JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Assessment of the Stability of Pesticides during Cryogenic Sample Processing. 1. Apples

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An assessment of the stability of a large number (106) of pesticides and related compounds during the cryogenic sample processing of apples has been undertaken. For the first time the procedure included an assessment of the losses during the freezing of the fruits, prior to processing. The stability of each pesticide during processing was assessed by comparing the mean recovery for the laboratory-spiked samples with the mean "survival" of the pesticides in cryogenically processed samples. The results clearly demonstrate that the vast majority, 94 of 106, of pesticides were stable during cryogenic processing. Of particular importance was that losses of several pesticides [bitertanol (95%), heptenophos (50%), isofephos (40%), and tolylfluanid (48%)] reported to occur during ambient processing of apples did not occur during cryogenic processing. Losses of dichlofluanid (54%), chlozolinate (22%), and etridiazole (40%), previously reported to occur during ambient processing of apples, were reduced to barely significant levels (10, 17, and 14%, respectively) by cryogenic processing. Small apparent losses for a few of the compounds were attributable to analytical and sample handling difficulties, rather than to losses during processing, and need further investigation.

KEYWORDS: Pesticide residues; apples; cryogenic milling; stability

INTRODUCTION

In the United Kingdom fruits and vegetables are routinely monitored for pesticide residues to check for compliance with maximum residue levels (MRL) and to assess consumer exposure to pesticides. Laboratories contributing to the monitoring program reported difficulties with the measurement of some pesticides due to losses during sample processing and analysis. Processing of laboratory samples is a prerequisite for representative subsampling for analysis. Several recent studies (*1*−*6*) into the processing of samples of fruits and vegetables have demonstrated that losses of some pesticides can occur when samples are comminuted at ambient temperature. The extent of losses was dependent on both the pesticide and commodity and even varied among different varieties and different samples of the same commodity. It is difficult to assess if the observed degradation of the pesticide residues was due to sample processing alone and/or some other factor, for example, heterogeneity of subsamples produced from the spiking procedure, degradation during extraction, degradation during storage of extracts prior to analysis, degradation during analysis, or imprecision of the measurement technique. Losses of pesticides at the sample processing stage and/or subsequent analytical steps will result in an underestimate of the residue level, with implications for both MRL compliance monitoring and consumer risk assessments. It is clearly desirable to develop and adopt sample-processing procedures that eliminate, or at least, minimize residue losses. Comminution of samples at low temperatures, in the presence of dry ice (cryogenic milling), should minimize the losses of many of the pesticides that occur during processing at ambient temperature. There is a growing body of evidence (*6*) to indicate that important reductions in losses can be achieved in practice. In principle, reducing the processing temperature, say, by 60 °C may reduce degradation some 60-fold. The U.K. regulatory agency, the Pesticides Safety Directorate (PSD), has already instructed laboratories to process samples of lettuce cryogenically prior to analysis, specifically to minimize losses of chlorothalonil. Before extending the use of cryogenic processing to other commodities, the regulator requires further evidence to demonstrate that cryogenic milling does not itself have detrimental effects on some pesticides. Although it is planned to validate this approach for apples, oranges, lettuce, tomatoes, and carrots, and for a suite of pesticides based on the current multiresidue suite, the procedure described in this study, for apples, was designed to reflect intended practice. Most importantly, spiking occurred prior to freezing of whole apples and the dry ice was allowed to evaporate at −20 °C postprocessing, prior to extraction. The final protocol, based on validation of the procedures on all five crops, may then be adopted by laboratories contributing to the U.K. monitoring program, to validate commodity/pesticide combinations on an ongoing basis.

MATERIALS AND METHODS

Overview of Experimental Protocol. Individual apples were spiked with a solution of pesticides and then placed in a freezer (−20 °C) for a minimum of 24 h. Two samples and one blank were processed in the presence of dry ice on each day of the experiment. The comminuted samples were then placed in a freezer at −20 °C to allow carbon dioxide to dissipate. The day after processing, the two samples were extracted

10.1021/jf010852y CCC: $22.00 Published 2002 by the American Chemical Society
Published on Web 12/28/2001

along with one method recovery determination. The sample extracts were measured by duplicate injection, and the method recovery was determined with a single injection. The whole procedure was repeated on seven different days. The individual results were corrected using the internal deposition standard and then averaged for each day.

Samples. A bulk sample of apples (~20 kg), of the variety Ida Red and labeled as "organically" produced, was purchased from a local retail outlet. Sample preparation prior to processing was minimal and involved removal of the stalks. Any damaged fruits were rejected. Individual apples were stored whole in "Stayfresh Longer" (Lakeland Ltd.) bags at 4 °C prior to use.

Reagents. Ethyl acetate (HPLC grade), acetonitrile (HPLC grade), water (HPLC grade), methanol (HPLC grade), ammonia solution, and glacial acetic acid (both of analytical reagent grade) were purchased from Fisher Scientific (Loughborough, U.K.). Anhydrous sodium sulfate, anhydrous sodium hydrogen carbonate, and anhydrous ammonium acetate (all of analytical reagent grade) were also supplied by Fisher Scientific. Certified reference pesticides were purchased from QMx Laboratories Ltd. (Saffron Walden, U.K.), Promochem Ltd. (Maidstone, U.K.) or Greyhound Chemicals (Birkenhead, U.K.). Tetraphenylethylene (TPE; 98%) was obtained from Aldrich Chemical Co. (Poole, U.K.).

Preparation of Standard Solutions. Mixed standard solutions (containing 20−40 μg/mL of each pesticide) and chlorpyrifos-methyl (internal deposition standard) were prepared in ethyl acetate. Solutions of TPE (10 μg/mL) and sulprofos (10 μg/mL) were also prepared in ethyl acetate for use as volumetric internal standards.

