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An evaluation of the stability of pesticides in fruit and vegetables during cryogenic sample processing (comminution of samples in the presence of dry ice) is reported. Pesticides were spiked onto the undamaged surface of individual units of fruit before freezing and comminution. The mean recoveries of pesticides spiked before and after comminution of the sample were compared to determine the relative stability of the individual pesticides during cryogenic sample processing. A stable internal deposition standard (IDS) was used to correct for physical losses and volumetric errors. Mean recovery results together with associated standard errors were obtained using Restricted Maximum Likelihood (REML) analysis. A total of 134 pesticides in 4 commodities (apples, grapes, lettuce and oranges) were evaluated. The results demonstrated that 120 pesticides were stable (i.e. the mean difference in recovery of pesticides spiked pre and post processing was <20 %) during cryogenic sample processing. Fourteen pesticides showed some instability or loss (i.e. the mean difference in recovery of pesticides spiked pre- and post- processing was >20 %) during cryogenic sample processing; biphenyl, cadusafos, captan, chlorothalonil, dichlorvos, disulfoton, ethoxyquin, etradiazole, heptenophos, malaoxon, phorate, tebuconazole, tecnazene and trifluralin.

Keywords : apples, grapes, lettuce, oranges, pesticides, cryogenic sample processing, stability

Introduction

Laboratories routinely monitor fruit and vegetables for pesticide residues to check for compliance with statutory Maximum Residue Limits (MRLs) and to provide the results for consumer exposure assessment. Fruit and vegetable samples for official monitoring programmes are usually taken according to CODEX Sampling Guidelines (Codex 1993), thus samples received at the laboratory should comprise a minimum of 5 or 10 units (individual fruit or vegetable units) with a minimum total weight of 2 or 1 kg respectively. The laboratory sample is then comminuted (chopped or blended) to produce a homogeneous sample from which representative sub-samples, typically 10-50g, are taken for subsequent laboratory analysis. The majority of laboratories continue to comminute samples at ambient temperature even though losses for a number of pesticides including chlorothalonil, folpet and tolylfluanid have been reported to occur during this procedure, (Hill *et al.* 2000; Lyn *et al.* 2003; El-Bidaoui *et al.* 2000). Losses of pesticides during sample processing will result in an underestimation of residue concentrations with implications for both MRL compliance monitoring and consumer risk assessments. In the UK, these losses resulted in public concern regarding the accuracy of the national monitoring programme (Hill *et al.* 2000). There is evidence to suggest that processing samples at low temperatures (cryogenic milling) can minimise the extent of these reported losses and thus produce more reliable results, (Hill *et al.* 2000; Fussell *et al.* 2002). Cryogenic milling requires the sample to be frozen, usually at -20 ºC, before being disintegrated into a fine, friable powder in the presence of dry ice (solid CO_2) or liquid nitrogen. By reducing the temperature at which the samples are comminuted, the potential reactions between any pesticide residues present in the samples and chemicals/enzymes released when plant cells are disrupted, can be slowed and hence the losses of pesticides minimised.

Chlorothalonil, which is not approved for use on protected Winter lettuce in the UK, is particularly prone to losses during sample processing at ambient temperature (Hill *et al.* 2000). Since 1996, samples of lettuce analysed as part of the UK pesticide surveillance and enforcement programmes have been comminuted in the presence of dry ice to minimise the loss of chlorothalonil and thus avoid an underestimation of residue concentrations. Before the use of cryogenic sample processing was extended to other commodities, it was considered necessary to check that the cryogenic milling process did not have adverse effects on stability of pesticide residues.

In 2002, results from the assessment of the stability of 106 pesticides and related compounds during the cryogenic processing of apples were reported (Fussell *et al.* 2002). The results from this initial study on apples were considered as indicative rather than definitive, as a full statistical analysis had not been carried out. The majority (94 out of 106) of the pesticides were stable (losses of ≤10 %), and only 3 out of 106 showed losses ≥20 %. The most significant findings were that losses of several pesticides (bitertanol, heptenophos, isofenphos and tolylfluanid) reported to occur during ambient processing of apples did not occur during cryogenic processing. Furthermore losses of dichlofluanid, chlozolinate, and etridiazole; also reported to occur during ambient processing of apples, were reduced to much lower levels (10 %, 17 % and 14 %, respectively) by cryogenic processing.

