averaged results for data from 7–13 laboratories (not using internal standardization) for the 18 blind duplicates at the 9 spiking levels in the 3 matrixes are as follows [%recovery and reproducibility relative standard deviation (RSD_R, %)]: atrazine, 92 (18); azoxystrobin, 93 (15); bifenthrin, 90 (16); carbaryl, 96 (20); chlorothalonil, 70 (34); chlorpyrifos, 89 (25); cyprodinil, 89 (19); *o,p′*-DDD, 89 (18); dichlorvos, 82 (21); endosulfan sulfate, 80 (27); imazalil, 77 (33); imidacloprid, 96 (16); linuron, 89 (19); methamidophos, 87 (17); methomyl, 96 (17); procymidone, 91 (20); pymetrozine, 69 (19); tebuconazole, 89 (15); tolylfluanid (in grapes and oranges), 68 (33); and trifluralin, 85 (20). For incurred pesticides, kresoxim-methyl (9.2 ± 3.2 ng/g) and cyprodinil (112 ± 18) were found in the grapes; permethrins (112 ± 41), λ-cyhalothrin (58 ± 11), and imidacloprid (12 ± 2) were determined in the lettuces; and ethion (198 ± 36), thiabendazole (53 ± 8), and imazalil (13 ± 4) were determined in the oranges. Chlorpyrifos-methyl (200 ng/g) was used as a quality control standard added during sample homogenization and yielded 86% recovery and 19% RSD_R. Intralaboratory repeatabilities for the method averaged 9.8% RSD for all analytes. The results demonstrate that the method is fit-for- purpose to monitor many pesticide residues in fruits and vegetables, and the Study Director recommends that it be adopted Official First Action.

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The recommendation was approved by the Methods Committee on Residues and Related Topics as First Action. *See* "Official Methods Program Actions," (2007) *Inside Laboratory Management*, January/February issue.

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This paper is a condensed version of the approved AOAC collaborative study report, which contains all analytical results without the use of internal standardization for quantitation. The collaborative study report is available in electronic format on the *J. AOAC Int.* Website as supplemental information to this paper (http://www.atypon-link.com/AOAC/loi/jaoi). If the reader is interested in the unabridged report, which includes results on the use of the internal standard and brief discussion of qualitative results, please contact the author. Certain points of discussion as noted in this paper have also been removed to reduce its length.

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A collaborative study was conducted to validate an efficient and effective method for the multiclass, multiresidue analysis of pesticides in fruits and vegetables. In the study, 20 representative pesticides fortified into 3 different fruit and vegetable matrixes were chosen for analysis in addition to any incurred residues present. Sample preparation entailed extraction with acetonitrile (MeCN) that contained 1% acetic acid (HOAc) and partitioning with a mixture of magnesium sulfate ($MgSO_4$) and sodium acetate (NaOAc) followed by a simple cleanup step using dispersive solid-phase extraction (dispersive-SPE). The analysis was done by both gas and liquid chromatography (GC and LC) coupled with mass spectrometry (MS) and tandem mass spectrometry (MS/MS) to quantify and identify the wide range of pesticide residues. Statistical evaluations of the results from the different laboratories were conducted to demonstrate quantitative performance of the method.

Collaborative Study

Need/Purpose

Multiresidue analysis of pesticides in fruits, vegetables, and other foods is a primary function of several regulatory, industrial, and contract laboratories throughout the world. More than 100 000 food samples are analyzed each year for pesticide residues to meet a variety of purposes, and this method can be applicable for nearly any of these purposes, including regulatory enforcement and surveillance monitoring.

Scope/Applicability

The approach is known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for multiclass, multiresidue analysis of pesticides in a variety of matrixes. As the name implies, the QuEChERS sample preparation approach has many practical advantages over existing methods without sacrificing quality of the results. The new method may be used to replace existing multiclass, multiresidue methods for a wide range of pesticides in a variety of food matrixes. The limit of quantitation (LOQ) of the method was designed to be <10 ng/g using this protocol for all analytes, and the linear dynamic range should permit analysis beyond 10 000 ng/g, depending on the analyte and instrumentation. This study tested the concentration range of 10 to 1000 ng/g.

In terms of analytical scope, nearly all pesticides except those relatively few that contain carboxylic acid groups can be monitored by the QuEChERS approach. The primary secondary amine (PSA) sorbent used in dispersive-SPE retains pesticides containing carboxylic acid groups, such as daminozide and 2,4-D. Chlorothalonil, dicofol, folpet, captan, captafol, dichlofluanid, and tolylfluanid tend to degrade in MeCN as pH increases and in the presence of light, thus results for those pesticides are more variable depending on the matrix and conditions used. Representative commodities and pesticides were chosen in this protocol to demonstrate the applicability of the method to a wide range of analytes and matrixes.

This protocol evaluated the QuEChERS method using PSA only as a cleanup sorbent. For fatty matrixes, high recoveries of semipolar and polar pesticides are still achieved, but recoveries of the most nonpolar pesticides decrease with respect to increasing fat content. The additional use of C_{18} sorbent in dispersive-SPE can provide additional cleanup of lipids. If no pesticides with planar structures (e.g., thiabendazole, terbufos, quintozene, hexachlorobenzene) are included among the analytes, then graphitized carbon black (GCB) can also be used in dispersive-SPE to provide additional cleanup of sterols, chlorophyll, and structurally planar matrix components.

As presented in this protocol, the method is devised for concurrent GC/MS and LC/MS/MS analysis of split final extracts, but it is very flexible and may be used for LC and GC analyses with other detectors. However, due to the presence of MeCN in the final extracts, this method cannot be used for GC analysis using the nitrogen-phosphorus detector (NPD) or other detectors adversely affected by high concentrations of nitrogen unless the detectors are equipped with a solvent bypass feature or precautions are made to thoroughly exchange the final extract to toluene.

Materials/Matrixes

The 3 selected representative matrixes consist of grapes, lettuces, and oranges (a mixture of different varieties was used in each case). The 20 pesticide analytes to be fortified in the chosen matrixes include atrazine, azoxystrobin, bifenthrin, carbaryl, chlorothalonil, chlorpyrifos, cyprodinil, *o,p′*-DDD, dichlorvos, endosulfan sulfate, imazalil, imidacloprid, linuron, methamidophos, methomyl, procymidone, pymetrozine, tebuconazole, tolylfluanid, and trifluralin. Incurred pesticides, which include imidacloprid, permethrins, and λ-cyhalothrin in lettuces; ethion, thiabendazole, and imazalil in oranges; and cyprodinil and kresoxim-methyl in grapes, were also determined as part of the study. Blind duplicates at 3 spiking concentrations varying from 10 to 1000 ng/g plus an unknown blank were performed in the study. The spiking levels for each matrix are shown in the tables and figures giving the results of the study. Tables 1–3 list some pertinent information about the pesticides included in the study.

Collaborators were provided with test portions and any other materials needed for the method that they requested. The Study Director (SD) provided all laboratories with the necessary amount of mixed standard solution to be used for preparing quality control (QC) and matrix-matched calibration standards. This saved costs and time even for experienced laboratories that would have had to prepare the mixtures and minimized the possibility of using degraded, mislabeled, or impure reference standards.

Grapes, lettuces, and oranges with incurred residues were obtained and mixed with other varieties of the same type of commodity. An aliquot of chlorpyrifos-methyl to make 200 ng/g was added to each bulk sample, which was

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Table 1. Information about the selected pesticides for the study

Pesticide	Use[a]	Class[b]	MW, g/mol[c]	Formula	Vp, mPa[d]	Solubility in water	pK_{ow}[e]	pK_a[f]	Analysis
Atrazine	H	Triazine	215.7	$C_8H_{14}ClN_5$	0.0385	33	2.5	1.7	GC and LC
Azoxystrobin	F	Strobilurin	403.4	$C_{22}H_{17}N_3O_5$	1.1×10^{-7}	6	2.5		LC[g] and GC
Bifenthrin	I	Pyrethroid	422.9	$C_{23}H_{22}ClF_3O_2$	0.024	<0.001	>6		GC
Carbaryl	I	Carbamate	201.2	$C_{12}H_{11}NO_2$	0.041	120	1.59		GC and LC[g]
Chlorothalonil	F	OC	265.9	$C_8Cl_4N_2$	0.076	0.81	2.92		GC
Chlorpyrifos	I	OP	350.6	$C_9H_{11}Cl_3NO_3PS$	2.7	1.4	4.7		GC[g] and LC
Chlorpyrifos-methyl	I	OP	322.5	$C_7H_7Cl_3NO_3PS$	3	2.6	4.24		GC[g] and LC
λ-Cyhalothrin	I	Pyrethroid	449.9	$C_{23}H_{19}ClF_3NO_3$	0.001	0.005	6.9		GC
Cyprodinil	F	Anilinopyrimidine	225.3	$C_{14}H_{15}N_3$	0.51	13	4.0	4.44	GC and LC[g]
Dichlorvos	I	OP	221.0	$C_4H_7Cl_2O_4P$	2100	≈18000	1.9		GC and LC[g]
o,p′-DDD	I	OC	320.05	$C_9H_{22}O_4P_2S_4$	0.18	0.09	6.02		GC
Endosulfan sulfate	I	OC	406.95	$C_9H_6Cl_6O_4S$	1.3	0.08	3.6		GC
Ethion	I	OP	384.5	$C_9H_{22}O_4P_2S_4$	0.2	2			GC[g] and LC
Imazalil	F	Imidazole	297.2	$C_{14}H_{14}Cl_2N_2O$	0.158	180	3.82	6.53	GC and LC[g]
Imidacloprid	I	Neonicotinoid	255.7	$C_9H_{10}ClN_5O_2$	4×10^{-7}	610	0.57		LC
Kresoxim-methyl	F	Strobilurin	313.4	$C_{18}H_{19}NO_4$	0.0023	2	3.4		LC[g] and GC
Linuron	H	Phenylurea	249.1	$C_9H_{10}Cl_2N_2O_2$	0.051	64	3.00		GC and LC[g]
Methamidophos	I	OP	141.1	$C_2H_8NO_2PS$	4.7	>200000	–0.8		GC and LC[g]
Methomyl	I	Oxime carbamate	162.2	$C_5H_{10}N_2O_2S$	0.72	57900	0.093		LC
Permethrins	I	Pyrethroid	391.3	$C_{21}H_{20}Cl_2O_3$	0.0015	0.006	6.1		GC
o-Phenylphenol	F	Phenol	170.2	$C_{12}H_{10}O$		700			GC
Procymidone	F	Dicarboximide	284.1	$C_{13}H_{11}Cl_2NO_2$	18	4.5	3.14		GC[g] and LC
Pymetrozine	I	Pyridine	217.2	$C_{10}H_{11}N_5O$	<0.004	290	–0.18		LC
Tebuconazole	F	Triazole	307.8	$C_{16}H_{22}ClN_3O$	0.0017	36	3.7		GC and LC[g]
Thiabendazole	F	Benzimidazole	201.3	$C_{10}H_7N_3S$	0.00046	30	2.39	4.73	GC and LC[g]
Tolylfluanid	F	*N*-trihalomethylthio	347.3	$C_{10}H_{13}Cl_2FN_2O_2S_2$	0.2	0.9	3.90		GC and LC[g]
Trifluralin	H	Dinitroaniline	335.3	$C_{13}H_{16}F_3N_3O_4$	6.1	0.221	4.83		GC

[a] I = Insecticide; F = fungicide; H = herbicide.
[b] OC = Organochlorine; OP = organophosphate.
[c] MW = Molecular weight.
[d] Vp = Vapor pressure.
[e] pK_{ow} = Partition coefficient between octanol and water.
[f] pK_a = Acid dissociation constant.
[g] Preferred technique.

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homogenized with a chopper. The chlorpyrifos-methyl served as a QC measure to check the quality of the mixing step by the SD. The sample was divided into portions and placed in individual containers for the 15.0 ± 0.1 g test and blank samples. The test portions were fortified at appropriate levels in the containers using 150 µL of appropriate mixed pesticide spiking solutions in MeCN containing 1% HOAc. The test portions were stored at –40°C in the labeled, sealed containers until they were shipped within a couple of days after being prepared.

The test samples, blanks, and requested materials were shipped frozen in coolers to the collaborating laboratories. The collaborators received the samples, returned the packing slips to note any problems, and stored the samples in their freezers. The collaborators were given 2 months to analyze the samples and another month to report the results.

Quality Assurance

The collaborators were expected to follow good laboratory practices (GLPs) in the operations of their laboratories. With

Table 2. General GC/MS information for the pesticides[a]

Pesticide	M^+	Base peak	Other ions	t_R, min
Atrazine	215	200	173, 217, 202	9.25
Azoxystrobin	403	344	372, 388	22.45
Bifenthrin	422	181	165, 166	17.77
Carbaryl	201	144	115, 116	11.14
Chlorothalonil	264	266	268	9.98
Chlorpyrifos	349	97	197, 199, 314, 316	12.03
Chlorpyrifos-methyl	321	286	288, 125, 197, 109	10.83
λ-Cyhalothrin	449	197	181, 208	18.38[b] + 18.54
Cyprodinil	225	224	210	12.96
Dichlorvos	220	109	185, 79, 145	5.32
o,p′-DDD	318	235	237, 165, 199	15.18
Endosulfan sulfate	420	272	274, 387, 229, 239	16.93
Ethion	384	231	153, 97, 125	16.21
d_6-α-HCH	294	224	222, 226, 185, 189	8.77
Imazalil	296	215	173, 217, 175	14.68
Kresoxim-methyl	313	131	116, 206	15.28
Methamidophos	141	94	95	5.26
d_{10}-Parathion	301	301	156, 187, 237, 269	12.11
Permethrins	390	183	163, 165	19.21 + 19.32
o-Phenylphenol	170	170	169, 141, 115	7.22
Procymidone	283	283	96, 285, 255	13.54
Tebuconazole	307	250	163, 125	17.24
Thiabendazole	201	201	174	13.54
Tolylfluanid	346	137	238, 240, 181	13.22
Triphenylphosphate	326	325	233, 215, 169, 170	17.32
Trifluralin	335	306	264, 290, 248	8.27

[a] Temperature oven program: 80°C held for 1.5 min, then a 25°C/min ramp to 180°C followed by a 5°C/min ramp to 230°C and a 25°C/min ramp to 290°C, held for 10 min.