Preparation of Spiked Samples. The skin of each individual apple was wiped with hexane and allowed to dry. The weight of each apple was recorded (~100 g unit weight) and the apple placed on a filter paper contained in a glass Petri dish. The mixed standard solution (250 μL), as prepared above, was then applied to the whole surface of the apple using a microsyringe, taking care to minimize runoff. This afforded a spiking level of ~0.05−0.1 mg/kg in the apple sample. The spiked apples were then placed in a freezer (−20 °C) for a minimum of 24 h prior to cryogenic processing. Untreated apples were also frozen for use as blanks to be processed at the same time as the spiked samples.

Approximately 10 blank apples were frozen and cryogenically comminuted to produce a bulk "blank" material for use in external recovery determinations. The pH of the processed apple was 3.9.

Cryogenic Processing. Each individual apple was placed in a mill (model 1094, Tecator AB, Hoganas, Sweden) and was comminuted for 1 min in the presence of dry ice (~200 g). As much as possible of the comminuted sample was recovered and immediately placed in a freezer (−20 °C) for 24 h to allow the dry ice to dissipate. Any remaining sample was recovered by washing the bowl, blade, and lid of the mill with ethyl acetate, and the "mill washes" were retained for analysis. The filter papers from the Petri dishes were also retained for analysis.

Extraction of Samples. Each comminuted apple was homogenized with ethyl acetate (200 mL) in the presence of anhydrous sodium sulfate (120 g) and sodium hydrogen carbonate (17 g) at 30 ± 3 °C. The resulting supernatant extracts were filtered through solvent-washed cotton wool.

For multiresidue analysis (analytical suites 1 and 2) an aliquot (10 mL) of ethyl acetate extract was cleaned up using a 500 mg Envi-carb solid phase extraction cartridge (Supelco Ltd., Poole, U.K.). The cleaned up extracts were concentrated, and an internal standard (tetraphenylethylene) was added before the volume was made up to 1 mL with ethyl acetate.

For analytical suite 3 individual apples were extracted with ethyl acetate as above. An aliquot (5 mL) of the ethyl acetate extract was concentrated, and a volumetric internal standard (sulprofos) was added before the volume was made up to 1 mL with ethyl acetate.

For analytical suite 4 individual apples were extracted with ethyl acetate as described above. An aliquot (10 mL) of the ethyl acetate was evaporated just to dryness and redissolved in methanol/acetic acid 95:5 (v/v) prior to cleanup using a 500 mg Isolute SCX solid phase extraction cartridge (Jones Chromatography, Hengoed, U.K.). The pesticide residues retained on the SCX cartridge were eluted with methanol/ammonia/water 95:2.5:2.5 (v/v/v), and the cleaned up extract was concentrated prior to HPLC analysis.

Extraction of Mill Washes. The beaker containing the mill washes was sonicated for 3 min prior to filtration/drying over anhydrous sodium sulfate. The filtrate was concentrated using rotary evaporation.

For multiresidue analysis (analytical suites 1 and 2) all of the extract was cleaned up using a 500 mg Envi-carb solid phase extraction cartridge. The cleaned up extracts were concentrated, and a volumetric internal standard (tetraphenylethylene) was added before the volume was made up to 2−4 mL with ethyl acetate. For analytical suite 3, a volumetric internal standard (sulprofos) was added before the volume was made up to 2−4 mL with ethyl acetate. For analytical suite 4, the extract was solvent exchanged into methanol/acetic acid 95:5 (v/v) prior to cleanup using an SCX solid phase extraction cartridge as described above.

Extraction of Filter Papers. The filter paper on which the apple was spiked and ethyl acetate rinses from the Petri dish were sonicated for 3 min in ethyl acetate. The extract was poured through anhydrous sodium sulfate, and the filtrate was then concentrated using rotary evaporation. The appropriate volumetric internal standard was added to the extract (tetraphenylethylene for suites 1 and 2, sulprofos for suite 3) in addition to double-concentrated blank to achieve matrix matching, before the volume was made up with ethyl acetate. For analytical suite 4 only, the filter paper extract was solvent exchanged into mobile phase for HPLC analysis.

Measurement of Pesticide Concentrations in Extracts. *Analytical Suite 1: GC-MSD Determination.* Determinations were made using capillary gas−liquid chromatography with a Hewlett-Packard mass selective detector (MSD model HP 5972) operated in SIM. Injection (3 μL) was splitless at 250 °C, and the detector temperature was set at 280 °C. Chromatography was performed using a DB-5 MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) with the carrier gas (helium) at a flow rate of 0.9 mL/min in constant flow mode. The oven temperature was programmed as follows: initial temperature, 100 °C, held for 1 min; programmed to 160 °C, at 15 °C /min, held for 1 min; then programmed to 230 °C at 2 °C/min, held for 1 min, then to 280 °C at 10 °C/min, and held for a further 5 min.

Analytical Suite 2: GC-MSD Determination. Determinations were made using capillary gas−liquid chromatography with a Hewlett-Packard mass selective detector (MSD model HP 5972) operated in SIM. Injection (3 μL) was splitless at 250 °C, and the detector temperature was set at 280 °C. Chromatography was performed using a DB-5 MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) with the carrier gas (helium) at a flow rate of 0.9 mL/min in constant flow mode. The oven temperature was programmed as follows: initial temperature, 100 °C, held for 1 min; programmed to 160 °C, at 15 °C/min, held for 1 min; then programmed to 230 °C at 2 °C/min, held for 1 min, then to 280 °C at 10 °C/min, and held for a further 8 min.