The aim of the work reported here was to assess the stability of a greater number of pesticides in representative commodities; lettuce, grapes and oranges. This work also included a statistical assessment of results reported previously for apples (Fussell *et al.* 2002). The proposed protocol did take into account the practice of freezing samples prior to comminution but did not follow normal procedures of comminuting 1-2 kg samples. Rather, the protocol was designed to assess the 'proof of principle' that pesticides are more stable when

comminuted at low temperatures. The procedure, previously evaluated for apples (Fussell *et al.* 2002), was based on the analysis of single crop units of fruit/vegetables. This simplified the spiking procedure and also enabled the analysis of the entire comminuted sample to minimise sub-sampling errors. In addition, analysis of single crop units was relatively cost-effective and allowed sufficient replication (duplicate analysis on 7 separate occasions), to provide conclusive evidence of relative stability or instability.

Experimental

Overview of Protocol

A detailed explanation of the development of the experimental protocol was reported in the original study on apples (Fussell *et al.* 2002). Most importantly, spiking was undertaken prior to freezing of whole apples, and the dry ice was allowed to evaporate at –20 °C after processing and prior to extraction of the comminuted sample. The stability of each pesticide during processing was assessed by comparing the mean recovery for the batch (method) recovery samples (spiked after sample processing and immediately before solvent extraction; 'post-processing') with the mean 'survival recovery' of the pesticides spiked before cryogenic processing i.e. 'pre-processing'. Pesticide method recovery and survival recovery results were 'corrected' using chlorpyrifos-methyl (CPM) or chlorpyrifos (CP) as an internal deposition standard. Chlorpyrifos-methyl and chlorpyrifos were selected for use as internal deposition standards (to correct for physical losses of pesticides and volumetric errors during sample processing and analysis procedures), because they are known to be stable and to yield good recovery using the analytical procedures employed in this study. CPM or CP deposited on the surface of the crop unit sample was subjected to the same conditions as the pesticides being assessed.

As the pesticides evaluated had a wide range of physico-chemical properties, a number of different extraction, clean-up and determination procedures were required to provide satisfactory recovery and precision for all analytes tested. Individual whole units or halved units (oranges and lettuce) weighing approximately 100 g were spiked at a concentration of approximately 0.05 - 0.1 mg/kg with mixed pesticide solutions.

The spiked 'units' were frozen individually in a freezer (-20 °C), stored, and then milled in the presence of dry ice on each day of the experiment. The comminuted samples were immediately placed in a freezer at -20 °C to allow carbon dioxide to dissipate before extraction. The samples spiked pre-processing, blank extract and the batch recovery extracts were analysed in discrete batches using GC-MSD, GC-FPD or LC-MS/MS. The whole procedure, was repeated on seven different days for each commodity-method combination. The individual results were corrected using the internal deposition standard, CPM or CP.

In these studies, the determination of pesticide concentrations in the mill washes (rinsing of the component parts of the mill with solvent) and filter papers (if used for spiking) was also carried out. These results were used to calculate the overall mass balance (recoveries) of pesticides spiked pre-processing. The mass balance for each pesticide was calculated as the combined survival recovery of the pesticide in the sample, filter papers (if used) and mill washes, uncorrected for IDS, and was approximately 70 –80 % for most experiments.

Samples

Apples (variety Royal Gala), oranges (varieties Valencia or Navel) and red grapes (variety unknown) were labelled as organically produced. Seedless green grapes and round lettuce (varieties not known) were not organically produced. All samples were purchased from local retail outlets and were used to prepare bulk blank samples of each commodity (for batch

recovery extractions), for individual batch blanks and for use in the preparation of individual spiked unit samples.