[b] Cyhalothrin peak, which should be reported separately if it appears.

respect to the method, each critical step was evaluated by the addition of a QC spike. A 200 ng/g addition of chlorpyrifos-methyl was made to check the homogeneity of the sample processing method. A pair of deuterated internal standard (IS) compounds, d_{10}-parathion and d_6-α-hexachlorocyclohexane (d_6-α-HCH), were added at 200 ng/g to each test sample to check extraction and potentially compensate for volume fluctuations and instrument variations. To check the quality of the analytical step, a 200 ng/g equivalent addition of triphenylphosphate (TPP) was to be made to all final extracts. Matrix-matched calibration standards at 6 levels (5, 10, 50, 100, 250, and 1000 ng/g equivalents) were used for quantitation in each commodity set (2 plots were created with 4 points each from 5–100 and 100–1000 ng/g). Unless otherwise noted, all results in this report were calculated without using the IS. For the IS normalized data, peak areas of the analytes in the sample divided by peak area of d_{10}-parathion served as the signal used for quantitation (d_6-α-HCH was available as a backup when needed in GC/MS). Recoveries of the ISs were also checked from the matrix-matched standards that also contained the ISs (which were added after sample preparation). Analysis of a matrix blank (0-standard) to determine potential interferences and sources of contamination in matrix, reagents, solvent, and the analytical column was also required. A reagent blank was also analyzed in each set to check for contamination and carryover.

Determination of Trueness/Precision

Trueness and precision were evaluated by measuring recoveries and repeatabilities/reproducibilities of the fortified pesticides at the 3 different levels in the 3 different matrixes among the collaborating laboratories. In single-laboratory validations (SLVs), typical recoveries by the QuEChERS method for pesticides in fruits and vegetables have been

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Table 3. General LC/MS/MS information for the pesticides[a]

Pesticides	$[M+H]^+$, *m/z*	MS/MS transition, *m/z*	t_r, min
Atrazine	216.0	216 → 174	16.46
Azoxystrobin	404.1	404 → 372	16.79
Carbaryl	201.8	202 → 145	15.46
Chlorpyrifos	349.9	350 → 200	20.20
Chlorpyrifos-methyl	321.8	322 → 125	19.37
Cyprodinil	225.9	226 → 108	18.47
Dichlorvos	220.8	221 → 127	14.91
Ethion	384.9	385 → 199	19.90
Imazalil	296.8	297 → 159	12.14
Imidacloprid	255.9	256 → 209	11.07
Kresoxim-methyl	314.0	314 → 206	18.51
Linuron	248.9	249 → 160	17.47
Methamidophos	141.8	142 → 112	4.17
Methomyl	163.0	163 → 88	8.77
d_{10}-Parathion	302.0	302 → 238	18.46
Procymidone	284.0	284 → 256	18.15
Pymetrozine	217.9	218 → 105	4.04
Tebuconazole	308.0	308 → 125	18.68
Thiabendazole	201.8	202 → 175	8.28
Tolylfluanid	346.8	347 → 238	18.52
Triphenylphosphate	327.0	327 → 77	18.62

[a] LC conditions: 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.

90–110%. The recoveries have been consistent [usually 5–10% relative standard deviation (RSD)] within extraction sets, and interlaboratory precision was expected to be higher. Typical check sample programs for pesticide residues in foods obtain rather variable results, even for laboratories that use rigorously validated methods and GLP. Reproducibility of check samples for pesticide residues in the European Unition (EU), Pesticide Data Program, Southern State Check Sample Program, and Food Analysis Performance Assessment Scheme (FAPAS®) are typically 20–40% RSD among 8–30 laboratories for pesticides that are usually spiked at >100 ng/g in frozen samples.

Previous Work

A number of papers on the QuEChERS method and related approaches have been published (1–15), and reports from e-mails and meetings indicate that numerous other chemists around the world are using the approach for pesticide residue analysis of foods. Other modifications and validation studies have been conducted in Germany as described at www.quechers.com.

The initial publication (1) provides the method development process in detail, and puts the new method in historical context. In particular, the commercial introduction of LC/MS/MS during the 1990s and current widespread usage of GC/MS for analysis gave the opportunity to use a simpler and faster sample preparation approach. A follow-up SLV study for >200 pesticides fortified in lettuce and orange matrixes showed excellent results for the large majority of pesticides, but some pH differences were observed for certain previously untested analytes by the method (7). Thus, the original QuEChERS method underwent a modification to entail buffering of the extracts by using HOAc in MeCN and NaOAc counter-ion (8). This led to higher and more consistent recoveries of the pH-sensitive pesticides (e.g., pymetrozine, and sethoxydim) independent of the pH of the matrix. Another study evaluated analytical aspects of difficult pesticides (e.g., chlorothalonil, captan, tolylfluanid, and deltamethrin) and demonstrated stability of the analytes in different solvents (4). The analysis of fatty foods by the buffered QuEChERS method was described and compared with matrix solid-phase dispersion (9). In November/December 2004, a U.S. Department of Agriculture/U.S. Environmental Protection Agency (USDA/EPA) training course was conducted to evaluate the method in dry matrixes, such as soybean, animal feeds, and nuts. The addition of water in those cases yielded high recoveries of the pesticides in fortified samples. A protocol of the QuEChERS approach based on the studies done during 2003–2004 is outlined in a book chapter (6). (*Note*: Further details of planning the collaborative study can be found in the unabridged report.)

Collaborators and Instrumentation

The QuEChERS sample preparation method is very easy to perform and takes only a little practice to learn, but it has nuances in the analytical steps that are different than traditional methods (e.g., use of MeCN as the final extract solvent), and much analyst familiarization is needed for best results. A difficulty was that some of the laboratories used large volume injection (LVI) in GC/MS, and others did not have an LVI device on their instruments. The protocol was written in such a way that the extract would be concentrated and solvent-exchanged to toluene so that the laboratories without LVI could still achieve 10 ng/g detection of the pesticides in GC/MS.

The samples were sent on February 9, 2004. Those received within 1 day (Laboratories 1, 8, 9, 12, and 13) remained frozen during shipment, and the others were thawed but still cold when received. Two of the original 15 collaborators who were shipped the samples did not perform the analyses, and 1 laboratory (No. 13) withdrew after completing only the grapes test samples. Although the QuEChERS method is fast and easy to conduct, the use of GC/MS and LC/MS/MS for so many analytes at low levels in the complicated matrixes takes extensive knowledge, skill, and experience. The list of 13 collaborators/laboratories appears in the title page and acknowledgments of this report.

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Choice of Pesticides, Concentrations, and Matrixes

The following revised text from a previous collaborative study by the same SD (16) is also suitable for this study. More than 350 pesticides are amenable for GC analysis (17, 18), and hundreds of nonfatty food commodities may be analyzed for these pesticides. Furthermore, the concentration range for analysis varies from approximately 1 to 50 000 ng/g, depending on the pesticide, commodity, purpose of analysis, and other factors. Millions of permutations exist in terms of possible pesticide combinations, commodities, and concentrations that may occur. The complete validation of multiclass, multiresidue methods to cover all of these permutations is impossible, and no method would achieve acceptable results for all permutations. Therefore, the choices of pesticides, concentrations, and matrixes in the study were made carefully to provide a diverse range of combinations to demonstrate the analytical capabilities of the method. Tables 1–3 give information about these aspects of the collaborative study.

To reduce the time and effort required of collaborators, the SD limited the number of samples to the amount that could be reasonably done within a week by a single proficient analyst. Unknown to the collaborators, the 7 test samples consisted of a blank and duplicate spikes of the representative pesticides at 3 different concentrations. This format was rather similar to the format of the only 2 other AOAC collaborative studies conducted on multiclass, multiresidue analysis of pesticides (16, 19), but this study was more extensive, probably making it the most involved AOAC collaborative study yet conducted.

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, λ-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

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	
λ-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.

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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	

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Table 2007.01B. (*continued*)

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]
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.

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 µL is typically needed, or the final extract can be concentrated and solvent exchanged to toluene (4 g/mL), in which case 2 µL 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 (20–22). 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.

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

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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	

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Table 2007.01C. (*continued*)

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]
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.

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), Restek (Bellefonte, PA) and Supelco (Bellefonte, PA) have introduced commercial dispersive-SPE products for QuEChERS and other applications. *See* Table 4 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 µL for a 1 g/mL MeCN extract (e.g., 75°C ramped to 275°C at 200°C/min) or 2 µL 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 µm 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 µL) 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 µm 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

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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	

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Table 2007.01D. (*continued*)

Analyte	Avg. C, ng/g	sr, ng/g	RSDr, %	sR, ng/g	Rec., %	RSDR, %	HorRat	No. of labs	Outlier labs[a]
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.

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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.

(**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 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 µm 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 µm 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

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.

solutions (e.g., 2000 ng/μL) 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/μL of both d_{10}-parathion and d_6-α-HCH in MeCN; triphenylphosphate (TPP) solution = 2 ng/μL TPP in 1% HOAc in MeCN solution; QC-spike solution = 40 ng/μL of the 27 pesticide analytes in 0.1% HOAc in MeCN; and individual test solutions = 10 ng/μL of each of the 30 compounds to be detected (except 40 ng/μL 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 μL volume into GC/MS and appropriate volumes of matrix spike, IS, and calibration standard solutions (12.5–300 μL).

(**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

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Step	Procedure
0.	Comminute >1 kg sample with vertical cutter. Homogenize ≈200 g subsample with probe blender.
1,2.	Transfer 15 g subsample to 50 mL Teflon tube.
3-5.	Add 15 mL 1%Hac in MeCN + 1.5 g anh. NaAc + 6 g anh. $MgSO_4$ + 75 µL I.S. solution.
6,7.	Shake vigorously for 1 min. Centrifuge >1500 rcf for 1 min.
8,9.	Transfer 1-8 mL to tube with 150 mg anh. $MgSO_4$ + 50 mg PSA per mL extract and shake for 30 s.
10.	Centrifuge >1500 rcf for 1 min.
11-15A.	Transfer 0.5-1 mL extract to GC vial and add TPP. Transfer 0.15-0.3 mL to LC vial and add *e.g.* 0.45-0.9 mL 6.7 mM formic acid.
11-14B.	Transfer 0.25 mL from Step 10 to LC vial. Add TPP and *e.g.* 0.86 mL 6.7 mM formic acid.
15-16B.	Transfer 4 mL from Step 10 to grad. cent. tube. Add 0.4 mL TPP Sol'n and 1 mL toluene.
17-19B.	Evaporate at 50°C with N_2 to 0.3-0.5 mL. Add toluene to make 1 mL. Add 0.2 mL anh. $MgSO_4$ and swirl >6 mL mark.
20B.	Centrifuge >1500 rcf for 1 min. Transfer ≈0.6 mL to GC vial.
16A/21B.	Analyze by (LVI/)GC/MS and LC/MS-MS

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

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/µL 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/µL of each pesticide + 4 ng/µL 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/µL of each pesticide + 4 ng/µL 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/µL of each pesticide + 4 ng/µL IS in MeCN + 0.1% HOAc. Add 500 µL 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/µL of each pesticide + 4 ng/µL IS in MeCN + 0.1% HOAc. Add 250 µL 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/µL of each pesticide + 4 ng/µL IS in MeCN + 0.1% HOAc. Add 50 µL 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/µL of each pesticide + 4 ng/µL IS in MeCN + 0.1% HOAc. Add 25 µL 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/µL 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/µL of each pesticide + 4 ng/µL IS in MeCN + 0.1% HOAc. Add 500 µL QC-spike solution + 100 µL IS solution + 100 µL 1% HOAc in MeCN + 320 µL MeCN.

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

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

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

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

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

(*7a*) *Cal-standard-5*.—0.1 ng/μL of each pesticide + 4 ng/μL IS in MeCN + 0.1% HOAc. Add 25 μL dilute QC-spike solution + 100 μL IS solution + 100 μL 1% HOAc + 795 μL 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/μL of each pesticide + 4 ng/μL 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/μL of each pesticide + 4 ng/μL 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/μL of each pesticide + 4 ng/μL IS in toluene. Add 500 μL QC-spike solution + 1 mL IS solution and fill to the mark with toluene.

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

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

(*13a*) *Cal-standard-5-tol*.—0.1 ng/μL of each pesticide + 4 ng/μL IS in toluene. Add 25 μL 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 μL QC-spike solution (40 ng/μL 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 μL 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 μL final extract from dispersive-SPE tubes to AS vials for LVI/GC/MS.

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

(*13A*) For the 6 calibration standards, add 25 μL 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 μL 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 μL extract, then add 450 μL 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 μL MeCN extract from dispersive-SPE tube to AS vial for LC/MS/MS.

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

(*13B*) For the 6 calibration standards, add 12.5 μL 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 μL 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 μL 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 μL 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 (μL) 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 (23). The SD

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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 (24). 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 (25). 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).

Results and Discussion

Collaborators' Comments

Comments were requested from collaborators by the SD on multiple occasions before and after the study was conducted. A few of the chemists provided much input in the planning and data analysis stages, but most collaborators made no comments. Laboratory 13 withdrew after the grapes analysis citing that they overestimated the amount of time needed to obtain and enter the results into the spreadsheet. They were the only laboratory to use LC/MS rather than LC/MS/MS (they made 8 injections in LC/MS per sample).

Laboratory 3 could give no explanation why their results for tolylfluanid in lettuces were so much better than the other laboratories. Laboratory 8 explained that they were aware of the bias in their LC/MS/MS results due to the use of a 50 μL volume added to a 0.5 mL autosampler vial for the analysis. The evaporation of solvent in the vial acted to concentrate the extract and led to a high bias when external calibration was used. They did nothing to avoid this bias because the IS corrected for it. Laboratory 9 reported that multiclass, multiresidue analysis was not done in their laboratory using GC/MS, and they did not have familiarity or the time to optimize the multiclass, multiresidue method.

Additional comments are included in the unabridged version of this report.

Quality Control Results and Instrument/Analyst Performance

Each step in the analytical method incorporated a QC spike to help isolate the degree of uncertainty in the steps, and try to ensure proper analyst performance. The SD added 200 ng/g chlorpyrifos-methyl prior to the comminution step of the bulk commodities. This helped determine the homogeneity of the sample processing step (incurred analytes also served this purpose). Each collaborator added 200 ng/g d_{10}-parathion and d_6-α-HCH to the mixed sample portions to ensure that extraction occurred for each sample and isolate the variability of sample preparation from the other steps. Finally, 200 ng/g TPP was added to all extracts prior to GC/MS and LC/MS/MS analyses to isolate the analytical step (including instrument performance) from the previous steps.

Including the matrix spike, calibration standards, and blanks, each sample set consisted of 7–14 data points for QC purposes, which provided enough replicates to evaluate the performance of each step within a laboratory. The QC approach used in the study was a valuable way to track the performance of each step in the method within and among the laboratories. Tables 6–13 provide the results of the QC standards for each sample set within each collaborating laboratory.

GC/MS.—Table 6 shows the variability in GC/MS results among the different laboratories, instruments, and techniques in terms of %RSD for TPP. The 8 quadrupole instruments, in which the selected ion monitoring (SIM) mode was used by all but Laboratory 3, averaged 8% RSD for the TPP QC-spike, independent of whether an IS was used or not in the analysis. The 3 ion trap instruments evaluated averaged 13–16% RSD, and the TOF instrument averaged 10–11% RSD for TPP. However, the results from a previous collaborative study in which a similar evaluation was made showed that no differences were observed between the use of quadrupole and ion trap instruments (16). The likely reason for differences found in the present study pertains to fewer collaborators, different injection techniques (e.g., LVI with Carbofrit), and use of MS/MS by 2 laboratories (No. 4 and 5).