Analytical Suite 3: GC-PFPD Determination. Determinations were made using capillary gas−liquid chromatography with a PFPD in the phosphorus mode. Injection (3 μL) was pulsed splitless at 210 °C and 25 psi for 1 min, and the detector temperature was set at 280 °C. Chromatography was performed using a DB-5 column (30 m × 0.53 mm i.d. × 1 μm film thickness) with the carrier gas (helium) at a flow rate of 4.2 mL/min in constant flow mode. The oven temperature was programmed as follows: initial temperature, 100 °C, held for 1 min, programmed to 200 °C, at 20 °C/min, held for 3 min; then programmed to 280 °C at 40 °C/min, and held for a further 7 min.

Analytical Suite 4: HPLC-FL Determination. Any carbendazim and thiabendazole residues in the extracts were determined by high-performance liquid chromatography (HPLC; model HP 1050) and fluorescence detection (model HP1046a) at λ_{ex} = 246 nm and λ_{em} = 315 nm for 6 min and then at λ_{ex} = 305 nm and λ_{em} = 345 nm. Chromatography was performed using a Phenomenex C_{18} column (150 × 4.6 mm, 3 μm), and the partial loop injection volume was 5 μL. The column was eluted using the gradient elution conditions shown in Table 1.

Analytical Quality Control. Prior to analysis of samples, the methods were validated for selected pesticides by analysis of seven replicates spiked at 0.05 mg/kg. All determinations were calibrated using

Table 1. Gradient Elution Conditions

time (min)	% A[a]	% B[b]	gradient
0	80	20	linear for 9 min
9	60	40	linear for 1 min
10	80	20	isocratic for 5 min

[a] A = 0.02 M ammonium acetate adjusted to pH 7 with acetic acid. [b] B = acetonitrile. The mobile phase flow rate was 1 mL/min, and the run time was 15 min.

Table 2. Method Validation Data Uncorrected for CPM

	recovery		corrected recovery		
pesticide	mean	% CV	mean	% CV	*n*
acephate	47.9	19.3	60.5	15.3	7
azinphos-methyl	80.1	9.6	101.6	1.8	7
bifenthrin	82.9	6.8	104.2	1.6	7
bitertanol	84.7	6.8	106.6	3.6	7
cadusafos	78.4	6.2	98.7	3.8	7
carbaryl	83.0	5.1	104.5	3.1	7
carbofuran	85.1	4.4	107.3	4.3	7
chlorpyrifos	82.6	5.7	103.9	2.3	7
chlozolinate	80.7	12.2	102.4	17.4	7
cyfluthrin (peak 2)	81.3	3.9	102.5	5.4	7
cypermethrin	80.7	4.3	101.7	4.3	7
deltamethrin	84.6	4.4	106.6	5.8	7
dichlofluanid	81.6	6.4	102.6	2.9	7
dichlorvos	66.5	10.8	84.3	4.2	7
dicloran	64.4	13.6	80.7	7.9	7
dimethoate	78.4	8.1	99.5	1.2	7
endosulfan (I)	84.0	3.9	105.8	4.5	7
endosulfan (II)	81.3	6.2	102.3	4.0	7
endosulfan sulfate	82.6	5.5	103.9	2.6	7
fenpropathrin	83.4	5.8	105.0	2.8	7
fenpropimorph	83.1	7.9	104.5	3.4	7
fenvalerate	83.3	5.1	104.9	3.4	7
flusilazole	84.4	5.4	106.3	2.6	7
imazalil	79.0	14.6	99.0	9.4	7
iprodione	83.9	5.9	105.5	2.3	7
isofenphos	83.9	6.2	105.5	2.1	7
λ-cyhalothrin	82.0	3.1	103.5	7.0	7
mecarbam	78.1	9.5	98.9	1.3	7
metalaxyl	85.7	3.4	108.1	5.4	7
methamidophos	49.6	16.5	62.8	12.5	7
methiocarb	85.7	3.9	108.0	4.0	7
monocrotophos	71.9	11.0	91.1	5.1	7
myclobutanil	84.6	6.1	106.4	2.4	7
omethoate	55.5	16.4	70.2	11.4	7
oxadixyl	85.9	3.3	108.3	6.2	7
paclobutrazole	84.7	6.0	106.6	2.3	7
parathion-methyl	82.6	5.7	103.9	3.6	7
pendimethalin	82.3	4.7	103.8	7.0	7
permethrin	81.3	4.5	102.4	4.3	7
phosmet	79.4	9.4	100.7	1.1	7
pirimiphos-methyl	76.7	9.7	97.2	1.8	7
p,p-DDD	82.9	5.9	104.3	2.3	7
p,p-DDE	84.4	5.2	106.3	2.8	7
propoxur	82.6	5.1	104.0	4.8	7
simazine	89.1	6.7	112.2	4.6	7
tecnazene	73.9	5.4	93.0	3.2	7
trifluralin	80.0	5.0	100.7	3.3	7

multipoint, matrix-matched standards, which bracketed the recovery extracts. The validation results are given in Table 2. The mean recoveries generally fell within the range 80−110% with coefficients of variation (CVs) below 10%. External recovery determinations consisted of ∼100 g of cryogencially milled apple matrix spiked with 250 μL of the mixed standard solution. A single recovery determination at a concentration of 0.05−0.1 mg/kg was extracted immediately after spiking and with each batch of samples. All determinations were calibrated using multipoint, matrix-matched standards, which bracketed the samples. Each sample extract was analyzed in duplicate and the recovery analyzed singly.

Calculations: Recoveries Not Corrected for CPM Internal Deposition Standard. The percent survival (mass balance) for each pesticide was calculated as follows:

$$[(\mu\text{g of pesticides in filter papers} + \mu\text{g of pesticides in the mill washes} + \mu\text{g of pesticides in the sample})/\mu\text{g pesticides added}] \times 100$$

The mean survival was the average of the recoveries (mean of duplicate injections of duplicate samples; i.e., four results) obtained on each of the 7 days. The percent recovery for each pesticide in laboratory-spiked recovery samples was calculated as follows:

$$[(\mu\text{g of pesticides measured in the recovery extract}/\mu\text{g of pesticides added in spiking solution}) \times 100] \quad (2)$$

The overall mean percent recovery for laboratory-spiked samples was the average of the individual recoveries (one injection) obtained on each of the 7 days.