Reagents

Ethyl acetate, acetonitrile (both HPLC grade), anhydrous sodium sulfate and anhydrous sodium hydrogen carbonate (both Analytical Reagent grade) were purchased from Fisher Scientific (Loughborough, UK). Certified reference pesticides were purchased from QMx Laboratories Ltd (Saffron Walden, UK), Sigma-Aldrich Chemical Co. (Poole, UK) or Greyhound Chemicals (Birkenhead, UK). Tetraphenylethylene (TPE, 98 % purity) and triphenyl phosphate (TPP, 99.5 % purity) were obtained from Sigma-Aldrich Chemical Co. (Poole, UK). ENVI^{TM}-Carb solid phase extraction cartridges were purchased from Supelco Ltd. Magnesium sulphate (anhydrous) was obtained from York Glassware Services Ltd. (York, UK). PSA solid phase extraction material (particle size 40 µm, part number 12213023) was purchased from Varian Ltd. (Walton on Thames, UK). Sodium chloride was obtained from ICN Pharmaceuticals Ltd. (Basingstoke, UK).

Equipment

Nalgene centrifuge tubes (part number CF615-45) and polypropylene microtubes (part number QP/509N) were purchased from Camlab (Cambridge, UK).

Preparation of standard solutions

A mixed standard solution (containing 20 or 40 µg/ml of each pesticide) and chlorpyrifos-methyl or chlorpyrifos (internal deposition standard at 20 or 40 µg/ml) was prepared in ethyl acetate. Solutions of TPE or TPP (10 µg/ml) were also prepared in ethyl acetate for use as volumetric internal standards.

Preparation of spiked samples

Whole apples were wiped with hexane to remove wax, placed on a filter paper contained in a glass Petri dish and the pesticide solution carefully applied, dropwise, onto the surface of the fruit. A sufficient number of individual grapes (total 100 g) were stacked (stalk end down) on a filter paper contained in a glass Petri dish and spiked. Oranges were wiped with hexane to remove wax, cut in half horizontally, placed cut side down onto a glass Petri dish and spiked. Lettuces were cut in half longitudinally, placed cut side down onto an aluminium foil covered plastic tray and spiked.

Spiking was carried out using a standard solution of pesticides in ethyl acetate. The mixed standard solution was applied dropwise using a micro-syringe to the whole, undamaged surfaces of the crop unit(s); taking care not to damage the fruit and to minimise 'run off'. The spiking solution also contained chlorpyrifos methyl (CPM) and/or chlorpyrifos (CP) to act as an internal deposition standard. The spiked crop unit samples were stored in a freezer (- 20 °C), for a minimum of 24 hours prior to cryogenic sample processing. Unspiked samples of each of the above commodities were prepared and stored in the same wayprior to use as blank control samples.

Methodology

Cryogenic processing

Each individual frozen crop sample unit (approximately 100 g) was placed in a Tecator Mill (Model 1094, Tecator AB, Hoganas, Sweden) and comminuted for one minute in the presence of dry ice (approximately 200 g). As much as possible of the comminuted sample was recovered and immediately placed in a pre-weighed Schott bottle. The bottle was immediately transferred to a freezer (- 20 °C) for 24 hours to allow the dry ice to dissipate. Two spiked crop unit samples and at least two blank samples were processed on each day of the

experiment. Two (or one) processed blank samples were spiked with the same mix of pesticides immediately prior to extraction, for use as post-processing method recovery determinations. The pH of cryogenically processed samples was 3.7 and 3.9 for apples, 3.4 (Valencia oranges), 3.1 (Navel oranges), 3.2 (grapes), 5.5 and 5.8 (lettuce).

Extraction of samples

Ethyl acetate based methods, Method Codes 1-2

Each comminuted crop unit sample was homogenised with ethyl acetate (200 ml) in the presence of anhydrous sodium sulfate (120 g) and sodium hydrogen carbonate (17 g) at 30 °C ± 3 °C. The resulting supernatant extracts were filtered through solvent washed cotton wool.

For analysis an aliquot (10 ml) of ethyl acetate extract was cleaned-up using a 500 mg ENVI-Carb™ solid phase extraction cartridge. The cartridge was eluted with ethyl acetate (*Method Code 1a*) or acetonitrile/toluene (3:1) (*Method Code 1b*). The cleaned-up extracts were concentrated and internal standard (TPP or TPE) added prior to quantification by GC-MSD.