When the data are divided among the different factors, splitless injection of toluene (Laboratories 6, 8, 9, 12, and 13) gave 11% RSD (without IS) and 8% RSD (with IS), whereas LVI of MeCN extracts (Laboratories 1–5, 7, 10, and 11) yielded 12% RSD (without IS) and 10% RSD (with IS). This is not a significant difference, and the results demonstrate that LVI of MeCN does not worsen the performance of the QC results when compared to traditional splitless injection of a nonpolar solvent such as toluene. The key to good

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Table 4. Manufacturers of analytical instruments used in the collaborative study[a]

Lab No.	LVI	GC	MS	Type	LC	MS/MS
1	Optic 3[b]	5890[c]	5972[c]	Quadrupole	1100[c]	API-3000[d]
2	1079[e,f]	3800[e]	Saturn 2000[e]	Ion trap	2690[g]	Quattro Ultima[g]
3	Best[h]	Trace[i]	Voyager[i]	Quadrupole	1100[c]	API-3000[d]
4	1079[e,f]	3800[e]	Saturn 2000[e]	Ion trap	Pro Star[e]	1200L[e]
5	1079[e,f]	3800[e]	Saturn 2000[e]	Ion trap	2795[g]	Quattro Micro[g]
6	NA[j]	Trace[i]	Polaris[i]	Ion trap	1100[c]	Quattro Ultima[g]
7	Optic 3[b,f]	6890[c]	5973[c]	Quadrupole	1100[c]	API-2000[d]
8	NA	6890[c]	5973[c]	Quadrupole	Alliance[g]	Quattro Micro[g]
9	NA	6890[c]	5973[c]	Quadrupole	1100[c]	API-3000[d]
10	ALEx[k]	6890[c]	Pegasus[l]	Time of flight	1100[c]	API-4000[d]
11	CIS-4[k]	6890[c]	5973[c]	Quadrupole	1100[c]	API-2000[d]
12	NA	6890[c]	5973[c]	Quadrupole	Surveyor[i]	Quantum[i]
13	NA	6890[c]	5973[c]	Quadrupole	1100[c]	MSD[c]

[a] All MS/MS instrument types for LC were triple quadrupoles, except a single quadrupole instrument was used in Laboratory 13, and ESI+ was used in all cases.
[b] Atas GL International (Veldhoven, The Netherlands).
[c] Agilent Technologies (Little Falls, DE).
[d] Applied Biosystems (Foster City, CA).
[e] Varian (Walnut Creek, CA).
[f] Carbofrit used in injection liner.
[g] Waters-Micromass (Milford, MA).
[h] Best/Thermo (via Interscience in Breda, The Netherlands).
[i] Thermo-Fisher Corp. (Waltham, MA).
[j] NA = Not applicable.
[k] Gerstel (Mülheim an der Ruhr, Germany).
[l] Leco Corp. (St. Joseph, MI).

Table 5. Sources for chemicals used in the collaborative study and other information[a]

	Source			Injection volume, μL (mg sample equivalent)	
Lab No.	$MgSO_4$[b] (%purity)	NaAc	GC solvent	GC	LC
1	Fisher (99)[c]	Aldrich[d]	MeCN	7.5 (7.5)	50 (12.5)
2	Merck (99)[c]	Merck[b]	MeCN	8 (8)	5 (5)
3	Merck (>98)[c]	Fluka[b]	MeCN	10 (10)	10 (10)
4	Prolabo (98)[c]	Panreac[b]	MeCN	10 (10)	10 (10)
5	Merck (98)[c]	Merck[b]	MeCN	10 (10)	50 (50)
6	J.T. Baker (>99)[c]	J.T. Baker[d]	Toluene	2 (8)	20 (20)
7	Fluka (>98)	Fisher[d]	MeCN	10 (10)	10 (10)
8	Fisher (99)[c]	Fisher[b]	Toluene	3 (12)	12 (12)
9	Mallinckrodt (99)[c]	EM Science[b]	Toluene	2 (8)	10 (10)
10	Merck (>98)	Merck[b]	MeCN	5 (5)	20 (2)
11	Fisher (99)[c]	Aldrich[b]	MeCN	40 (40)	20 (20)
12	Fisher (99)[c]	EM Science[d]	Toluene	2 (8)	10 (10)
13	BDH (99)[c]	BDH[d]	Toluene	2 (8)	5 (5)

[a] All collaborators used PSA from Varian in the dispersive-SPE cleanup step.
[b] Anhydrous.
[c] Heated to >500°C for >5 h.
[d] 2.5 g $NaOAc \cdot 3H_2O$ added per 15 g sample rather than 1.5 g anhydrous NaAc.

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performance is good system suitability, well-chosen ions, and proper integration, whereas injection technique, instrument used, or MS approach was less important.

With respect to evaluating the system suitability of the instruments, Laboratories 1, 7, 8, 12, and 13 achieved <15% RSD for TPP in all cases, and Laboratories 3, 4, and 9 only had 1 GC/MS sequence each in which RSD for TPP exceeded 15%. Laboratories 5, 6, and 10 yielded >15% RSD of TPP in multiple sequences (depending on whether an IS was used or not), and Laboratories 2 and 11 did not add TPP to the extracts, thus, their instrument's system suitability could not be assessed in this way.

LC/MS/MS.—Table 7 shows the TPP results related to the LC/MS/MS analytical step (Laboratory 2 did not add TPP to the extracts, and 3 other laboratories could not detect the d_{10}-parathion). The overall variability in the TPP results for each laboratory averaged 11–14% RSD in LC/MS/MS. (*Note*: Laboratory 13 used LC/MS with 8 injections per sample and Laboratory 3 used diethatyl-ethyl rather than TPP.) Any differences observed based on the various instruments used in the study were not likely related to the instrument model, but were more likely due to system suitability and operational performance of the conditions used.

Use of the IS.—The use of an IS often improves trueness and precision in analytical chemistry, but the appropriateness of this tool is still debated among pesticide chemists in regulatory circles in the United States. In GC/MS, whether an IS was used or not led to small differences in most of the 31 measured cases shown in Table 6, but the use of an IS reduced the TPP variability by at least a factor of 2 in 8 instances, whereas the IS increased %RSD of TPP by more than a factor of 2 only once (Laboratory 5 for grapes). The IS rarely affected the GC/MS QC results adversely, but the use of the IS sometimes dramatically improved the results, thus it was deemed to be useful.

However, an appropriate IS must be used to yield accurate results. In GC/MS, the analysts had 2 good IS compounds from which to choose, depending on interferences and other factors. Unfortunately, d_{10}-parathion was a poor choice as the IS in LC/MS/MS, and no alternate compound was included in the protocol. Four laboratories did not analyze the IS in LC/MS/MS, and the consistency of the IS results were not very good in 5 other laboratories (4 laboratories still had good results for d_{10}-parathion). In retrospect, at least 3 IS compounds should have been included: 1 suitable for GC/MS only, 1 suitable for LC/MS/MS only, and 1 suitable for both. The SD employed deuterated IS compounds to avoid the chance that the chemicals would appear in the samples, but the selection was limited due to availability and price. Further consideration of the appropriate IS for LC/MS/MS should be made, but in the final analysis, an IS is not required to achieve good results with the method.

The data used in the final, official results in the collaborative study did not employ the IS, and the statistician only processed data without use of the IS. This decision was made by the SD mainly because the LC/MS/MS and GC/MS results were often included together in the analyte/matrix data sets, depending on which analytes the collaborators chose to monitor by the 2 analytical techniques, and in several cases, fewer than 8 laboratories provided LC/MS/MS results using the IS.

System suitability.—Considering that QC guidelines indicate that TPP variability should be <15% RSD, Laboratories 6 and 10 yielded unacceptable GC/MS system suitability (Table 6) for all matrixes, Laboratory 3 did not meet GC/MS performance criteria for oranges, and Laboratory 4 did not meet the criteria for lettuces. Laboratories 2 and 11 (for GC/MS only in the latter case) did not analyze the TPP. For LC/MS/MS (Table 7), Laboratory 5 did not meet QC criteria for any matrix, Laboratory 10 failed for lettuces and oranges, and Laboratory 9 did not meet standards for oranges. Furthermore, Laboratory 2 did not meet the analytical deadline for any GC/MS analysis, Laboratory 6 did not meet the deadline for GC/MS of grapes, and Laboratory 9 did not meet the deadline for any matrix, which may have been the case for Laboratory 12, too, which did not report the dates.

If the results from all of these laboratories were eliminated, then there would not be enough collaborators to meet the criterion that at least 8 laboratories should be included in the collaborative study assessment. Thus, no results were eliminated for not following the protocol exactly, QC reasons, or tardiness. This is justified because in reality, the <15% RSD cutoff is an arbitrary value, and the TPP result does not necessarily correlate to other analytes.

Sample preparation.—Tables 8–10 give the results for the d_6-α-HCH and/or d_{10}-parathion, which were added just prior to sample preparation (which is defined as the extraction and cleanup steps in the method). Table 8 shows their consistency of detection in GC/MS and LC/MS/MS. In these results, the variability of both sample preparation and instrumental analysis are combined, thus it is expected that the IS would give worse consistency than TPP. Indeed, this was often the case, but only to a small extent based on the comparison of averages shown in Table 8 with those in Tables 6 and 7.

As described more fully in the unabridged version of this report, volumetric biases from measuring small volumes and pipetting differences are typically the most significant source of error in the QuEChERS method within a single laboratory. This assertion is supported by the fact that the bias in all results from the stable pesticides from individual laboratories in the study tended to track together with respect to trueness (as measured by recoveries). Due to the simplicity of the QuEChERS method, which avoids uncertainties from multiple steps, such details of how pipetting is done when making calibration standards become a significant source of bias in the sample preparation method, thus analysts have to take care to avoid these biases by using appropriate pipets and volumetric measurements, especially when not using an IS.

Tables 9 and 10 show the results of the QC compounds averaged among the 7 test sample extracts versus those from the calibration standards. Overall, the average results indicate that IS recoveries were essentially 100% (1.0) in the QuEChERS protocol, and only a few instances occurred in which a bias was noted in the QC results. For GC/MS results

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of grapes, these include Laboratory 3 (85% "recovery"), Laboratory 7 (111%), and Laboratory 11 (113%). In lettuces and oranges, Laboratory 11 also showed 123 and 125% "recoveries," respectively, of the IS in GC/MS (and the LC/MS/MS results for Laboratory 11 in Table 10 also hint that up to a 25% positive bias in the IS may have occurred). Interestingly, Laboratory 8 exhibited a high bias of the IS and TPP in oranges with good precision. This is indicative of a systematic 13–24% volume difference between the calibration standards and test sample extracts in the GC/MS analysis of oranges. This collaborator also showed biases in the LC/MS/MS results for the IS, especially for grapes and lettuces, which can be observed in the analytical results (as noted in *Collaborators' Comments*).

In reality, it is impossible for the "recovery" of the IS (or TPP) to exceed 100%. Therefore, test samples yielding >1.0 results in Tables 9 and 10 relate to a matrix effect or volumetric bias. In the LC/MS/MS results shown in Table 10, clear biases of 31% (in oranges) and 54% (in grapes) for Laboratories 1 and 8 for the IS do not occur in GC/MS of the same extracts (Table 9). Thus, this arises from a matrix effect in the LC/MS/MS analysis of the IS in the extracts. It is possible that the "matrix-matched" calibration standards induced ion suppression of the d_{10}-parathion more strongly in the calibration standards at the conditions used than the matrix used as the test samples.

The results from these tables answer a key question that indeed the QuEChERS method essentially achieves 100% recovery of the IS, and any concerns with using the IS must pertain to the analytical aspects (particularly for individual laboratories), not sample preparation.

Sample processing.—Sample processing in this case entails the test sample homogenization and weighing steps, which were done by the SD. This step was measured by the consistency in the results of chlorpyrifos-methyl (and incurred pesticides) in the test samples, as shown in Table 11. Average RSDs were 12% without using the IS, and 10% when using the IS. This matches the variability observed in the sample preparation and/or analytical steps. Thus, the sample processing step did not significantly contribute to the overall uncertainty in the collaborative study for stable analytes, such as chlorpyrifos-methyl.

Conclusions for QC results.—The reproducibility of the results for the pesticide analytes in the collaborative study was limited by the combination of reproducibilities for each step in the method, which could be estimated by the QC standards. As further described in the full report, the summation of squares ($RSD_{proc.}^2 + RSD_{prep.}^2 + RSD_{anal.}^2 = RSD_{total}^2$) estimates the

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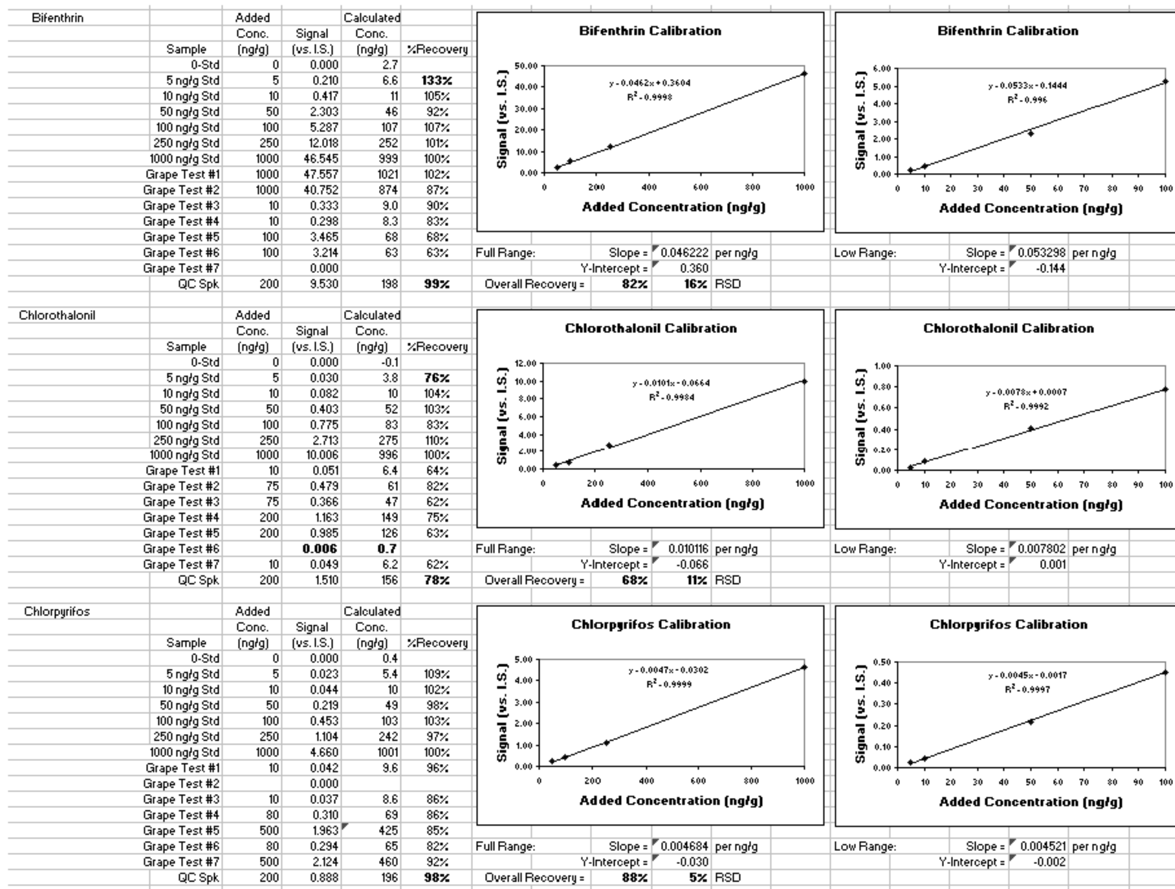