Calculation: Recoveries Corrected for CPM Internal Deposition Standard. The CPM corrected mean survival for each pesticide in cryogenically processed samples were calculated as, and using the same data, in eq 1, but after the individual recoveries (for samples, excluding mill washes and filter papers) were normalized against CPM. The CPM corrected mean recoveries for each pesticide in laboratory-spiked samples were calculated as, and using the same data, in eq 2, but after the individual recoveries were normalized against CPM.

RESULTS AND DISCUSSION

Assessment of Stability of the Pesticides during Cryogenic Processing. The uncorrected, and internal deposition standard (CPM) corrected, results are summarized in Tables 3 and 4, respectively. These tables have been constructed from results derived from >5000 residue determinations. Generally, the corrected results demonstrate excellent accuracy and precision, especially when the relatively low spiking level (∼0.05−0.1 mg/kg) is taken into account. The mean corrected recoveries obtained for the majority of pesticides were within the range of 95−105%. Analytical difficulties resulted in three to six, rather than seven, replicates being obtained in certain cases. A GC-MS hardware failure caused the loss of one complete set of data for analytical suite 1, and the work could not be repeated within the project deadline. In other cases intermittent poor sensitivity (notably for endosulfan and DDT groups) or retention time drift outside the SIM windows was responsible for incomplete data sets. Recoveries of <90% and CVs >10% may indicate either a problem with the method or that chlorpyifos-methyl may not have been a suitable internal standard for that particular pesticide because of significant differences in the physicochemical properties. Factors such as polarity and volatility must also be taken into account when the results are interpreted.

The stability of each pesticide was assessed by comparing the mean recovery for the laboratory-spiked samples with the mean survival for the cryogenically processed samples. The results clearly demonstrate that the vast majority (94 of 106) of pesticides were stable during cryogenic processing. Furthermore, losses reported to occur for several pesticides [bitertanol (95%), heptenophos (50%), isofenphos (40%), and tolylfluanid (48%)] during ambient processing of apples (*3*) did not occur during cryogenic processing in the present experiment. Possible small losses of dichlofluanid (10%), chlozolinate (17%), and etridiazole (14%) were much lower, compared with losses of 54% for dichlofluanid, 22% for chlozolinate, and 40% for etridiazole previously reported to occur during the ambient processing of apples (*3*). Apparent small losses observed for acephate (11%), methamidophos (13%), and omethoate (10%)