For polar organophosphorus pesticides, an aliquot (5 ml) of the ethyl acetate extract was concentrated and a volumetric internal standard (sulprofos or triphenyl phosphate) was added before making up to volume (1 ml) with ethyl acetate. Quantification was by GC-FPD (*Method Code 2a*)

Alternatively an aliquot (1 ml) of the ethyl acetate extract was taken, without further clean up or concentration, and an aliquot of internal standard (TPP) was added prior to analysis by GC-MSD (*Method Code 2b*).

Acetonitrile based methods, Method Codes 3-4

Each comminuted crop unit sample was shaken with acetonitrile (100 ml) and then sonicated for 1 minute. Anhydrous magnesium sulfate (40 g) and sodium chloride (10 g) were added and the mixture was shaken immediately. Then 0.5 ml of a 20 µg/ml solution of TPP in acetonitrile was added and the mixture was shaken for a further 30 seconds. PSA sorbent (25 mg) and

anhydrous magnesium sulfate (150 mg) were added to a 1.5 ml micro-centrifuge vial, followed by a 1 ml aliquot of the acetonitrile extract. The mixture was shaken for 30 seconds and then centrifuged at 6000 rpm for 1 minute. The supernatant extract was transferred to vials for GC-FPD analysis (*Method Code 3a*) or GC-MSD analysis (*Method Code 3b*). Alternatively a 500 µl aliquot was taken and 10 µl of 10 % acetic acid in acetonitrile was added prior to addition of PSA and anhydrous magnesium sulphate. The supernatant extract was transferred to vials for GC-MSD analysis (*Method Code 3c*).

For quantification using LC-MS/MS, an aliquot (1 ml) of the initial acetonitrile extract was dried using anhydrous magnesium sulphate (150 mg) (*Method Code 4*).

Measurement of pesticide concentrations in extracts

Typical conditions for GC-MSD determination

Determinations were made using an Agilent 6890 gas chromatograph connected to an Agilent 5973 Mass Selective Detector (MSD) operated in SIM mode. Injection (3 µl) was splitless at 250 ºC and the detector temperature was set at 280 ºC. Chromatographic separation was performed using a DB-5 MS capillary column (30 m x 0.25 mm i.d. x 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 ºC, held for 1 min; programmed to 150 ºC, at 30 ºC/min; then programmed to 220 ºC at 3 ºC/min, held for 1 min, and then finally programmed to 280 ºC at 2 ºC/min with a final hold time of 3 min.

Typical conditions for GC-FPD determination

Determinations were made using an Agilent 6890 gas chromatograph fitted with a flame photometric detector (FPD). Injection (1 µl) was pulsed splitless at 210 ºC and the detector temperature was set at 250 ºC. Chromatographic separation was performed using a DB1701 megabore column (30 m x 0.53 mm i.d. x 1.0 µm film thickness) with the carrier gas (helium) at a flow rate of 4.0 ml/min in constant flow mode. The oven temperature was programmed as follows: initial temperature 100 ºC, held for 0.5 min; programmed to 200 ºC, at 20 ºC/min, held

for 3 min; then programmed to 240 ºC at 5.0 ºC/min, held for 2 min, and then finally programmed to 280 ºC at 5 ºC/min with a final hold time of 12 min.

Typical conditions for LC-MS/MS determination

LC-MS/MS determinations were achieved using an Applied Biosystems Sciex API 2000 triple quadrupole mass spectrometer interfaced with an Agilent 1100 HPLC system. The mass spectrometer was used in positive electrospray mode. Two selected reaction monitoring (SRM) transitions were acquired per compound using a single time window. Chromatography used a Jones Genesis C18 analytical column (150 x 2.1 mm i.d.) fitted with a Phenomenex Security Guard C18 cartridge (4 x 2 mm i.d.). Mobile phase A was 10 mM aqueous ammonium acetate, mobile phase B was methanol. Gradient elution started at 10 % B increasing linearly over 10 min to 90 % B, held at 90 % B for a further 12 min before returning to the initial conditions. The total injection cycle time was 28 min, the flow rate was 0.2 ml/min and the injection volume was 5 µl.