Bifenthrin Sample	Added Conc. (ng/g)	Signal (vs. I.S.)	Calculated Conc. (ng/g)	%Recovery
0-Std	0	0.000	2.7	
5 ng/g Std	5	0.210	6.6	**133%**
10 ng/g Std	10	0.417	11	105%
50 ng/g Std	50	2.303	46	92%
100 ng/g Std	100	5.287	107	107%
250 ng/g Std	250	12.018	252	101%
1000 ng/g Std	1000	46.545	999	100%
Grape Test #1	1000	47.557	1021	102%
Grape Test #2	1000	40.752	874	87%
Grape Test #3	10	0.333	9.0	90%
Grape Test #4	10	0.298	8.3	83%
Grape Test #5	100	3.465	68	68%
Grape Test #6	100	3.214	63	63%
Grape Test #7		0.000		
QC Spk	200	9.530	198	**99%**

Full Range: Slope = 0.046222 per ng/g; Y-Intercept = 0.360; Overall Recovery = **82%** **16%** RSD
Low Range: Slope = 0.053298 per ng/g; Y-Intercept = -0.144

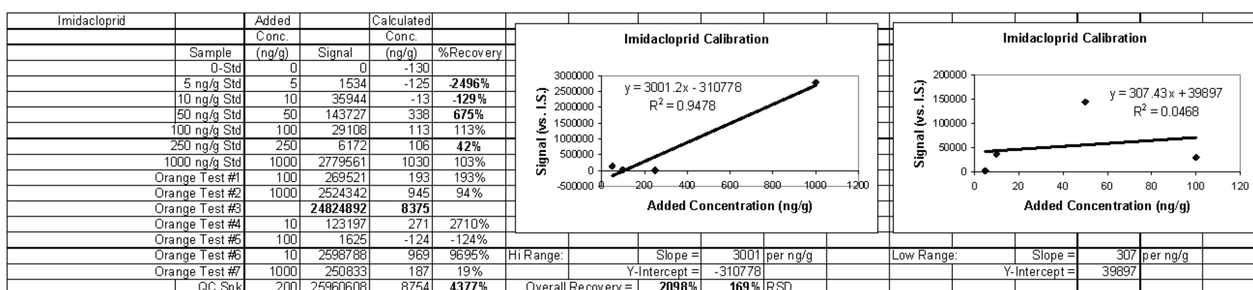
Chlorothalonil Sample	Added Conc. (ng/g)	Signal (vs. I.S.)	Calculated Conc. (ng/g)	%Recovery
0-Std	0	0.000	-0.1	
5 ng/g Std	5	0.030	3.8	**76%**
10 ng/g Std	10	0.082	10	104%
50 ng/g Std	50	0.403	52	103%
100 ng/g Std	100	0.775	83	83%
250 ng/g Std	250	2.713	275	110%
1000 ng/g Std	1000	10.006	996	100%
Grape Test #1	10	0.051	6.4	64%
Grape Test #2	75	0.479	61	82%
Grape Test #3	75	0.366	47	62%
Grape Test #4	200	1.163	149	75%
Grape Test #5	200	0.985	126	63%
Grape Test #6		**0.006**	**0.7**	
Grape Test #7	10	0.049	6.2	62%
QC Spk	200	1.510	156	**78%**

Full Range: Slope = 0.010116 per ng/g; Y-Intercept = -0.066; Overall Recovery = **68%** **11%** RSD
Low Range: Slope = 0.007802 per ng/g; Y-Intercept = 0.001

Chlorpyrifos Sample	Added Conc. (ng/g)	Signal (vs. I.S.)	Calculated Conc. (ng/g)	%Recovery
0-Std	0	0.000	0.4	
5 ng/g Std	5	0.023	5.4	109%
10 ng/g Std	10	0.044	10	102%
50 ng/g Std	50	0.219	49	98%
100 ng/g Std	100	0.453	103	103%
250 ng/g Std	250	1.104	242	97%
1000 ng/g Std	1000	4.660	1001	100%
Grape Test #1	10	0.042	9.6	96%
Grape Test #2		0.000		
Grape Test #3	10	0.037	8.6	86%
Grape Test #4	80	0.310	69	86%
Grape Test #5	500	1.963	425	85%
Grape Test #6	80	0.294	65	82%
Grape Test #7	500	2.124	460	92%
QC Spk	200	0.888	196	**98%**

Full Range: Slope = 0.004684 per ng/g; Y-Intercept = -0.030; Overall Recovery = **88%** **5%** RSD
Low Range: Slope = 0.004521 per ng/g; Y-Intercept = -0.002

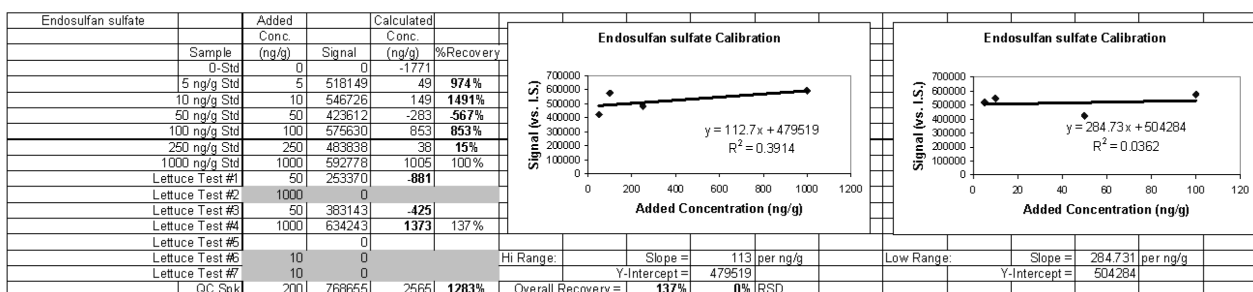
Figure 1. Excerpt from the spreadsheet used for calculation of Laboratory 1 results for GC/MS data from the grapes samples (d_{10}-parathion used as IS).



Imidacloprid	Added Conc. (ng/g)	Signal	Calculated Conc. (ng/g)	%Recovery
0-Std	0	0	-130	
5 ng/g Std	5	1534	-125	**-2496%**
10 ng/g Std	10	35944	-13	**-129%**
50 ng/g Std	50	143727	338	**675%**
100 ng/g Std	100	29108	113	113%
250 ng/g Std	250	6172	106	**42%**
1000 ng/g Std	1000	2779561	1030	103%
Orange Test #1	100	269521	193	193%
Orange Test #2	1000	2524342	945	94%
Orange Test #3		**24824892**	**8375**	
Orange Test #4	10	123197	271	2710%
Orange Test #5	100	1625	-124	-124%
Orange Test #6	10	2598788	969	9695%
Orange Test #7	1000	250833	187	19%
QC Spk	200	25960608	8754	**4377%**

Hi Range:	Slope =	3001	per ng/g	Low Range:	Slope =	307	per ng/g
	Y-Intercept =	-310778			Y-Intercept =	39897	
Overall Recovery =	**2098%**	**169%**	RSD				

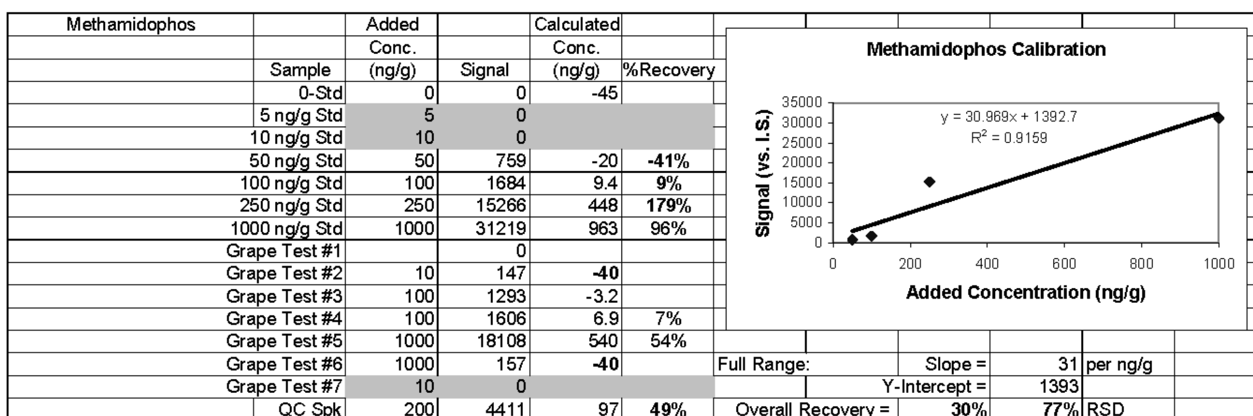
LC-MS/MS results for imidacloprid in oranges for Lab #9 without an I.S.



Endosulfan sulfate	Added Conc. (ng/g)	Signal	Calculated Conc. (ng/g)	%Recovery
0-Std	0	0	-1771	
5 ng/g Std	5	518149	49	**974%**
10 ng/g Std	10	546726	149	**1491%**
50 ng/g Std	50	423612	-283	**-567%**
100 ng/g Std	100	575630	853	**853%**
250 ng/g Std	250	483838	38	**15%**
1000 ng/g Std	1000	592778	1005	100%
Lettuce Test #1	50	253370	**-881**	
Lettuce Test #2	1000	0		
Lettuce Test #3	50	383143	**-425**	
Lettuce Test #4	1000	634243	**1373**	137%
Lettuce Test #5		0		
Lettuce Test #6	10	0		
Lettuce Test #7	10	0		
QC Spk	200	768655	2565	**1283%**

Hi Range:	Slope =	113	per ng/g	Low Range:	Slope =	284.731	per ng/g
	Y-Intercept =	479519			Y-Intercept =	504284	
Overall Recovery =	**137%**	**0%**	RSD				

GC-MS results for endosulfan sulfate in lettuces for Lab #9 without an I.S.



Methamidophos	Added Conc. (ng/g)	Signal	Calculated Conc. (ng/g)	%Recovery
0-Std	0	0	-45	
5 ng/g Std	5	0		
10 ng/g Std	10	0		
50 ng/g Std	50	759	-20	**-41%**
100 ng/g Std	100	1684	9.4	**9%**
250 ng/g Std	250	15266	448	**179%**
1000 ng/g Std	1000	31219	963	96%
Grape Test #1		0		
Grape Test #2	10	147	**-40**	
Grape Test #3	100	1293	-3.2	
Grape Test #4	100	1606	6.9	7%
Grape Test #5	1000	18108	540	54%
Grape Test #6	1000	157	**-40**	
Grape Test #7	10	0		
QC Spk	200	4411	97	**49%**

Full Range:	Slope =	31	per ng/g
	Y-Intercept =	1393	
Overall Recovery =	**30%**	**77%**	RSD

GC-MS results for methamidophos in grapes for Lab #4 without an I.S.

Figure 2. Examples of calibration errors that did not allow trustworthy quantitation of the results for that pesticide/matrix/laboratory in the study.

variability of each individual step, as shown for each laboratory in Tables 12 and 13. Due to discrepancies, imaginary numbers appeared frequently in the estimations, but these can be assumed to be zero. Poor system suitability for some of the laboratories also led to poor estimations, as indicated in the footnotes in Tables 8–13. Despite this, the overall estimations of 11–14% RSD contribution from the analytical step, 5–9% RSD from the sample preparation step, and 0% RSD from the sample processing step in the study are very likely to be correct. The analytical step was found to be the limiting source of uncertainty within each laboratory.

Test Sample Results

Tables 14–73 appear in the supplemental information of this paper on the *J. AOAC Int*. Website when accessing this article electronically or are available upon request from the author. The tables list the results from the determinations for the fortified analytes in test samples from each laboratory for each matrix. Other factors included in the tables pertain to the analysis of 200 ng/g matrix spikes for recovery, acceptability of the two 4-point calibration curves used to determine the analyte concentrations, and matrix blanks to test for interferences or false positives. The statistical outliers are also noted in the tables, as are the laboratories that did not achieve <15% RSD of the analytical and sample preparation QC standards (shaded areas). Due to calibration range, LOQ, and carry-over concerns, only those determinations yielding concentration ≥1 ng/g were included in the statistical analysis. Moreover, due to analytical uncertainties and calibration curves not always going through zero, negative concentrations also occurred in some cases.

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Table 6. QC characteristics of the GC/MS step (as determined by %RSD of 200 ng/g TPP over the course of 14 injections in a sequence) with respect to the different matrixes, laboratories, and instruments

	Grapes		Lettuces		Orange		Average		
Lab No.	Without IS	With IS	Without IS	With IS	Without IS	With IS	Without IS	With IS	Model
Quadrupole									
1[a,b]	6	8	8	7	5	6	6	7	5972
3[b,c]	8	8	5	7	16[d]	7	10	7	Voyager
7[a,b]	7	6	8	5	12	6	9	6	5973
8[a,e]	3	5	6	5	13	4	7	5	5973
9[a,e]	9	9	9	17[d]	11	10	10	12	5973
11[a,b]	ND[f]	ND	ND	ND	ND	ND	ND	ND	5973
12[a,e]	5	2	5	7	6	8	5	6	5973
13[a,e]	8	10	NA[g]	NA	NA	NA	8	10	5973
Avg.	7	7	7	8	10	7	8	8	Quad
Ion trap									
2[b,c]	ND	ND	ND	ND	ND	ND	ND	ND	Saturn
4[b,h]	10	15	18[d]	9	11	10	13	11	Saturn
5[b,h]	7	17[d]	13	20[d]	14	16[d]	11	18[d]	Saturn
6[c,e]	30[d]	6	18[d]	14	24[d]	10	24[d]	10	Polaris
Avg.	16[d]	13	16[d]	14	16[d]	12	16[d]	13	Trap
Time of flight									
10[b,c]	16[d]	8	18[d]	11	28[d]	21	21	13	Pegasus
Avg.	10	8	11	10	14	10	11	10	Overall

[a] Selected-ion monitoring mode.
[b] Large volume injection of MeCN extracts.
[c] Full scan.
[d] RSD >15%.
[e] Splitless injection of concentrated toluene extracts.
[f] ND = Not done.
[g] NA = Not applicable.
[h] MS/MS.