Table 3. Recovery and Survival Data (Uncorrected) for Replicate Days

pesticide	analytical suite	recovered (μg)				survival		recovery		n	difference survival vs recovery %
		sample	mill wash	filter	total	mean	% CV	mean	% CV		
acephate	3	2.35	0.23	0.30	2.40	48	30	56	22	7	−7.7
azinphos-methyl	3	3.69	0.41	0.42	3.74	75	7	77	12	7	−2.5
bendiocarb	2	3.53	0.10	0.06	3.65	73	6	82	10	7	−9.4
bifenthrin	1	3.40	0.14	0.00	3.55	71	50	76	50	5	−5.1
biphenyl	1	1.84	0.12	0.00	1.96	39	21	68	15	6	−28.7
bitertanol	1	4.05	0.07	0.02	4.13	83	4	96	7	6	−13.4
bitertanol	2	3.55	0.12	0.08	3.67	73	9	87	7	7	−13.2
bromopropylate	1	3.97	0.17	0.02	4.16	83	7	91	10	6	−7.5
bupirimate	1	3.91	0.00	0.02	3.93	79	9	94	10	6	−15.3
buprofezin	2	3.53	0.00	0.07	3.57	71	7	84	8	7	−13.1
cadusafos	1	3.96	0.24	0.01	4.21	84	8	94	8	6	−9.4
carbaryl	1	3.98	0.20	0.01	4.19	84	4	89	8	6	−5.0
carbaryl	2	3.60	0.14	0.04	3.77	75	6	84	8	7	−8.8
carbendazim	4	7.88	0.39	0.30	8.09	81	13	93	11	7	−12.4
carbofuran	1	3.35	0.17	0.01	3.53	71	50	74	49	5	−3.0
chlorfenvinphos (z)	2	3.61	0.14	0.07	3.77	75	6	84	8	7	−8.4
chlorpyrifos	1	3.45	0.14	0.01	3.60	72	50	75	50	5	−3.5
chlorpyrifos-methyl	2	3.62	0.13	0.07	3.79	76	4	85	8	7	−9.4
chlorpyrifos-methyl	1	4.01	0.21	0.01	4.23	85	7	92	10	6	−7.1
chlorpyrifos-methyl	3	3.60	0.26	0.43	3.74	75	7	76	13	7	−1.5
chlozolinate	1	3.51	0.17	0.01	3.70	74	28	95	5	6	−20.8
cyfluthrin (sum)	1	4.06	0.35	0.02	4.43	89	7	93	5	6	−4.7
cypermethrin (sum)	2	3.64	0.16	0.08	3.85	77	7	87	7	7	−9.6
p,p-DDD	1	3.47	0.17	0.00	3.65	73	49	76	49	5	−3.5
p,p-DDE	1	4.02	0.24	0.02	4.28	86	8	90	8	6	−4.5
o,p-DDT	2	3.88	0.09	0.06	4.01	80	3	86	10	4	−6.2
p,p-DDT	2	4.04	0.11	0.06	4.19	84	15	86	7	3	−2.5
deltamethrin	1	4.07	0.14	0.02	4.23	85	10	93	10	6	−8.7
diazinon	1	3.83	0.06	0.02	3.92	78	6	91	8	6	−12.3
dichlofluanid	1	2.95	0.05	0.02	3.02	60	49	77	50	5	−16.4
dichlofluanid	2	3.26	0.12	0.06	3.39	68	11	84	10	7	−16.6
dichlorvos	3	2.03	0.60	0.33	1.95	39	24	61	32	7	−22.1
dicloran	1	3.35	0.47	0.02	3.84	77	10	70	6	6	6.9
dimethoate	3	3.54	0.36	0.32	3.68	74	10	77	13	7	−3.4
diphenylamine	2	3.34	0.06	0.05	3.42	68	9	80	11	7	−11.5
endosulfan (I)	1	3.44	0.17	0.00	3.61	72	50	78	50	5	−5.6
endosulfan (II)	1	2.61	0.09	0.02	2.72	54	78	61	78	4	−6.4
endosulfan sulfate	1	4.01	0.24	0.02	4.27	85	7	91	8	6	−6.1
EPN	1	4.20	0.19	0.01	4.41	88	8	91	12	6	−3.0
ethion	2	3.60	0.14	0.07	3.78	76	4	86	8	7	−10.2
ethofumesate	2	3.65	0.10	0.08	3.74	75	10	86	12	7	−11.3
ethoprofos	2	3.53	0.14	0.07	3.71	74	7	84	10	7	−9.7
etridiazole	2	2.65	0.11	0.01	2.76	55	6	75	10	7	−19.4
etrimfos	2	3.54	0.12	0.06	3.70	74	7	83	10	7	−9.1
fenitrothion	1	4.06	0.17	0.02	4.25	85	8	94	9	6	−8.9
fenpropathrin	1	4.06	0.24	0.02	4.33	87	10	92	9	6	−5.9
fenpropidin	2	3.26	0.06	0.03	3.28	66	21	81	17	7	−15.7
fenpropimorph	1	4.02	0.08	0.01	4.11	82	9	96	7	6	−13.7
fenvalerate (peak 1)	1	3.90	0.18	0.02	4.10	82	16	95	8	6	−13.2
fenvalerate (peak 1)	2	3.55	0.22	0.07	3.81	76	7	86	6	7	−10.0
flurochloridone	2	3.73	0.14	0.06	3.91	78	7	85	9	6	−7.2
flusilazole	1	3.89	0.00	0.01	3.91	78	12	89	9	6	−10.8
fonophos	2	3.53	0.12	0.05	3.68	74	5	85	8	7	−11.3
furalaxyl	2	3.56	0.14	0.07	3.73	75	5	84	9	7	−9.0
α-HCH	2	3.54	0.12	0.05	3.69	74	5	84	9	7	−10.1
β-HCH	2	3.99	0.13	0.05	4.14	83	12	94	18	6	−10.8
γ-HCH	1	3.85	0.21	0.01	4.08	82	7	88	7	6	−6.5
heptenophos	1	3.63	0.17	0.18	3.98	80	10	87	7	6	−7.6
heptenophos	2	3.37	0.11	0.05	3.52	70	8	82	9	7	−12.1
iprodione	1	4.16	0.20	0.02	4.39	88	7	96	5	6	−7.9
isazophos	2	3.56	0.13	0.07	3.73	75	6	85	9	7	−10.0
isofenphos	1	4.01	0.24	0.02	4.27	85	5	92	11	6	−6.4
λ-cyhalothrin	1	4.03	0.22	0.02	4.27	85	6	92	8	6	−6.5
malaoxon	1	3.18	0.07	0.01	3.26	65	13	91	10	6	−25.8
malathion	2	3.50	0.17	0.08	3.69	74	6	85	7	7	−11.2
mecarbam	1	2.55	0.14	0.02	2.70	54	77	59	78	4	−4.9
mecarbam	3	3.55	0.27	0.45	3.76	75	7	78	12	7	−2.9
mephosfolan	2	3.58	0.01	0.05	3.61	72	16	87	13	6	−14.8
metalaxyl	1	3.98	0.14	0.03	4.15	83	4	91	7	6	−8.1
methamidophos	3	2.28	0.27	0.17	2.37	47	24	56	19	7	−8.3
methidathion	2	3.63	0.11	0.06	3.75	75	4	87	8	7	−11.5
methiocarb	1	4.11	0.20	0.02	4.32	86	5	92	6	6	−6.0
monocrotophos	3	3.36	0.00	0.46	3.38	68	21	74	15	7	−6.5
myclobutanil	1	3.54	0.06	0.02	3.62	72	50	75	49	5	−3.0

Table 3 (Continued)