Analytical Quality Control

Validation of the analytical methods

Prior to the analysis of the samples, the extraction and clean-up methods described in the experimental section were validated for the pesticides of interest by analysis of seven 100 g replicate samples spiked at 0.05 - 0.1 mg/kg, using the procedures described.

Post-processing batch recoveries were obtained from processed blank samples spiked at 0.05 – 0.1 mg/kg, and analysed at least individually, or more commonly in duplicate. These were analysed together with the pre-processing survival recoveries on each occasion. All determinations (including method validations) were calculated using multi-point, matrix-matched standards, which bracketed the samples. TPP or TPE was used as an internal volumetric standard.

Calculations

Recoveries corrected for internal deposition standard

The CPM or CP corrected mean survival or pre-processing recovery (%) in the cryogenically processed samples is the average of the individual corrected survival results expressed as percent for each of the cryogenically milled samples.

Individual % uncorrected survival or pre-processing recovery results (SU) were calculated for each pesticide as follows:

$$\frac{\mu g \text{ pesticide in the sample} \times 100}{\mu g \text{ pesticide added}} = SU$$

Individual CPM/CP % survival or pre-processing recovery correction factors (IS) were calculated as follows:

$$\frac{\mu g \text{ CPM (or CP) in the sample} \times 100}{\mu g \text{ CPM (or CP) added}} = IS$$

Therefore individual corrected % survival or pre-processing recovery results (SC) were calculated as follows:

$$SC = SU/IS \times 100$$

The CPM/CP corrected mean batch extraction or post-processing recoveries for each pesticide were calculated on the same basis.

Statistical analysis

The stability of pesticides during sample processing (comminution) was assessed by comparing the mean concentration of pesticides in samples spiked before comminution (mean survival or pre-processing recovery) with the mean concentration of pesticides in batch recovery samples spiked immediately after comminution (mean method post-processing recovery).

Prior to the statistical analysis, the results were 'corrected' using the response of the internal deposition standards CPM or CP. Statistical analysis of the collated data was performed, using

the statistical software package Genstat8, by Restricted Maximum Likelihood Analysis (REML) due to the 'unbalanced design' of the data (e.g. not all pesticides were measured for all commodities). Variation due to the analytical system, day, experiment, repetition value and commodity, where appropriate, were taken into account when calculating the error estimates of the main effects of interest.

The mean differences between recoveries from pre- and post- sample processing were then calculated by commodity for each of the pesticides analysed, together with 95% confidence intervals. This was performed to investigate if the effect of sample processing for each pesticide varied with the type of commodity.

Results and Discussion

Method Validation

Validation results for nearly all pesticide/commodity combinations were satisfactory. The mean recoveries (not corrected for IDS) were generally within the range 70-110 % with coefficients of variation (% CVs) below 10 %. A definitive and quantitative assessment of pesticide stability during sample processing as well as an accurate determination of residue concentrations; can only be carried out on the basis of good quality analytical data. Thus any protocol devised for this type of study is dependent on the availability of reliable and robust analytical methods for the full range of pesticides evaluated.

Stability

Data were collated for 134 pesticides over the four different commodities, from 21 sets of experiments. Each of the experiments had measurements over 6-7 days. Results for some pesticides were obtained using two or more sets of experiments and/or two different analytical systems.

The mass balance for each pesticide was calculated as the combined survival recovery of the pesticide in the sample, filter papers (if used) and mill washes, uncorrected for IDS, and was approximately 70 –80 % for most experiments. It is difficult to explain these losses of 20 – 30% of the pesticides. As the whole crop unit sample was comminuted and extracted, these losses could not be explained by gravimetric error caused by condensation. Schmidt *et al* (2006) conducted experiments using radiolabelled pesticides and reported that losses up to 20 % occurred during the spiking and freezing process but no significant losses were observed during the cryogenic homogenisation step. The use of internal deposition standards (CPM or CP) which are known to be stable under cryogenic processing conditions, to correct for recovery, removed the effect of physical losses of pesticide from the results, thus enabling a more accurate assessment of stability to be made.Therefore all further assessments were based on the relative stability of the pesticide to chlorpyrifos or chlorpyrifos-methyl.