Calibration errors occurred periodically in the study, which happened more often at the 5 and 10 ng/g calibration standard levels than at other concentrations. The problem was often avoided by using the 50–1000 ng/g calibration range. Figure 2 shows some examples of calibration errors.

Table 74 provides the results in all 3 matrixes for the chlorpyrifos-methyl QC standard, which is treated as an "incurred" pesticide in the study with a known added concentration. Actual incurred pesticides appear in Tables 75–79 (*o*-phenylphenol was also analyzed as an incurred pesticide in oranges, but its quantitation was unreliable because the matrix blanks used for preparation of calibration standards either had a GC/MS interferant or inconsistent *o*-phenylphenol concentrations in all but 5 laboratories).

GC/MS versus LC/MS/MS.—Certain pesticides were analyzed by both GC/MS and LC/MS/MS in several of the laboratories. When both sets of results were provided, only 1 of the sets from each laboratory for all 7 test samples per analyte/matrix pair was included in the statistical analysis. The choice of GC/MS or LC/MS/MS results depended on the analyte, QC results, calibration plots, and LOQ (as observed from the calibration plots and undetected analytes in the test samples).

Table 80 compares the statistical analysis using only GC/MS or LC/MS/MS for different incurred pesticides in the collaborative study. The most nonpolar analytes, such as λ-cyhalothrin and permethrins, are not able to be analyzed by LC/MS/MS at the same conditions as the other pesticides, and the most polar pesticides, such as imidacloprid, are not compatible in GC/MS. For those in between, relatively apolar analytes, such as chlorpyrifos-methyl and ethion, are at the edge of LC/MS/MS compatibility, and GC/MS generally provides more trustworthy results for them. For relatively polar analytes, such as thiabendazole, LC/MS/MS yields better results. In the case of semipolar analytes, such as kresoxim-methyl and cyprodinil, quality of GC/MS and LC/MS/MS results are much the same. The LC/MS/MS method is usually more selective and sensitive than GC/MS,

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Table 7. QC characteristics of the LC/MS/MS step (as determined by %RSD of 200 ng/g TPP over the course of 14 injections in a sequence) with respect to the different matrixes, laboratories, and instruments

	Grapes		Lettuces		Oranges		Average	
Lab No.	Without IS	With IS	Without IS	With IS	Without IS	With IS	Without IS	With IS
1	2	5	6	6	6	15	5	9
2	ND[a]	ND	ND	ND	ND	ND	ND	ND
3[b]	9	20[c]	8	8	8	13	8	14
4	9	ND	4	ND	6	ND	6	ND
5	61[c]	45[c]	26[c]	17[c]	16[c]	37[c]	34[c]	33[c]
6	15	19[c]	11	14	10	9	12	14
7	7	10	6	12	4	9	6	10
8	8	20[c]	10	9	15	21[c]	11	17[c]
9	4	ND	5	ND	19[c]	ND	9	ND
10	7	8	24[c]	12	25[c]	9	19[c]	10
11	3	16[c]	5	8	6	12	5	12
12	6	ND	8	ND	6	ND	7	ND
13	7	6	NA[d]	NA	NA	NA	7	6
Overall	12	17[c]	10	11	11	14	11	14

[a] ND = Not done.
[b] Laboratory 3 used diethatyl-ethyl instead of TPP.
[c] RSD is >15%.
[d] NA = Not applicable.

Table 8. QC characteristics in the combination of the extraction/cleanup and analytical steps as determined by %RSD of the 200 ng/g d_{10}-parathion (unless noted otherwise) in the analyses over the course of 14 injections in a sequence

	Grapes		Lettuces		Oranges		Average	
Lab No.	GC	LC	GC	LC	GC	LC	GC	LC
1	6	5	9	6	5	14	7	8
2	8	ND[a]	7	ND	14	ND	10	ND
3	10	12	6	12	17[b,c]	13	11	12
4	28[b,c]	ND	15[c]	ND	16[b]	ND	20[b]	ND
5	12	65[b,c]	13	24[b,c]	16[b]	25[b,c]	14	38[b,c]
6	28[b,c]	10	14[c,d]	11	24[b,c]	11	22[b,c]	11
7	6	7	9	9	11	12	9	9
8	5	24[b]	5	14	13	14	8	17[b]
9	12[d]	ND	16[b,d]	ND	12[d]	ND[c]	13[d]	ND
10	16[b,c]	8	11[c]	13[b]	16[b,d]	23[b,c]	14[c]	15[c]
11	7	17[b]	13	8	14	14	11	13
12	5	ND	9	ND	6	ND	7	ND
13	16[b]	8	NA[e]	NA	NA	NA	16[b]	8
Avg.	12	17[b]	11	12	14	16[b]	12	15

[a] ND = Not done.
[b] RSD is >15%.
[c] Quality control criteria were not met for TPP (>15% RSD in Tables 6 and 7).
[d] 200 ng/g d_6-HCH result.
[e] NA = Not applicable.

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Table 9. Ratio between the average areas of the test samples ($n = 7$) divided by the average areas of the calibration standards ($n = 7$) for the IS (d_6-α-HCH or d_{10}-parathion) and QC standard (TPP) for each laboratory as determined in the GC/MS sequences[a]

	Grapes			Lettuces			Oranges		
Lab No.	d_6-HCH	d_{10}-Parathion	TPP	d_6-HCH	d_{10}-Parathion	TPP	d_6-HCH	d_{10}-Parathion	TPP
1	0.93 (8)[b]	1.00 (8)	0.91 (5)	1.02 (12)	1.06 (11)	0.96 (12)	1.07 (7)	1.07 (6)	0.98 (6)
2	0.90 (14)	1.08 (9)	ND[c]	1.05 (9)	1.09 (7)	ND	0.98 (15)	1.16 (16)	ND
3	0.94 (3)	0.85 (9)	0.93 (10)	1.00 (5)	0.98 (8)	1.07 (6)	0.99[d] (17)[d]	0.99[d] (24)[d,e]	0.98[d] (22)[d,e]
4	0.91 (13)	0.71[d,e] (38)[d,e]	0.87 (10)	1.04[d] (17)[d]	0.96[d] (22)[d,e]	1.07[d] (22)[d,e]	0.94[d] (27)[d,e]	1.05[d] (22)[d,e]	1.03 (15)
5	0.95[d] (35)[d,e]	0.98 (26)[e]	0.94 (10)	1.20[e] (12)	1.02 (18)	1.02 (19)	1.28[d,e] (16)[d]	1.32[e] (9)[d]	1.12[d] (17)[d]
6	1.00[d] (16)[d]	0.95[d] (42)[d,e]	0.99[d] (42)[d,e]	0.95[d] (21)[d,e]	0.52[d,e] (83)[d,e]	0.89[d] (27)[d,e]	0.80[d,e] (55)[d,e]	0.83[d] (36)[d,e]	0.90 (38)[e]
7	1.04 (6)	1.11 (3)	1.08 (7)	1.17[d] (17)[d]	1.10 (11)	1.03 (11)	1.08 (12)	1.08 (13)	1.04 (16)
8	0.93 (8)	0.96 (6)	1.03 (4)	0.84 (11)	1.03 (6)	1.09 (6)	1.13 (10)	1.23[e] (10)	1.24[e] (9)
9	1.00 (18)	1.08[d] (13)[d]	0.98 (13)	0.99[d] (22)[d,e]	1.44[d,e] (21)[d,e]	1.06 (11)	0.90 (17)	1.40[d,e] (8)[d]	1.06 (14)
10	1.06[d] (10)[d]	1.04[d] (21)[d,e]	1.11[d] (19)[d]	1.13[d] (9)[d]	1.15[d] (11)[d]	1.33[d,e] (13)[d]	0.95[d] (23)[d,e]	1.18[d] (35)[d,e]	1.26[d,e] (29)[d,e]
11	ND	1.13 (5)	ND	ND	1.23[e] (10)	ND	ND	1.25[e] (10)	ND
12	ND	1.01 (8)	1.01 (7)	ND	1.12 (8)	1.05 (6)	ND	1.08 (6)	1.04 (8)
13	0.86[d] (26)[d,e]	0.86[d] (21)[d,e]	0.98 (11)	NA[f]	NA	NA	NA	NA	NA
Avg.	0.96 ± 0.06	0.98 ± 0.12	0.98 ± 0.07	1.04 ± 0.11	1.06 ± 0.21	1.06 ± 0.11	1.01 ± 0.13	1.14 ± 0.15	1.06 ± 0.11

[a] This is potentially a measurement of recoveries of the IS, but volume and pipetting differences are also involved.

[b] Values in parentheses are the %RSD of the ratios in the calculations ($n = 14$). These factors indicate if biases and/or imprecisions occurred in the results for the analytical sequence.

[c] ND = Not done.

[d] Variability of quality control standards were >15% RSD (*see* Tables 6–8).

[e] Results with >20% bias or >20% RSD.

[f] NA = Not applicable.

and the SD chose LC/MS/MS for statistical treatment in most cases when QC results showed both sets to be of equal quality.

The ability to cover a wide analytical scope with both instruments and compare results for doubly analyzed pesticides is an important advantage for both qualitative and quantitative purposes. The QuEChERS method achieves high recoveries of a wide polarity range of GC and LC compatible analytes. The compatibility of the method for both GC and LC analysis of the same extract in a fast and simple procedure, especially when LVI is used in GC, is a key feature of the QuEChERS method.

Performance of Individual Laboratories in Analysis

Table 81 presents the overall QC assessment of each laboratory in the study based on the GC/MS and LC/MS/MS results for each matrix. The table summarizes the number of times that the factors listed in Tables 14–79 occurred for each combination of laboratory, technique, and matrix. As one would expect, the most QC problems occurred for those sample sequences shaded gray (in the supplemental information of this paper on the *J. AOAC Int.* Website) or as indicated in the footnotes of the tables shown in this collaborative study, which indicate high variability of the QC standards. For example, Laboratory 4 did not meet the <15% RSD QC criteria for GC/MS analysis in any of the matrixes, and this laboratory had 41 instances of missed analytes, calibration errors, poor QC spike recoveries, or false positives and negatives in GC/MS. This laboratory yielded 9 outliers, all from the GC/MS analyses. A major reason for the problems with this laboratory's results was their reliance on GC/MS to cover so many analytes, including those that were better analyzed by LC/MS/MS. The opposite situation occurred for Laboratory 11, which gave many outliers for pesticides that should have been analyzed by GC/MS (e.g., chlorpyrifos, chlorpyrifos-methyl, and ethion), rather than LC/MS/MS.

The IS corrected high biases in many of the LC/MS/MS grapes and lettuces results for Laboratory 8 (10 outliers were reduced to 1 by using the IS for those matrixes), but this was not always the case. Laboratories 9 and 10 gave 10 and 9 outliers, respectively, without use of an IS, but using an IS doubled the number of outliers in both cases. Laboratory 9 also had calibration errors in GC/MS for all but 2 analytes in lettuces, which had 2 false negatives and 2 outliers even among those few data points. However, the LC/MS/MS results for Laboratory 9 were very good, with only 4 outliers (all in oranges, mainly due to a spurious source of error for imidacloprid). This demonstrated that the sample preparation steps were fine, even when an analysis gave poor results, and the analytical step was the limiting factor in the protocol.

The summation of results in Table 81 shows that Laboratories 1–3, 6, 7, 12, and 13 generally gave reliable results

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Table 10. Ratio between the average areas of the test samples (n = 7) divided by the average areas of the calibration standards (n = 7) for d_{10}-parathion and TPP for each laboratory as determined in the LC/MS/MS sequences[a]

	Grapes		Lettuces		Oranges	
Lab No.	d_{10}-Parathion	TPP	d_{10}-Parathion	TPP	d_{10}-Parathion	TPP
1	1.05 (7)[b]	1.02 (2)	0.95 (8)	0.90 (3)	1.31[c] (5)	1.00 (9)
2	ND[d]	ND	ND	ND	ND	ND
3	1.08 (15)	0.96 (17)	1.03 (16)	0.97 (10)	1.04 (18)	0.94 (12)
4	ND	0.98 (12)	ND	0.97 (5)	ND	1.03 (8)
5	0.75[c,e] (123)[c,e]	1.29[c,e] (66)[c,e]	0.76[c,e] (33)[c,e]	0.72[c,e] (36)[c,e]	1.08[e] (32)[c,e]	0.99[e] (22)[c,e]
6	1.09 (12)	1.00 (21)[c]	1.14 (11)	0.95 (17)	1.03 (16)	1.07 (17)
7	1.01 (10)	1.04 (9)	1.05 (12)	0.97 (8)	1.12 (8)	1.04 (5)
8	1.54[c,e] (12)[e]	1.13 (7)	1.26[c] (10)	1.17 (8)	0.94 (19)	0.79[c] (16)
9	ND	1.04 (6)	ND	0.97 (7)	ND[e]	0.83[e] (29)[c,e]
10	0.99 (11)	1.03 (10)	0.77[c,e] (6)[e]	0.64[c,e] (22)[c,e]	0.91[e] (35)[c,e]	0.77[c,e] (41)[c,e]
11	1.25[c,e] (15)[e]	1.02 (5)	0.99 (11)	0.93 (5)	1.15 (14)	1.11 (5)
12	ND	0.98 (9)	ND	1.10 (7)	ND	1.00 (8)
13	0.99 (10)[f]	0.95 (9)[f]	NA[g]	NA	NA	NA
Avg.	1.08 ± 0.22	1.04 ± 0.09	0.99 ± 0.17	0.94 ± 0.15	1.07 ± 0.13	0.96 ± 0.12

[a] This is potentially a measurement of recoveries of the IS, but volume and pipetting differences are also involved.
[b] Values in parentheses are the %RSD of the ratios in the calculations (n = 14). This factor indicates if biases and/or imprecisions occurred in the results for the analytical sequence.
[c] Results with >20% bias or >20% RSD.
[d] ND = Not done.
[e] Variability of the quality control standards were >15% RSD (*see* Tables 7 and 8).
[f] Average of 8 sequences (n = 112).
[g] NA = Not applicable.

in all cases (few QC problems and outliers). As also shown in Table 10, LC/MS/MS results for Laboratory 8 exhibited a high bias in the grapes and lettuces samples. This collaborator knew of the problem due to a volume change of the extract prior to LC/MS/MS analysis. They expected that only the results using the IS mattered in the study, which created a difficulty for the SD in how to evaluate their non-normalized results. Laboratory 9 gave reliable LC/MS/MS results, but GC/MS was a problem for that laboratory, for reasons noted in *Collaborators' Comments*. Laboratory 10 did well in the grapes and lettuces analyses, but its performance was worse for the oranges. Laboratories 4, 5, and 11 gave acceptable results in many cases, but sporadic QC problems and outliers made their overall results less trustworthy.