pesticide	analytical suite	recovered (μg)				survival		recovery			difference survival
		sample	mill wash	filter	total	mean	% CV	mean	% CV	*n*	vs recovery %
napropamide	1	3.98	0.06	0.02	4.05	81	6	92	10	6	−11.2
ofurace	2	3.59	0.07	0.05	3.66	73	7	86	10	7	−12.9
omethoate	3	2.53	0.21	0.36	2.54	51	21	62	16	7	−10.9
oxadixyl	1	4.08	0.07	0.02	4.18	84	6	92	5	6	−8.7
paclobutrazol	1	3.30	0.01	0.02	3.32	66	50	74	50	5	−7.8
parathion-ethyl	2	3.63	0.17	0.07	3.84	77	4	83	10	7	−6.4
parathion-methyl	2	3.64	0.15	0.06	3.82	76	4	86	13	7	−9.4
penconazole	2	3.68	0.06	0.06	3.75	75	10	85	8	6	−9.7
pendimethalin	2	3.50	0.19	0.07	3.72	74	6	81	12	7	−7.0
permethrin (sum)	2	3.59	0.18	0.08	3.81	76	9	86	9	7	−9.6
phenthoate	2	3.52	0.14	0.07	3.70	74	4	83	7	7	−8.6
phosalone	2	3.09	0.11	0.07	3.23	65	7	71	24	7	−6.1
phosmet	2	3.47	0.11	0.06	3.60	72	16	79	15	6	−6.8
phosmet	3	3.61	0.18	0.43	3.80	76	6	77	12	7	−1.3
phosphamidon (sum)	2	3.54	0.06	0.05	3.58	72	5	86	8	7	−14.2
pirimicarb	2	3.55	0.00	0.05	3.58	72	8	85	9	7	−13.6
pirimiphos-ethyl	2	3.42	0.03	0.06	3.46	69	7	79	10	7	−9.9
pirimiphos-methyl	2	3.56	0.00	0.07	3.60	72	7	83	8	7	−11.3
pirimiphos-methyl	3	3.53	0.28	0.42	3.70	74	7	77	13	7	−3.3
procymidone	2	3.78	0.15	0.05	3.98	80	6	89	12	5	−9.0
profenofos	1	3.40	0.15	0.00	3.55	71	49	79	51	5	−7.8
prometryn	2	3.64	0.00	0.05	3.67	73	10	86	8	6	−12.5
propanil	1	4.11	0.20	0.01	4.33	87	8	94	6	6	−7.6
propargite	2	3.62	0.10	0.07	3.74	75	5	86	4	7	−11.7
propiconazole (sum)	2	3.59	0.06	0.07	3.66	73	7	85	8	7	−11.4
propoxur	1	4.01	0.22	0.01	4.25	85	6	90	6	6	−5.3
propyzamide	2	3.65	0.12	0.05	3.80	76	8	86	11	7	−9.5
prothiofos	2	3.65	0.15	0.07	3.84	77	4	84	9	7	−7.7
pyridaphenthion	1	4.06	0.07	0.01	4.15	83	11	94	10	6	−11.4
pyrimethanil	1	1.81	0.00	0.01	1.82	36	25	38	23	6	−1.3
quinalphos	2	3.67	0.09	0.08	3.79	76	8	85	12	7	−9.6
simazine	1	3.97	0.01	0.01	3.99	80	7	95	8	6	−15.6
tebuconazole	1	4.00	0.06	0.02	4.08	82	5	95	12	6	−13.4
tecnazene	1	3.39	0.19	0.00	3.58	72	12	86	7	6	−14.1
tetrachlorvinphos	1	4.01	0.12	0.02	4.15	83	4	90	7	6	−6.9
tetradifon	2	3.68	0.14	0.07	3.86	77	7	86	9	7	−8.4
thiabendazole	4	8.03	0.27	0.22	8.25	83	12	93	12	7	−10.9
tolclofos-methyl	2	3.58	0.17	0.07	3.79	76	5	83	9	7	−7.4
tolylfluanid	1	4.07	0.19	0.01	4.27	85	18	97	17	6	−11.2
tolylfluanid	2	3.56	0.14	0.05	3.74	75	12	84	9	7	−8.8
triazophos	1	4.05	0.07	0.00	4.12	82	8	93	8	6	−10.9
trifluralin	1	3.98	0.27	0.01	4.26	85	6	92	6	6	−7.0
vinclozolin	2	3.62	0.13	0.06	3.78	76	9	82	13	7	−6.7

may be attributable to deficiencies in the analytical method rather than losses during cryogenic processing. The losses are similar, but it is unlikely that all three would degrade at the same rate. If acephate had been used as an internal deposition standard for methamidophos or omethoate, or vice versa, the results would almost certainly have shown that no losses had occurred during processing. The most likely explanation is that the temperature control of the relatively large volume (200 mL) of solvent during extraction was inadequate. Even a small change in temperature can affect the solubility of sodium sulfate and consequently affect the partition of the polar pesticides into ethyl acetate from the aqueous sample. This requires further investigation before any further assessments are undertaken for these polar compounds.

Likewise, small apparent losses for carbendazim (14%) and thiabendazole (12%) are also probably due to deficiencies in the analytical method rather than to cryogenic processing. The CPM internal deposition standard was not amenable to the HPLC method of analysis, so the data could not be corrected. As a result, the accuracy and precision were not as good as for other compounds. An alternative internal standard (2-phenylbenzamidazole) was assessed but was prone to chromatographic interference. Inclusion of a suitable internal standard for the assessment of carbendazim and thiabendazole stability during processing will almost inevitably require the use of LC-MS for the determination step.

The apparent loss of malaoxon (20%) during cryogenic processing could be real and is reported for the first time. There are no corresponding data available on the stability of malaoxon during ambient milling. It is possible that the loss was the result of a specific interaction between malaoxon and the sample of apples used. The observed significant losses of biphenyl (28%) and dichlorvos (26%) are almost certainly due to their relatively high volatility. The losses could have occurred during evaporation of the solvent after spiking and/or during freezing of the whole apples, as well as during cryogenic processing. Dichlorvos will volatilize even at −20 °C. Such losses are difficult to assess and control, and the residue levels of these volatile pesticides are likely to be continuously changing before and after sampling and prior to analysis.

Assessment of the Protocol. Despite attempts to recover the total amounts of the pesticides spiked onto the apples, by analysis of the filter papers and mill washes in addition to the samples, mass balances were on the order of 80%. It appears that ~20% (equivalent to 1 μg) was lost during the spiking, storage, processing, extraction, cleanup, concentration, and measurement steps. The use of chlorpyrifos-methyl as an internal deposition standard compensated for these losses very effectively