[Insert Figures 1-4 here]

The relative stability results (difference between pre- and post- processing recoveries) for all of the pesticide/commodity combinations are summarized in Figures 1-4. The results obtained from GC-MSD and GC-FPD measurements are presented in Figures 1-3, results derived from LC-MS measurements are shown in Figure 4. The data in Figures 1-4 show a plot of mean differences between pre- and post-processing recoveries with 95 % confidence limits for all of the individual pesticides. They are also coded by commodity. Pesticides which have 95 % confidence intervals not containing zero, i.e. 95 % confidence limits entirely <0 % indicate that the mean difference between pre- and post- sample processing results are statistically significantly different. However for practical analytical purposes, a mean difference of $\leq$-20 % is regarded as evidence of a significant level of instability or loss during sample processing.

The results for some of the LC-MS pesticides (Figure 4) showed apparent survival (pre-processing recoveries) of >100 %. These analytes are generally less volatile and more polar than CP, therefore CP is not the optimum internal deposition standard for these pesticides. Stable isotope analogues could be used, however these are expensive and only available for a limited number of pesticides.

The data in Figures 1-4 show that 120 pesticides were stable (mean difference between pre- and post- processing recoveries was >-20 %) during cryogenic sample processing. Fourteen of the pesticides showed some instability or loss during processing, and results for these compounds are listed in detail in Table 1.

[Insert Table 1 here]

The pesticides showing processing losses were dichlorvos, etradiazole (all commodities), biphenyl (apples, grapes, oranges), tecnazene (grapes, lettuce and oranges), captan and ethoxyquin (apples, oranges), malaoxon (apples), chlorothalonil and tebuconazole (grapes), cadusafos, disulfoton, heptenophos, phorate and trifluralin (oranges). The 95 % confidence limits for these mean results are also listed in Table 1. Figures 1–4 show that there were also a further 4 pesticides with results for mean difference >-20 %, but where the upper and lower 95 % confidence limits were <0 % and <-20 % respectively. These compounds were deltamethrin and dicofol (grapes), aldicarb and dichlofluanid (oranges); however as the mean difference between pre- and post-processing recoveries was >-20 %, for practical analytical purposes they were regarded as stable.

Cadusafos, captan, chlorothalonil, heptenophos, malaoxon, tebuconazole, tecnazene and trifluralin all showed losses for at least one commodity, but not in other commodities tested. Although oranges were the individual commodity most frequently showing losses, it was not possible to make a definitive comparison as (i) only 8 pesticides showed apparent commodity-specific losses and (ii) not all pesticides were evaluated in all commodities.

Overall, the data showed most (120 out of 134) pesticides were stable during cryogenic sample processing. This is especially significant as the pesticides evaluated included those suspected to be unstable during processing. For apples, lettuce and oranges, some comparison can be made with previously reported data for ambient processing, although ambient processing data was acquired using different experimental protocols and thus an exact comparison is not possible. The ambient processing results were also based on the simple difference between mean results from pre- and post- processing spiked samples, with no further statistical treatment of the data.

In the case of apples, losses (>20 %) of a number of pesticides (bitertanol, chlorothalonil, dichlofluanid, heptenophos, isofenphos, oxadixyl, prochloraz, tebuconazole and tolylfluanid) previously reported to occur during ambient processing (Hill *et al.* 2000), were minimised using cryogenic processing. These finding are consistent with those of Poulsen and Jonassen (2006), who reported higher recoveries for some pesticides in apples after cryogenic processing compared to processing apples at ambient temperature.

A comparison of lettuce showed a similar pattern. Losses of pesticides (captan, chlozolinate, dichlofluanid, dicofol, iprodione, metalaxyl and tolylfluanid; all >20 %) previously reported to occur during ambient processing of lettuce (Hill *et al.* 2000), were not observed using cryogenic processing.

Processing oranges at ambient temperature also resulted in losses (>20 %) for several pesticides by comparison with cryogenic processing. Losses observed for dichlofluanid, dicloran, and tolylfluanid during ambient sample processing (Fussell *et al.* 2006), were not found in this evaluation of oranges.