Qualitative Analysis

The unabridged report discusses qualitative factors with respect to false positives and negatives for each pesticide/commodity pair, which are summarized in Table 81. In brief, quantitation is meaningless without qualitative knowledge of what chemical is being reported. In many pesticide monitoring applications, it is more important to be right about the presence or absence of a wide scope of pesticides in the samples, and accurately determining the concentration is of secondary importance. As the reported results of this interlaboratory study demonstrate, despite the use of state-of-the-art GC/MS and LC/MS/MS for analysis, the rates of false positives and negatives in real-world monitoring laboratories is higher than desirable, and all monitoring laboratories should take care to address this often ignored issue. Despite the simplicity of the QuEChERS sample preparation method, multiclass, multiresidue analysis with sophisticated instruments is not a simple task, and good quality of results comes from analyst knowledge, skill, and experience. In defense of the collaborators, they were volunteers who had other work to do in their laboratories and were limited with respect to the time, money, and care that they could put into this study.

Overall Performance of the Method for Incurred Pesticides

Tables **2007.01A–D** give the statistical performance of the method for the incurred and fortified pesticides in the collaborative study in each matrix. The statistical values in the tables consist of C (average concentration), s_r (standard deviation of repeatability for the results within single laboratories), $\%RSD_r$ (relative standard deviation of repeatability), s_R (standard deviation of overall reproducibility for the results), $\%RSD_R$ (relative standard deviation of

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Table 11. QC characteristics of the overall method including sample homogenization and weighing steps (done by the SD) as determined by %RSD of 7 injections of 200 ng/g chlorpyrifos-methyl in each sequence using GC/MS (unless noted otherwise) for analysis

Lab No.	Grapes	Lettuces	Oranges	Average
1	2	13	6	7
2	6	6	11	8
3	6	10	16[a,b]	11
4	25[a,b]	41[a,b]	14[b]	27[a,b]
5	5	5	9[b]	6
6	20[a,b]	10[b]	15[b]	15[b]
7	4	8	12	8
8	5	5	6	5
9	16[a,b]	40[a,b]	9[b]	22[a]
10	9[b]	13[b]	21[a,b]	14[b]
11[c]	8[b]	6	20[a]	11
12	9	4	9	7
13	10[b]	NA[d]	NA	10[b]
Avg.	10	14	12	12

[a] RSD is >15%.
[b] Quality control standards were >15% RSD (*see* Tables 6–8).
[c] LC/MS/MS results used for Laboratory 11.
[d] NA = Not applicable.

Table 12. Compilation of the approximate %RSD contributions from the extraction/cleanup and sample processing steps in the protocol using results for each laboratory and matrix[a]

	Extraction/cleanup						Processing		
	Grapes		Lettuces		Oranges		Grapes	Lettuces	Oranges
Lab No.	GC	LC	GC	LC	GC	LC	GC	GC	GC
1	0	5	4	0	0	13	0	9	—[b]
2	<8	UC[c]	<7	UC	<14	UC	—	—	—
3	6	8	3	9	6[d]	10	—	8	—[d]
4	26[d,e]	UC	—[d]	UC	12[d]	UC	—[d]	37[d,e]	—[d]
5	10	22[d,e]	0	—[d]	8[d]	19[d,e]	—	—	—[d]
6	—[d]	—	40[d,e]	0	0[d]	5	—[d]	—[d]	—[d]
7	—	0	4	7	—	11	—	—	0
8	4	23[d,e]	—	10	0	—	0	—	—
9	4	UC	41[d,e]	UC	17[e]	UC[d]	13	—[d]	—
10	0[d]	4	—[d]	—	—[d]	—[d]	—[d]	—[d]	—[d]
11	<7	17[d,e]	<13	6	<14	13	—	0[f]	14[f]
12	0	UC	7	UC	0	UC	7	—	7
13	14[d]	4	NA[g]	NA	NA	NA	—[d]	NA	NA

[a] Values were estimated by summation of squares from quality control data.
[b] — = Estimation gave imaginary number.
[c] UC = Unable to be calculated.
[d] <15% RSD of quality control standards not achieved.
[e] RSD > 15%.
[f] LC/MS/MS data used.
[g] NA = Not applicable.

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reproducibility), %recovery when applicable, HorRat (Horwitz ratio), number of laboratories included in the assessment, and notes about outliers.

The assessment of the method for incurred pesticides (plus chlorpyrifos-methyl, the QC standard for homogenization of the test samples) appears in Table **2007.01A**. The concentrations of these pesticides were unknown except for chlorpyrifos-methyl, of which 86% with 19% RSD_R was recovered on average from the 3 matrixes. The equally good RSD_r and RSD_R data for the incurred samples (accounting for concentration differences) indicate that they behaved similarly to chlorpyrifos-methyl during homogenization. This further demonstrates that the sample processing was acceptable in the study, and no appreciable losses of the QC standard occurred.

Incurred versus fortified pesticides.—Table **2007.01E** provides the average overall results of the study. The overall recovery of the fortified pesticides averaged 87 ± 11%. The analysis of incurred analytes is very important to demonstrate the real-world applicability of a method. Although the actual concentration of these analytes other than chlorpyrifos-methyl are unknown, statistical analysis of results from multiple laboratories in an AOAC collaborative study give strong evidence about the method performance and quality. The fact that the statistical analysis of incurred pesticides gave similar repeatabilities (10–12%), reproducibilities (21–22%), and HorRat values (0.86–0.92) as those from the fortified pesticides, which were typically recovered at 87%, is strong support that the method achieves equivalent trueness for incurred pesticides as those that are fortified. The 86% average recovery of the "incurred" chlorpyrifos-methyl QC standard further buttresses this point.

Permethrins.—All of the incurred analytes gave HorRat <1.2 except for permethrins in lettuces (HorRat = 1.63 without IS). As shown in Table 77, the higher variability of permethrins was mainly the result of consistently low GC/MS determinations by Laboratory 2, but which was not able to be removed as an outlier (HorRat would be 1.20 if Laboratory 2 results were not included). The removal of Laboratories 1 and 6 as Cochran outliers improved repeatability, but with average concentrations similar to Laboratories 3–5, 7, 11, and 12, it made matters worse in terms of the reproducibility and HorRat. The analysis of permethrins was more complicated than the other analytes due to the combination of *cis*- and *trans*-permethrins in the standard and integrated peaks. Differences in the ratios of *cis*- and *trans*-permethrins in the sample versus the ratio in the standard yield greater variability in the results. Thus, the higher variability for permethrins than the other incurred analytes is not unexpected.

Permethrins are also among the last pesticides to elute from the GC column, and they are more sensitive to the temperatures in the injector, column, and ion source. Constant flow rate rather than constant inlet pressure can make significant improvements in permethrin results. Unlike the last eluting analyte, azoxystrobin, they could not be detected by LC/MS/MS for comparison purposes. The use of higher temperatures to improve analysis for permethrins tends to

Table 13. Compilation of the approximate %RSD results for each step in the protocol using averaged results among the 3 matrixes[a]

Lab No.	Analysis		Extraction/cleanup		Processing (GC data)	Overall (GC data)
	GC	LC	GC	LC		
1	6	5	4	6	0	7
2	<10	UC[b]	<10	UC	—[c]	8
3	10	8	5	9	0	11
4	13[d]	6	15[d]	UC	18[d,e]	27[d,e]
5	11	34[d,e]	9	17[d,e]	—	6
6	24[d,e]	12	—[d]	—	—[d]	15[d]
7	9	6	0	7	—	8
8	7	11	4	13	—	5
9	10	9	8	UC	18[d,e]	22[e]
10	21[d,e]	19[d,e]	—[d]	—[d]	0[d]	14[d]
11	<11	5	<11	12	—	11
12	5	7	5	UC	0	7
13	8[d]	7	14	4	—[d]	10[d]
Avg.	11	14	9	5	—	12

[a] Values were estimated by summation of squares from quality control data.
[b] UC = Unable to be calculated.
[c] — = Estimation gave imaginary number.
[d] <15% RSD of quality control standards not achieved.
[e] RSD > 15%.

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Table 74. Overall results for chlorpyrifos-methyl in the test samples

				Test sample/concn, ng/g								
Matrix	Lab No.	Hi-Cal R^2 value	Spike rec., %	1	2	3	4	5	6	7	Avg. rec., %	RSD, %
Grapes	1	1.000	105	169	167	160	171	168	175	166	84	2
	2	1.000	109	172	162	171	192	185	193	187	90	6
	3	1.000	90	117	120	103	108	111	114	106	56[a]	5
	4[b]	0.992	85	56[c]	142[c]	105[c]	107[c]	114[c]	138[c]	125[c]	56[b]	24[b]
	5	1.000	92	130	139	137	133	145	150	134	69[a]	5
	6[b]	0.999	84	135[c]	174[c]	197[c]	183[c]	138[c]	216[c]	125[c]	83	19[a]
	7	1.000	103	190	187	183	176	178	201	185	93	4
	8	1.000	93	163	185	162	172	161	170	163	84	5
	9	1.000	91	195	190	155	129	174	151	175	83	13
	10[b]	0.999	112	195	184	168	191	186	159	191	91	7
	11[b,d]	0.996	122[a]	197	231	252	266	225	220	246	117	9
	12	1.000	104	136	123	160	134	149	143	156	72	8
	13[b]	0.998	108	116	150	123	128	156	159	164	71	13
Lettuces	1	1.000	98	139	212	200	185	150	173	195	90	14
	2	1.000	98	163	179	151	171	181	163	164	84	6
	3	0.999	84	170	144	128	138	133	125	142	70	10
	4[b]						Calibration errors					
	5	1.000	87	179	203	201	197	182	195	179	95	5
	6[b]	0.999	69[a]	209	170	146	175	174	166	194	88	11
	7	0.989[a]	89	240	221	220	226	199	195	181	106	9
	8	1.000	91	195	182	184	172	189	172	171	90	5
	9[d]	1.000	109	162	210	135	153	218	159	152	85	17[a]
	10[b]	0.999	63[a]	213	197	210	277	204	190	198	106	13
	11[d]	1.000[e]	165[a]	363[f]	288[f]	341[f]	335[f]	317[f]	337[f]	322[f]	165[a]	7
	12	1.000	120	153	156	146	156	141	145	143	74	4
Oranges	1	0.999	94	195	182	175	170	186	161	164	88	6
	2	0.999	104	189	213	182	186	174	161	153	90	10
	3[b]	0.997	71	208	166	144	144	157	148	123	78	16[a]
	4[b]	0.992	30[a]	166	174	113	115	138	149	162	73	16[a]
	5[b]	0.983[a]	81	175	177	172	190	175	139	144	84	10
	6[b]	0.998	77	143	127	111	126	132	101	141	63[a]	11
	7	0.973[a]	80	231	228	224	215	168	164	153	99	16[a]
	8	1.000	117	230	215	219	201	207	200	192	105	6
	9[b]	0.997	86	227	212	225	195	219	177	170	102	11
	10	1.000	56[a]	173	155	163	192	122	100	174	77	19[a]
	11[c]	0.996	98	222	218	289	201	145	203	197	105	19[a]
	12	1.000	102	173	179	157	169	148	169	133	81	9

[a] $R^2 < 0.99$, recovery <70 or >120%, positive finding in a blank sample, value outside of calibration range by more than 20%, or RSD >15%.
[b] <15% RSD of quality control standards not achieved.
[c] Cochran outlier.
[d] LC/MS/MS results.
[e] Quadratic.
[f] Single Grubbs outlier.

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Table 75. Overall results for kresoxim-methyl in the grapes test samples

				Test sample/concn, ng/g								
Lab No.	Analysis	Lo-Cal R^2 value	Spike rec., %	1	2	3	4	5	6	7	Avg., ng/g	RSD, %
1	LC	1.000	108	10	8.9	8.8	9.4	8.2	10	10	9.4	8
2	LC	1.000	99	13	14	7.3	7.8	13	9.8	15	11	25[a]
3	LC	1.000	97	6.5	6.2	5.5	5.9	4.8	2.3[a]	5.8	5.3	25[a]
4	GC	0.999	100	ND[b]	ND	ND	ND	ND	ND	ND	—[c]	—
5	GC	1.000	115	7.1	8.3	8.0	9.1	6.5	7.9	8.6	7.9	10
6	LC	0.997	94	8.6	12	8.9	10	8.6	12	10	10	14
7	LC	0.999	115	8.5	9.5	10	9.6	8.7	8.6	8.6	9.1	6
8[d]	LC	0.982[a]	138[a]	9.3	17	14	15	11	18	14	14	19[a]
9	LC	1.000	104	11	11	8.2	5.9	9.3	8.2	10	9.1	19[a]
10	LC	0.975[a]	90	9.9	5.3	5.9	4.8	7.4	6.1	9.0	6.9	26[a]
11[d]	LC	0.998	121[a]	19	12	12	15	11	13	11	13	20[a]
12	LC	0.999	94	6.7	5.2	5.7	6.5	5.6	5.1	7.2	6.0	12
13[d]	GC	1.000	101	11	7.6	6.1	6.0	6.7	7.3	9.9	7.8	23[a]

[a] R^2 < 0.99, recovery <70 or >120%, value outside of calibration range by more than 20%, or RSD >15%.
[b] ND = Not done.
[c] — = Unable to calculate.
[d] <15% RSD of quality control standards not achieved.

worsen the results for the many less volatile pesticides in the GC/MS method. Thus, analytical quality of late-eluting analytes, such as permethrins, are usually sacrificed for better overall quality in multiclass, multiresidue analysis. Interestingly, Laboratory 7 made a 2nd injection for each sample using optimized conditions just for permethrins, but of course, this increases time, effort, and cost of the analysis.

Nothing about the anomalously more variable result for permethrins indicates a problem with the sample preparation method, and the very good result for other nonpolar pyrethroids, λ-cyhalothrin (incurred) and bifenthrin (fortified), in the same samples shows that the QuEChERS method works well for the pyrethroids (previous validation studies also did not indicate a problem). In any event, the HorRat for permethrins is still acceptable by AOAC criteria.

Method Performance for Fortified Pesticides

Tables **2007.01B–D** list the results for the pesticides at 3 fortified levels in the 3 representative fruit and vegetable matrixes. The recoveries outside the range 70–120%, repeatabilities >15%, reproducibilities >25%, HorRat >1.20, and $n < 8$ are indicated in the footnotes of the tables. The outliers listed here are compiled within Table 81. As mentioned previously, certain laboratories (No. 4, 5, and 8–11) tended to have the most outliers (depending on the particular analytical sequence), and although their values were not always statistically removable, those laboratory results were usually the source of HorRat >1.20 when that occurred. Further discussion appears in the supplemental information about the reasons for those occurrences.