Table 4. Recovery and Survival Data (CPM Corrected) for Replicate Days

pesticide	analytical suite	recovery		survival		*n*	difference survival vs recovery %
		mean	% CV	mean	% CV		
acephate	3	73.2	17.6	61.9	20.8	7	−11.4
azinphos-methyl	3	101.4	2.9	101.3	1.5	7	−0.1
bendiocarb	2	96.5	3.8	97.6	3.8	7	1.1
bifenthrin	1	99.6	4.0	99.6	2.9	5	0.0
biphenyl	1	73.8	8.9	45.8	16.9	6	−28.1
bitertanol	1	104.9	3.2	101.2	3.9	6	−3.8
bitertanol	2	101.6	3.4	98.0	5.1	7	−3.6
bromopropylate	1	99.1	7.5	99.2	3.9	6	0.1
bupirimate	1	102.4	3.5	97.6	2.6	6	−4.8
buprofezin	2	99.1	3.9	97.5	3.2	7	−1.6
cadusafos	1	102.4	8.9	98.7	6.4	6	−3.8
carbaryl	1	97.2	8.5	99.7	7.5	6	2.4
carbaryl	2	98.9	5.3	99.6	7.3	7	0.7
carbendazim*	4	93.3	11.5	80.4	13.4	7	−12.9
carbofuran	1	98.4	5.9	100.9	4.5	5	2.4
chlorfenvinphos (z)	2	98.4	6.9	99.8	4.2	7	1.4
chlorpyrifos	1	98.7	4.2	100.8	3.7	5	2.1
chlozolinate	1	103.7	6.4	86.7	24.4	6	−17.1
cyfluthrin (sum)	1	102.2	5.6	101.3	4.2	6	−0.8
cypermethrin (sum)	2	101.6	5.1	100.5	4.2	7	−1.2
p,p-DDD	1	100.1	4.9	101.8	4.7	5	1.6
p,p-DDE	1	98.3	4.0	100.1	3.3	6	1.7
o,p-DDT	2	101.9	4.1	98.4	2.9	4	−3.5
p,p-DDT	2	101.8	5.0	101.2	9.1	3	−0.5
deltamethrin	1	101.6	3.1	101.6	3.8	6	0.0
diazinon	1	99.0	4.1	95.8	4.8	6	−3.3
dichlofluanid	1	98.3	3.4	88.3	8.3	5	−10.1
dichlofluanid	2	98.9	5.4	89.9	10.1	7	−8.9
dichlorvos	3	79.5	27.1	53.6	20.7	7	−25.9
dicloran	1	76.6	7.0	83.6	7.9	6	7.0
dimethoate	3	100.9	1.1	99.7	1.9	7	−1.2
diphenylamine	2	93.6	4.7	92.2	4.1	7	−1.4
endosulfan (I)	1	101.8	4.6	100.7	7.0	5	−1.1
endosulfan (II)	1	103.2	5.0	102.2	5.8	4	−1.0
endosulfan sulfate	1	99.8	3.6	99.9	2.5	6	0.1
EPN	1	100.0	13.6	104.9	9.3	6	4.9
ethion	2	100.8	4.0	99.5	3.4	7	−1.3
ethofumesate	2	100.9	7.0	100.8	7.2	7	−0.1
ethoprofos	2	98.3	4.9	97.3	3.1	7	−0.9
etridiazole	2	87.4	3.3	73.2	5.7	7	−14.2
etrimfos	2	97.3	5.5	97.7	3.8	7	0.4
fenitrothion	1	102.4	3.1	101.4	5.2	6	−0.9
fenpropathrin	1	100.8	3.2	101.3	2.9	6	0.5
fenpropidin	2	96.2	12.2	86.8	17.0	7	−9.4
fenpropimorph	1	105.0	6.8	100.8	10.7	6	−4.2
fenvalerate (peak 1)	1	104.2	6.3	96.8	12.5	6	−7.3
fenvalerate (peak 1)	2	101.3	5.4	98.1	6.0	7	−3.2
flurochloridone	2	100.4	3.5	100.2	3.8	6	−0.2
flusilazole	1	97.4	8.3	96.9	8.7	6	−0.4
fonophos	2	99.5	2.5	97.4	2.4	7	−2.2
furalaxyl	2	98.3	8.0	98.3	3.3	7	0.0
α-HCH	2	98.4	2.7	97.8	2.3	7	−0.6
β-HCH	2	110.1	13.0	104.9	7.6	6	−5.2
γ-HCH	1	96.1	2.9	96.1	2.8	6	−0.1
heptenophos	1	95.4	4.6	90.7	5.3	6	−4.6
heptenophos	2	96.7	3.6	93.0	4.8	7	−3.7
iprodione	1	104.8	7.9	104.2	6.6	6	−0.6
isazophos	2	99.3	3.7	98.3	2.8	7	−1.0
isofenphos	1	100.3	9.5	100.3	4.3	6	0.0
λ-cyhalothrin	1	100.3	3.4	100.8	2.9	6	0.4
malaoxon	1	99.4	5.9	79.3	9.9	6	−20.1
malathion	2	99.9	4.1	96.9	3.1	7	−3.0
mecarbam	1	99.7	5.6	98.3	8.3	4	−1.5
mecarbam	3	102.4	1.5	99.9	0.7	7	−2.5
mephosfolan	2	103.7	7.8	98.1	10.8	6	−5.6
metalaxyl	1	99.4	2.9	99.3	3.2	6	−0.1
methamidophos	3	73.2	13.6	60.6	17.3	7	−12.6
methidathion	2	101.5	4.0	100.4	4.3	7	−1.1
methiocarb	1	101.3	7.9	102.9	9.1	6	1.6
monocrotophos	3	97.0	3.4	90.3	8.0	7	−6.7
myclobutanil	1	101.7	11.1	108.7	13.2	5	7.0
napropamide	1	100.7	5.3	99.4	3.4	6	−1.2
ofurace	2	101.0	7.0	99.0	3.2	7	−2.0
omethoate	3	80.8	7.7	70.6	15.5	7	−10.2