There is limited data on the stability of pesticides during the ambient milling of grapes and therefore a relevant comparison of the stability of pesticides during cryogenic and ambient processing is not possible. However, losses of biphenyl, dichlorvos, tecnazene, ethoxyquin and etridiazole were found in all commodities for which these pesticides were tested. The losses of the volatile pesticides, biphenyl, dichlorvos and tecnazene; may be due to the large amount of carbon dioxide gas released during comminution, as these pesticides may be volatilised, despite the low temperatures. The reason for the losses of ethoxyquin and etridiazole is not known. As previous data on ambient processing also showed large losses, this indicates that an accurate determination of residue concentrations for the volatile pesticides may not be possible, until the problem of volatilisation loss is solved.

Conclusion

Overall, the data showed most (120 out of 134) pesticides were stable during cryogenic sample processing. This is especially significant as the pesticides evaluated included those previously suspected to be unstable during processing at ambient temperature. Cryogenic milling reduces the likelihood of underestimating residue concentrations (and thus possible MRL non-compliances) of some widely used pesticides. The improved stability of pesticide residues together with improved homogeneity of sub-samples supports the increasing use of cryogenic sample processing in the UK pesticides monitoring programme. It is recommended that

laboratory reports should include a description of the sample processing procedure employed to avoid misinterpretation of pesticide residue results.

Acknowledgements

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Table Legend

Table 1. Difference in pre- and post-spiking methods with approximate 95% confidence interval, where the difference is less than -20 (i.e more than 20 % loss) for at least one commodity per pesticide.

Figure Captions

Figure 1. Difference Between Pre and Post Spiking Concentration with Approximate 95% Confidence Limits, Part 1

Figure 2. Difference Between Pre and Post Spiking Concentration with Approximate 95% Confidence Limits, Part 2

Figure 3. Difference Between Pre and Post Spiking Concentration with Approximate 95% Confidence Limits, Part 3

Figure 4. Difference Between Pre and Post Spiking Concentration with Approximate 95% Confidence Limits, for Pesticides Tested Using LCMS

[Table 1]