Effect of matrix on results.—As shown in Table **2007.01E**, no significant differences were observed in the overall results among the matrixes. Grapes achieved the best overall results probably because it had a moderately acidic pH (≈3.5) and is a cleaner matrix than both lettuces and oranges (presence of large amounts of chlorophyll and pectins, respectively). Lettuces have pH ≈6, and oranges have pH ≈4 (vm.cfsan.fda.gov/~comm/lacf-phs.html). Due to the buffering in the QuEChERS protocol, the pH had only a minor effect on the extraction and cleanup, but there were observable effects in interferences (or contamination) and degradation prior to the analysis. Endosulfan sulfate results, for example, may have been affected by interferences in lettuces, and the early eluting methamidophos and dichlorvos in GC/MS were difficult to analyze in the oranges due to the many coextractives appearing in that part of the chromatogram.

Overall Results for Individual Pesticides

Figure 3 is an example (atrazine in this case) of the plots made for all pesticides fortified in the different matrixes from 10–1000 ng/g. All 20 figures (Figures 3–22) appear in the supplemental information in this manuscript. The plots graphically exhibit the compiled and overall results for each pesticide and matrix across the 10–1000 ng/g fortification range in the collaborative study. Only results from Tables **2007.01B–D** with 7 or more laboratories were included in the overall calculations. The slope and R^2 values of the log-log plots are also given in the figures to indicate if there was a concentrational dependence on recoveries or high deviation of results. A perfectly linear relationship of the determined

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Table 76. Overall results for λ-cyhalothrin in the lettuces test samples

				Test sample/concn, ng/g								
Lab No.	Analysis	R^2 value	Spike rec., %	1	2	3	4	5	6	7	Avg., ng/g	RSD, %
1	GC	0.999	107	56	65	71	76	61	69	62	66	10
2	GC	1.000	113	64	62	59	66	74	62	65	65	7
3	GC	0.993	68[a]	48	45	52	43	41	40	43	45	9
4[b]	GC	0.991	76	51	49	58	59	64	45	43	53	14
5	GC	1.000	96	59	59	67	58	61	61	63	61	5
6[b]	GC	0.995	58[a]	59	43	39	48	40	50	57	48	15
7						Not analyzed						
8	GC	0.999	86	58	53	61	54	69	61	67	60	10
9						Calibration errors						
10[b]	GC	0.999	68[a]	82	72	74	82	76	65	64	74	9
11	GC	0.999	106	77[c]	65[c]	92[c]	101[c]	70[c]	71[c]	98[c]	82[c]	17[a]
12	GC	1.000	116	55	47	53	51	41	42	44	48	11

[a] R^2 < 0.99, recovery <70 or >120%, value outside of calibration range by more than 20%, or RSD >15%.
[b] <15% RSD of quality control standards not achieved.
[c] Cochran outlier.

versus added concentrations would yield a slope of 1.00 with R^2 = 1.000. The results from the different matrixes can be observed from the different symbols on the plots, and the error bars represent s_R.

The large majority of analytes presented no issues in the study. Thirteen of the 20 fortified pesticides performed very well in the method with average recoveries = 86–98%, RSD_r = 7–11%, RSD_R = 15–21%, and HorRat = 0.63–0.87. Listed in order of increasing HorRat, these consist of azoxystrobin, imidacloprid, methamidophos, tebuconazole, methomyl, bifenthrin, *o,p′*-DDD, atrazine, linuron, procymidone, trifluralin, cyprodinil, and carbaryl. The QuEChERS sample preparation method and GC/MS and/or LC/MS/MS analysis performed very well for all of those analytes independent of matrix, laboratory, or concentration. Note that polar (e.g., methomyl and imidacloprid) and nonpolar (e.g., *o,p′*-DDD and bifenthrin) pesticides, as well as volatile (methamidophos) and nonvolatile (azoxystrobin) analytes, are included in the group of best actors. Methamidophos is one of the most difficult pesticides to extract and detect in traditional methods, which entail GC analysis, but with the QuEChERS method, it is among the easiest analytes, particularly when LC/MS/MS is used for analysis.

The next group of pesticides gave good results, but either had somewhat lower recoveries or higher variability. These analytes consist of dichlorvos (82, 9.6, 21, 0.88 overall averages of %rec., $\%RSD_r$, $\%RSD_R$, and HorRat, respectively); chlorpyrifos (89, 12, 25, 1.04); and pymetrozine (69, 8.9, 19, 0.72). The worst actors consisted of endosulfan sulfate (80, 20, 27, 1.29); imazalil (76, 7.1, 32, 1.36); tolylfluanid (68, 14, 33, 1.32); and chlorothalonil (70, 24, 34, 1.41). These pesticide results will be discussed individually in the following paragraphs. More details are provided in the supplemental information.

Dichlorvos.—Dichlorvos is the most volatile pesticide commonly analyzed in monitoring programs. It may be lost during sample processing even when dry ice is used. The SD had some concern that some of the analyte would be lost during the solvent evaporation and exchange step to toluene for Laboratories 6, 8, 9, 12, and 13, but the few QC spikes from GC/MS results of dichlorvos generally showed 100% recoveries. This concern was unfounded also because the laboratories mostly used LC/MS/MS for the analysis of dichlorvos, which did not require solvent evaporation. Dichlorvos could have been included in the group of best actors, except its slightly lower recovery (82%) and higher variability (21% RSD_R) was probably a consequence of its having a higher LOQ (≈5 ng/g) than the other analytes. Also, dichlorvos was adversely affected in a few instances when laboratories that commonly gave biases were not able to be removed as statistical outliers.

Chlorpyrifos and imazalil.—The somewhat worse (but easily acceptable) results for chlorpyrifos were a surprise to the SD. The even worse results for imazalil were very surprising, especially considering that it has given excellent results in previous experiments. Chlorpyrifos is a stable, semipolar, common pesticide analyzed by GC/MS with no unique difficulties. It is not much different from procymidone (or chlorpyrifos-methyl), for example, which gave excellent results. On the other hand, imazalil is one of the most basic analytes included in monitoring schemes, and the SD initially hypothesized that the basic nature of the analyte posed a problem in the buffered QuEChERS method. However, closer inspection of the data revealed that both of these analytes fell

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victim to analytical biases and reduced quality from certain laboratories in a few instances that could not be removed as statistical outliers. In the case of imazalil, the very odd relationship that average RSD_r was merely 7.1%, which was the 2nd lowest value among all analytes, whereas RSD_R was 32% (3rd highest) points to a false assessment. The SD maintains that both analytes, along with dichlorvos, are determined by the QuEChERS method with equal quality as some of the best actors in the collaborative study, but the statistics are based on the assumption that the results arise from random factors, whereas the reality is that nonsystematic analytical shortcomings (e.g., use of less than optimal conditions, integration errors, and other mistakes) are the main cause of problems. The SD relied on the statistical treatments to take care of the biases and errors, but they were not always caught. However, such is the nature of multiclass, multiresidue pesticide analysis, and this study was not outside the norm of real-world laboratory analysis, so removal of these data sets is not justifiable. It is also likely true that some of the results for individual pesticides were unrealistically improved by the removal of some outliers based on the statistics.

Endosulfan sulfate.—In grapes, those examples listed in the previous section account for all HorRat values >1.20 except for endosulfan sulfate, which also gave even higher variability in the lettuces and oranges (Tables **2007.01B–D**). The LOQ was >10 ng/g endosulfan sulfate in most of the laboratories; thus $n < 8$ in 5 of the 9 sets of results. The reason for greater variability in its analysis does not relate to sample preparation, but is a consequence of unusual electron ionization properties of the molecule. Endosulfan sulfate has dozens of ions with >10% relative abundance produced in its mass spectrum at the standard –70 eV filament setting. The multitude of ions produced leads to much greater variability in the chromatographic peaks based on only 1–3 quantitation ions. Only a small fraction of the injected endosulfan sulfate ends up as a quantitated ion, which is the reason for its higher LOQ and worse variability. Overall, however, endosulfan sulfate still meets AOAC criteria when its results are averaged, which yielded 80% recovery and HorRat = 1.29. Furthermore, its results from the most reliable laboratories (e.g., Laboratory 1) were quite good even at the 10 ng/g level in all matrixes.

Pymetrozine.—Pymetrozine is not typically included in multiclass, multiresidue methods, but it is a critical analyte to indicate that the acetate-buffered QuEChERS method works well for samples over a wide pH range. Pymetrozine is even more basic than imazalil, and extensive validation of the original (nonbuffered) QuEChERS method for oranges and lettuces led to the conclusion that sample pH indeed affected recoveries of certain pH-sensitive pesticides (7). Pymetrozine, which is registered for use in citrus, gave low recoveries in oranges, but high recoveries in lettuces, due to the pH differences. Modification of the method to use acetate buffering at pH 4.75 resolved this dilemma for pymetrozine and other pesticides with the opposite pH dependence (e.g., tolylfluanid).

The collaborative study results for pymetrozine demonstrate the elegance of the buffering modification. No significant differences were observed in the recoveries or precision between the grapes, lettuces, or oranges, and even though the recoveries were 69% on average overall, the repeatability (8.9%), reproducibility (19%), and HorRat (0.72) values were among the best in the study. Another important aspect is that the pymetrozine did not appreciably degrade in the grapes or oranges at the lower pH than lettuces; it simply was consistently recovered nearly the same as the QC spikes for each matrix. The lower recovery of

Table 77. Overall results for permethrins in the lettuces test samples

Lab No.	Analysis	R^2 value	Spike rec., %	Test sample/concn, ng/g							Avg., ng/g	RSD, %
				1	2	3	4	5	6	7		
1	GC	0.999	96	87[a]	146[a]	142[a]	110[a]	92[a]	99	149[a]	118[a]	21[b]
2	GC	1.000	123[b]	39	38	36	40	43	37	39	39	6
3	GC	0.999	88	116	103	99	106	105	106	115	107	6
4[c]	GC	0.992	84	108	103	128	125	143	90	90	112	17[b]
5	GC	0.999	96	117	116	123	131	124	128	125	124	4
6[c]	GC	0.984[b]	53[b]	155[a]	72[a]	60[a]	114[a]	93[a]	88[a]	101[a]	98[a]	29[b]
7	GC	0.997	101	158	146	133	147	145	136	132	142	6
8	GC	0.999	98	65	61	64	60	64	61	61	62	3
9	Calibration errors											
10[c]	GC	1.000	71	172	164	176	162	168	150	131	160	9
11	GC	0.995	58[b]	148	142	153	155	147	146	146	148	3
12	GC	0.999	125[b]	114	123	111	119	108	110	107	113	5

[a] Cochran outliers.
[b] $R^2 < 0.99$, recovery <70 or >120%, value outside of calibration range by more than 20%, or RSD >15%.
[c] <15% RSD of quality control standards not achieved.

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Table 78. Overall results for ethion in the oranges test samples

				Test sample/concn, ng/g								
Lab No.	Analysis	Hi-Cal R^2 value	Spike rec., %	1	2	3	4	5	6	7	Avg., ng/g	RSD, %
1	GC	1.000	102	215	198	191	183	200	175	184	192	6
2	GC	1.000	111	205	234	229	211	190	186	176	205	10
3[a]	GC	0.999	67[b]	208	168	140	148	165	166	133	161	14
4[a]	GC	0.999	40[b]	215	222	189	183	184	186	193	196	8
5[a]	GC	0.989[b]	94	204	200	190	218	198	172	184	195	7
6[a]	GC	0.999	74	138	129	185	187	133	183	142	157	16[b]
7	GC	0.986[b]	93	283	267	257	235	208	203	195	236	14
8	GC	1.000	119	265	233	236	220	246	214	221	233	7
9	GC	1.000	95	231	243	247	225	245	205	ND[c]	233	6
10[a]	GC	1.000	54[b]	181	160	179	218	122	110	194	166	22[b]
11	LC	1.000	141[b]	319[d]	311[d]	413[d]	408[d]	254[d]	314[d]	330[d]	335[d]	16[b]
12	GC	1.000	107	181	183	174	180	165	185	152	174	6

[a] <15% RSD of quality control standards not achieved.
[b] R^2 < 0.99, recovery <70 or >120%, value outside of calibration range by more than 20%, or RSD >15%.
[c] ND = Not done.
[d] Cochran outlier.

pymetrozine versus the other analytes is not to be viewed negatively, but as a positive outcome of the buffering modification. In fact, this shows that the method was successfully tailored to yield a consistent 70% recovery of pymetrozine as a compromise between the ≈90% recoveries in lettuces and <40% recoveries in oranges with the original method.

Tolylfluanid.—The only pesticide to show a clear difference in the results depending on the matrix was tolylfluanid in lettuces, which unlike pymetrozine in oranges degraded at the higher pH in the lettuces test samples prior to their analysis. This conclusion is supported by the ≈100% recoveries of tolylfluanid in the lettuces QC spikes and known degradation chemistry of tolylfluanid (4). A main reason that some laboratories choose to use cryogenic conditions with dry ice or liquid nitrogen (and addition of acidic carbonate salts) is to avoid degradation of tolylfluanid, chlorothalonil, and similarly unstable pesticides (26). Tolylfluanid is the representative of the *N*-trihalomethylthio class of fungicides, which also includes captan, captafol, folpet, and dichlofluanid. Like chlorothalonil (and dicofol), the parent forms of these pesticides are not always included in multiclass, multiresidue methods, and some laboratories choose to only monitor their degradation products by GC/MS (tolylfluanid and dichlofluanid can be detected by LC/MS/MS, too). They give poor stability in acetone and acetonitrile, especially in light, at higher pH and temperature, but again, certain lots of acetonitrile were shown to reduce their degradation, and addition of 0.1% acetic (or formic) acid improves their stability (4).

In the case of tolylfluanid, the acetate-buffered QuEChERS method achieved 68% recovery with HorRat = 1.32 on average in the grapes and oranges matrixes, in which it is more stable. Tolylfluanid results were more variable than pymetrozine, which was a likely outcome of its worse stability in MeCN solvent. Like pymetrozine, the choice of conditions for tolylfluanid is a compromise among a wider scope of analytes and matrixes. Although its results were not as good as the other analytes, they are still acceptable in most multiresidue monitoring applications.

This is true even in the lettuces matrix if care is taken to avoid degradation in the sample prior to extraction. Laboratories 3 and 8 (to a lesser extent) exhibited very interesting tolylfluanid results. The 100 and 1000 ng/g results for those laboratories were statistical outliers because they were too good among the low recoveries for the other laboratories. After consultation with the collaborator, it remained a mystery how Laboratory 3 avoided degradation of tolylfluanid in the lettuces.

Chlorothalonil.—Chlorothalonil is one of the most problematic analytes in multiclass, multiresidue analysis of pesticides. It requires cryogenic sample processing to avoid degradative losses during homogenization (26), and it also degrades rapidly in acetone, and in MeCN to a lesser extent, especially in light at higher pH (4). Ethyl acetate is a better extraction and analytical solvent for chlorothalonil, but it has disadvantages for many other pesticides and matrixes. However, chlorothalonil may degrade in the hot inlet during injection in GC, and it yields a relatively common mass spectral pattern of 264–268 *m/z* for multichlorinated molecules. Some laboratories do not bother to even analyze for chlorothalonil in multiresidue monitoring, or only detect its presence by looking for its common degradation product.