Table 4 (Continued)

pesticide	analytical suite	recovery		survival		*n*	difference survival vs recovery %
		mean	% CV	mean	% CV		
oxadixyl	1	101.1	7.1	101.9	5.1	6	0.7
paclobutrazol	1	99.0	4.7	99.3	2.8	5	0.3
parathion-ethyl	2	97.4	4.0	100.5	5.6	7	3.1
parathion-methyl	2	100.6	6.8	100.7	4.4	7	0.1
penconazole	2	99.9	8.1	98.9	4.5	6	–1.0
pendimethalin	2	95.3	5.8	97.0	8.8	7	1.7
permethrin (sum)	2	100.8	6.1	99.0	4.4	7	–1.8
phenthoate	2	97.0	5.2	97.3	3.1	7	0.3
phosalone	2	82.4	19.3	85.2	7.6	7	2.8
phosmet	2	92.2	7.3	92.5	8.0	6	0.3
phosmet	3	101.5	2.9	101.2	1.7	7	–0.3
phosphamidon (sum)	2	100.8	4.7	98.0	3.4	7	–2.9
pirimicarb	2	99.9	3.3	98.2	3.7	7	–1.7
pirimiphos-ethyl	2	92.7	5.1	94.5	3.8	7	1.7
pirimiphos-methyl	2	97.9	5.7	98.4	2.3	7	0.5
pirimiphos-methyl	3	101.2	1.0	99.5	0.4	7	–1.7
procymidone	2	102.8	7.7	100.3	5.0	5	–2.6
profenofos	1	102.9	4.6	99.4	3.0	5	–3.4
prometryn	2	101.1	5.1	97.6	3.8	6	–3.4
propanil	1	103.0	4.4	102.5	7.6	6	–0.5
propargite	2	101.8	7.3	100.2	4.1	7	–1.6
propiconazole (sum)	2	99.3	4.9	99.0	3.3	7	–0.3
propoxur	1	98.7	4.4	100.3	2.7	6	1.6
propyzamide	2	101.9	5.5	98.9	3.4	7	–3.0
prothiofos	2	99.1	5.0	100.9	4.1	7	1.9
pyridaphenthion	1	103.0	6.7	101.3	5.2	6	–1.7
pyrimethanil	1	41.2	22.7	44.8	22.6	6	3.6
quinalphos	2	100.0	5.6	101.3	4.1	7	1.2
simazine	1	104.1	3.8	99.1	3.6	6	–5.0
tebuconazole	1	103.4	6.0	99.7	5.2	6	–3.7
tecnazene	1	93.5	4.0	84.4	6.5	6	–9.1
tetrachlorvinphos	1	98.2	4.0	100.2	3.0	6	1.9
tetradifon	2	100.5	5.8	101.7	4.5	7	1.2
thiabendazole*	4	93.4	12.1	81.6	13.0	7	–11.8
tolclofos-methyl	2	97.5	2.5	99.0	2.2	7	1.5
tolylfluanid	1	105.4	15.3	103.0	28.0	6	–2.4
tolylfluanid	2	99.8	3.4	96.4	5.4	7	–3.4
triazophos	1	101.9	5.5	101.2	5.8	6	–0.7
trifluralin	1	100.7	4.3	99.2	3.3	6	–1.5
vinclozolin	2	99.7	15.0	97.6	3.9	7	–2.1

for the majority of pesticides. Attempts to measure the mass balance of spiked pesticides in the other commodities to be studied should therefore be considered a costly and unnecessary complication.

The maximum amount of spiking solution that could be applied to a single apple, without significant runoff, was 250 μL. The higher the concentration of the compounds in the spiking solution, the fewer compounds that can be included. In this experiment, a compromise was made of a maximum of 50 compounds at 20−40 μg/mL to produce realistic residue levels at 0.05 mg/kg.

The fact that insignificant quantities of the pesticides were found on the filter papers suggests that the spiking procedure was satisfactory, with little or no measurable runoff during application.

The results clearly demonstrate that the vast majority (∼90%) of pesticides included in this study were completely stable during cryogenic processing. In particular, losses reported to occur for several pesticides (bitertanol, heptenophos, isofephos, and tolylfluanid) during ambient processing of apples (*3*) did not occur during cryogenic processing. Small "apparent losses" for dichlofluanid, chlozolinate, and etridiazole were much reduced compared to the higher losses previously reported to occur during ambient processing of apples (*3*). The use of an internal deposition standard proved to be successful, and this approach should be adopted for the other commodities. Alternative internal deposition standards will be required for more accurate assessments of the more polar and nonvolatile pesticides. It is unnecessary to recover the total amount of pesticides spiked (mass balance).

Other compounds previously reported to suffer losses during ambient processing (*3*), chlorothalonil (60%), ethoxyquin (90%), prochloraz parent (100%), and tebuconazole (37%), were not included in this study. These, along with compounds that were subject to relatively poor analytical performance in this study (acephate, carbendazim, dicloran, the DDT group, the endosulfan group, etridiazole, methamidophos, omethoate, phosalone, pyrimethanil, and thiabendazole), will be subjected to a further study of their stabilities during cryogenic processing of apples.

ABBREVIATIONS USED

CPM, chlorpyrifos-methyl; CV, coefficient of variation; FPD, flame photometric detector; GC, gas chromatography; GC-FPD, gas chromatography−flame photometric detector; GC-MSD, gas chromatography−mass selective detector; HPLC, high-performance liquid chromatography; HPLC-FL, high-performance liquid chromatography−fluorescence detection; MRL, maximum residue level; MSD, mass selective detector; PRC, Pesticides Residues Committee; PSD, Pesticides Safety Directorate; SIM,

selected ion monitoring; SRM, single residue method; TPE, tetraphenylethylene; WPPR, Working Party on Pesticide Residues.

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Received for review July 3, 2001. Revised manuscript received October 29, 2001. Accepted October 30, 2001. We acknowledge the financial support of the U.K. Ministry of Agriculture, Fisheries and Food and the Pesticides Safety Directorate for this work.

JF010852Y