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Table 79. Overall results for thiabendazole in the oranges test samples

Lab No.	Analysis	Lo-Cal R^2 value	Spike rec., %	Test sample/concn, ng/g							Avg., ng/g	RSD, %
				1	2	3	4	5	6	7		
1	LC	1.000	102	55	55	56	55	54	55	55	55	1
2	LC	0.998	96	53	51	51	50	53	53	53	52	2
3	LC	1.000	96	45	46	42	44	42	42	43	43	3
4	LC	1.000	88	44	47	51	48	49	46	52	48	5
5[a]	LC	0.989[b]	106	63	60	56	56	56	54	53	57	6
6	LC	1.000	87	59	57	53	61	47	46	47	53	11
7	LC	0.995	107	52	64	60	55	54	56	57	57	6
8	LC	0.983[b]	123[b]	58	50	48	49	46	43	43	48	10
9[a]	LC	1.000	88	55	57	55	55	53	51	55	54	3
10[a]	LC	0.998	90	40	38	34	48	35	40	44	40	11
11	LC	0.995	129[b]	69	59	51	62	66	66	65	63	9
12	LC	1.000	116	62	62	61	62	58	62	59	61	3

[a] <15% RSD of quality control standards not achieved.
[b] $R^2 < 0.99$, recovery <70 or >120%, value outside of calibration range by more than 20%, or RSD >15%.

Realizing that chlorothalonil is problematic in all multiclass methods (much of it is lost during normal sample processing anyway), the SD included chlorothalonil as a difficult test of the method and out of curiosity about its results.

One interesting aspect in the collaborative study was that 8 laboratories used LVI of MeCN extracts (Laboratories 1–5, 7, 10, and 11), and 5 laboratories used hot splitless injection of 2 µL concentrated toluene extracts. The variety of different system suitabilities and injection, and GC and MS conditions, introduces too many variables for a valid comparison to be made, but chlorothalonil is the most sensitive analyte to solvent effects (MeCN versus toluene), thus it is the most prominent analyte to evaluate in this respect (other sensitive analytes in GC include methamidophos, dichlorvos, and tolylfluanid, but unlike chlorothalonil, they could be better analyzed by LC/MS/MS).

The comparison of laboratories using LVI versus those using toluene showed no differences in the chlorothalonil results. All laboratories had more variable results in the calibration, QC spike recoveries, and test sample recoveries for chlorothalonil than for other GC/MS pesticides. However, its overall average recovery of 70% and HorRat of 1.41 when 7 or more laboratories contributed results demonstrates that the buffered QuEChERS method still achieves acceptable results for general multiresidue monitoring purposes in the fruits and vegetable for the problematic pesticide, chlorothalonil.

Use of IS or not in quantitation.—In the unabridged report of the QuEChERS method, all results were presented when both the IS was used or not. There were quite large differences among some individual laboratory results, with improvements for some laboratories by using the IS and deteriorations in other cases. The main conclusion is that overall results were largely the same whether the IS was used or not. The poor performing analytes (chlorothalonil, tolylfluanid, and endosulfan sulfate) remained poor, and the best actors remained good performers, except for methamidophos. In the case of methamidophos, average $\%RSD_r$ doubled from 11 to 22, $\%RSD_R$ went from 17 to 28, and HorRat increased from 0.68 to 1.18 when the IS was used. This is partly because methamidophos is more appropriately analyzed by LC/MS/MS than GC/MS, and good LC/MS/MS results from Laboratories 2, 9, and 12 with the IS were lost or replaced by worse GC/MS results. Interestingly, imazalil also had this problem, thus the quality gains from using the IS to compensate for bias in Laboratory 8 were offset by the worse GC/MS results from Laboratories 2 and 12.

The d_{10}-parathion and d_6-α-HCH IS compounds generally improved the results in GC/MS for nonpolar and semi-polar analytes (relatively polar ones are better analyzed by LC/MS/MS), but the d_{10}-parathion is not a good choice as the IS in LC/MS/MS. This conclusion is further supported by the significant improvements in the average HorRats of the "GC/MS only" analytes, *o,p′*-DDD (0.73 to 0.59), procymidone (0.81 to 0.62), and trifluralin (0.81 to 0.62) when the IS was used. In the case of "LC/MS/MS only" analytes, the opposite occurred, as indicated for imidacloprid (0.65 to 0.85), methomyl (0.69 to 1.00), and azoxystrobin (0.63 to 0.81).

Further work should be conducted to find the best IS for LC/MS/MS, but actually, no IS is needed unless matrix effects and biases are clearly observed. The use of an IS can simplify sample preparation by avoiding the care needed in the method to avoid volumetric biases, but it also complicates the situation if it yields low and/or variable recovery in the method, or there is a problem in any analyses of the IS in a sample set. A mistake in the IS result affects the results for all other analytes in the sample, which is why it is helpful to have an alternate IS. Keeping track

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Table 80. Comparison of GC/MS and LC/MS/MS compiled results for the analysis of the incurred pesticides in the test samples

		GC/MS					LC/MS/MS				
Matrix	Pesticide	Concn, ng/g	RSDr, %	RSD_R, %	*n*	HorRat	Concn, ng/g	RSD_r, %	RSD_R, %	*n*	HorRat
Grape	Chlorpyrifos-methyl	155	11	20	12	0.92	187	11	31[a]	6[a]	1.49[a]
	Cyprodinil	108	NA[b]	20	8	0.88	115	NA	15	10	0.68
	Kresoxim-methyl	8.7	34[a]	38[a]	9	1.17	9.4	21[a]	36[a]	10	1.13
Lettuces	Chlorpyrifos-methyl	179	10	17	9	0.81	176	12	52[a]	5[a]	2.48[a]
	λ-Cyhalothrin	60	12	23	10	0.95	NA	NA	NA	NA	NA
	Imidacloprid	NA	NA	NA	NA	NA	12	NA	14	11	0.44
	Permethrins	112	8.7	36[a]	9	1.63[a]	NA	NA	NA	NA	NA
Oranges	Chlorpyrifos-methyl	170	13	20	11	0.93	156	45[a]	54[a]	6[a]	2.58[a]
	Ethion	198	12	18	11	0.89	162	25[a]	62[a]	6[a]	2.92[a]
	Imazalil	13	NA	NA	1[a]	NA	13	NA	35[a]	8	1.15
	Thiabendazole	56	28[a]	29[a]	5[a]	1.17	53	7.2	14	12	0.58

[a] RSD_r > 15%, RSD_R > 25%, HorRat > 1.2, or fewer than 8 laboratories in an assessment.
[b] NA = Not applicable.

of the many details in the results becomes somewhat more complicated when using an IS, but intelligent, trained, and experienced analysts are needed when using sophisticated GC/MS and LC/MS/MS techniques and instruments, and knowing how to properly employ an IS is a minor issue compared to developing the optimal analytical conditions and making good decisions when choosing quantitation ions, integrating peaks, and making analyte identifications. No single set of rules can cover all circumstances, and good system suitability and sound human judgment is always the most important factor in achieving excellent qualitatively and quantitatively accurate analytical results.

Conclusions

This AOAC collaborative study evaluated the acetate-buffered QuEChERS method for 26 important incurred and fortified representive pesticides at 3 blind duplicate levels per matrix between 10–1000 ng/g in grapes, lettuces, and oranges among 13 laboratories in 7 countries. LC/MS/MS and GC/MS were both conducted for each sample extract to cover the wide scope of analysis, and quantitation was done with and without the use of an IS. QC standards were included in the study to isolate each step in the method (sample processing, sample preparation, and analysis) and monitor performance of each laboratory. In all, 7 test samples and 8 QC/calibration standards were injected for each matrix per instrument for the determination of 30 analytes including QC standards in each laboratory. Considering that the IS was used or not in the assessment and about half of the 30 analytes, were detected by both GC/MS and LC/MS/MS, nearly 50 000 data points were generated in this study.

The statistical analysis of the results according to AOAC standards demonstrated that the QuEChERS method met the acceptance criteria for all analytes. There were a few particular analyte/matrix/concentration combinations that gave HorRat >2, or fewer than 8 laboratories contributed results, but these were discrepancies when compared to the overall results for those analytes. Among the 26 analytes, 21 gave average HorRat <1.1. Chlorothalonil (fortified, HorRat = 1.41) and permethrins (incurred, 1.63) gave the worst results in the study, and imazalil (1.36), endosulfan sulfate (1.29), and tolylfluanid (1.32) were also relatively problematic. Incurred pesticides gave the same overall quality of results as fortified pesticides. Dependences were not found with respect to matrix or concentration in any analyte's results, including pH-sensitive pesticides, but tolylfluanid partially degraded in lettuces prior to the analyses.

In other evaluation criteria, all analytes met the <10 ng/g LOQ criterion in nearly all matrixes and laboratories except for endosulfan sulfate in lettuces and oranges. Average recovery was 87% with a range of 68–98% in the study, and only tolylfluanid (68%) and pymetrozine (69%) averaged <70%, which was a designed compromise based on known pH effects. Average RSD_r was 10% with only kresoxim-methyl (incurred at 10 ng/g in grapes), endosulfan sulfate, and chlorothalonil exceeding 15%. Average RSD_R was 21% in the study, and all analyte/matrix pairs achieved overall RSD_R ≤25% except permethrin, kresoxim-methyl, chlorothalonil, tolylfluanid, imazalil, and endosulfan sulfate.

The QuEChERS method lives up to its name as being quicker, easier, and cheaper than any other interlaboratory validated multiclass, multiresidue method for pesticide analysis of foods, and the results from this extensive validation study demonstrate that it achieves excellent quality

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Table 81. Overall performance of the 13 laboratories in the collaborative study for the 21 test samples analyzed in 3 analytical matrixes for a total of 68 pesticide/matrix pairs (known to the collaborators), 57 pesticide negatives, 433 pesticide positives, and 191 pesticide/concentration/matrix combinations (unknown to the collaborators)

		No. of times factor occurred per laboratory, technique, and matrix												
Factor	Matrix	1	2	3	4	5	6	7	8	9	10	11	12	13
Calibration errors or not analyzed	Grapes-GC	—[a]	—	—	2[b]	2	—[b]	—	—	1[b]	—[b]	2	—	—[b]
	Grapes-LC	—	—	—	3	1[b]	—	—	1[b]	—	—	—[b]	—	—
	Lettuces-GC	—	—	2	7[b,c]	1	—[b]	3	—	7[b,c]	—[b]	2	—	NA[d]
	Lettuces-LC	—	—	—	4	—[b]	—	—	1	—	—[b]	—	—	NA
	Oranges-GC	—	—	1[b]	3[b]	2[b]	—[b]	1	1	1[b]	—[b]	2	—	NA
	Oranges-LC	—	—	—	3	1[b]	—	—	1	1[b]	—[b]	—	—	NA
70 > quality control %rec. > 120	Grapes-GC	—	1	1	1[b]	1	2[b]	—	—	1[b]	1[b]	—	—	3[b]
	Grapes-LC	1	—	—	—	1[b]	1	—	10[b,c]	—	1	11[b,c]	2	—
	Lettuces-GC	—	4	1	1[b]	3	10[b,c]	1	2	—[b,e]	7[b,c]	2	5[c]	NA
	Lettuces-LC	2	1	—	—	1[b]	—	—	5[c]	—	1[b]	11[c]	2	NA
	Oranges-GC	1	2	3[b]	9[b,c]	1[b]	1[b]	1	—	—[b]	6[b,c]	1	2	NA
	Oranges-LC	3	—	—	—	—[b]	—	—	6[c]	—[b]	1[b]	13[c]	3	NA
False positives	Grapes-GC	—	—	—	1[b]	—	—[b]	4	—	1[b]	1[b]	—	2	—[b]
	Grapes-LC	—	—	—	—	—[b]	8[c]	2	—[b]	4	—	5[b,c]	1	—
	Lettuces-GC	—	2	1	—[b]	1	—[b]	1	—	—[b,e]	1[b]	1	3	NA
	Lettuces-LC	—	—	3	1	1[b]	7[c]	1	—	3	—[b]	2	1	NA
	Oranges-GC	1	2	1[b]	—[b]	—[b]	—[b]	—	—	—[b]	—[b]	—	3	NA
	Oranges-LC	—	—	1	—	1[b]	—	3	—	3[b]	2[b]	2	1	NA
False negatives	Grapes-GC	—	—	6[c]	12[b,c]	5[c]	2[b]	—	2	1[b]	—[b]	—	—	—[b]
	Grapes-LC	—	—	—	1	—[b]	—	—	—[b]	—	—	2[b]	1	2
	Lettuces-GC	—	1	—	1[b]	11[c]	2[b]	—	6	—[b,e]	4[b]	4	3	NA
	Lettuces-LC	—	2	—	1	—[b]	—	—	2	—	4[b]	2	1	NA
	Oranges-GC	—	4	—[b]	4[b]	16[b,c]	—[b]	—	2	10[b,c]	5[b,c]	2	—	NA
	Oranges-LC	—	—	—	4	—[b]	—	—	3	—[b]	2[b]	—	—	NA
Outliers	Grapes-GC	—	1	1	4[b]	4	4[b]	—	1	1[b]	4[b]	2	—	—[b]
	Grapes-LC	—	—	—	—	—[b]	—	—	5[b,c]	—	—	1[b]	—	—
	Lettuces-GC	1	1	—	2[b]	2	1[b]	—	1	—[b,e]	1[b]	1	2	NA
	Lettuces-LC	—	3	2	—	1[b]	1	—	5[c]	—	—[b]	6[c]	—	NA
	Oranges-GC	—	4	1[b]	3[b]	—[b]	1[b]	—	—	5[b,c]	4[b]	—	3	NA
	Oranges-LC	1	—	—	—	—[b]	1	4	—	4[b]	—[b]	7[c]	1	NA

[a] — = 0.
[b] >15% RSD of quality control standards.
[c] ≥5.
[d] NA = Not applicable.
[e] Calibration errors eliminated possible results.

and reliability of results for a wide range of pesticides at 10–1000 ng/g in fruit and vegetable matrixes.

Recommendations

The SD recommends that the evaluated analytical method for pesticides in nonfatty foods be accepted as a First Action Official Method of AOAC.

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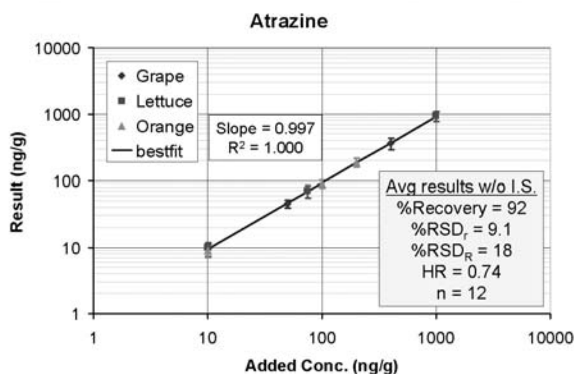

Figure 3. Overall results for atrazine in the collaborative study.

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