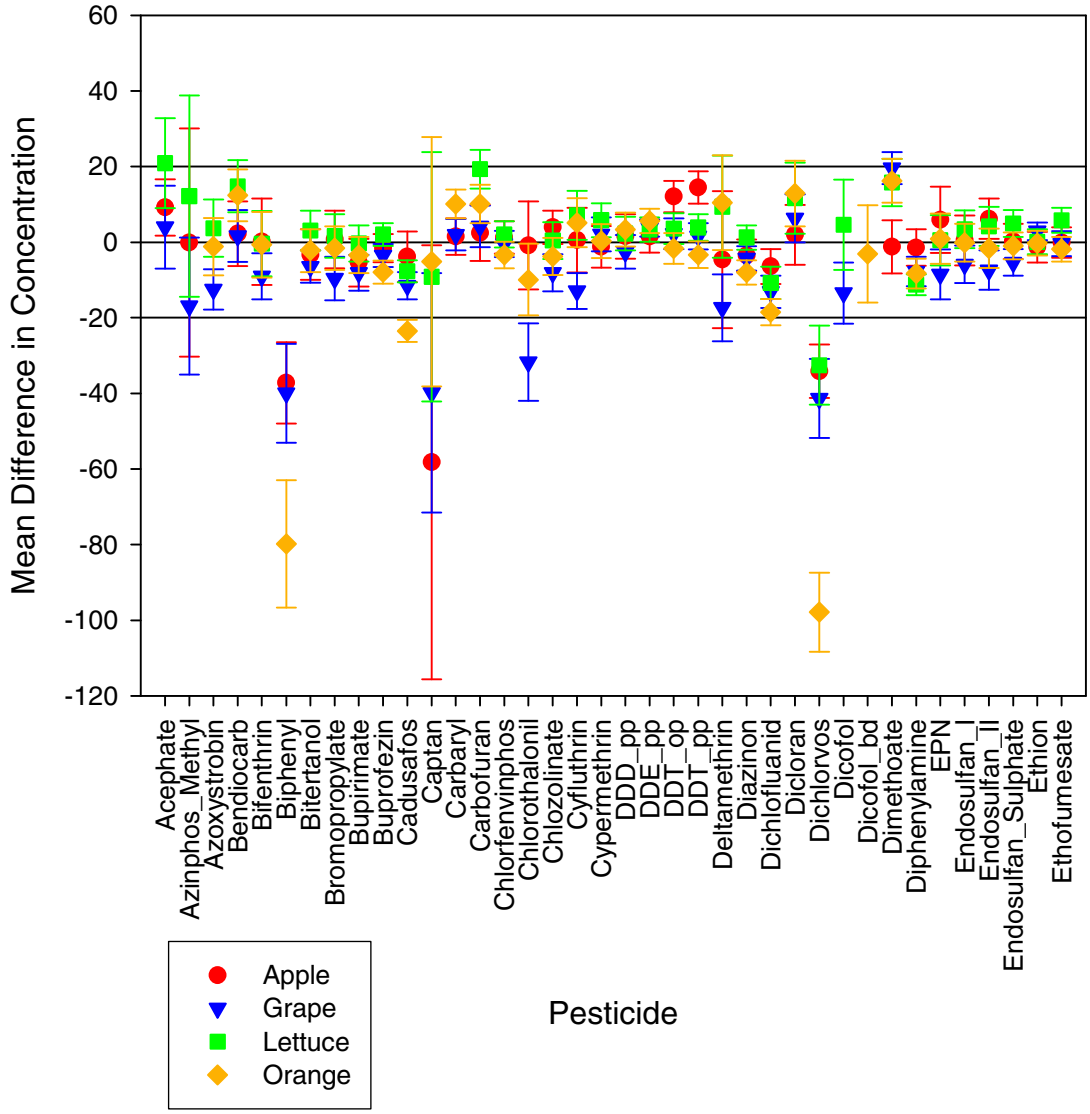
Pesticide	Commodity	Pre Mean	Post Mean	Mean Diff	LCI*	UCI**	Method Code
Biphenyl	apple	48.8	86.0	-37.2	-47.98	-26.43	1a, 2b
	grapes	40.2	80.2	-40.0	-53.00	-26.91	1b
	orange	18.5	98.3	-79.8	-96.65	-62.96	1a, 3c
Cadusafos	apple	96.4	100.2	-3.9	-10.59	2.82	1a
	grapes	88.4	99.9	-11.5	-15.10	-7.94	1a, 1b
	lettuce	93.6	101.3	-7.7	-10.62	-4.78	1a, 3a, 3b
	orange	76.2	99.7	-23.5	-26.41	-20.57	1a, 3a, 3c
Captan	apple	22.5	80.7	-58.2	-115.60	-0.82	
	grapes	39.6	79.4	-39.8	-71.48	-8.13	1a, 1b
	lettuce	114.1	123.3	-9.2	-42.13	23.81	1a
	orange	76.7	81.9	-5.2	-38.14	27.80	1a
Chlorothalonil	apple	84.7	85.6	-0.9	-12.47	10.72	2b
	grapes	48.7	80.5	-31.7	-41.96	-21.51	1b
	orange	15.1	25.0	-9.9	-19.38	-0.45	3c
Dichlorvos	apple	48.4	82.5	-34.1	-41.17	-27.07	1a, 2b, 3a
	grapes	51.8	93.2	-41.3	-51.76	-30.87	3a
	lettuce	63.6	96.1	-32.5	-42.99	-22.10	3a
	orange	-3.0	94.9	-97.9	-108.33	-87.44	3a
Disulphoton	orange	77.6	107.0	-29.4	-39.29	-19.48	4

Pesticide	Commodity	Pre Mean	Post Mean	Mean Diff	LCI*	UCI**	Method Code
Ethoxyquin	apple	50.1	129.4	-79.3	-96.63	-61.94	2b
	orange	41.9	98.6	-56.6	-69.75	-43.54	3c
Etridiazole	apple	55.1	87.2	-32.1	-40.32	-23.91	1a, 2b
	grapes	50.9	78.8	-27.9	-34.83	-20.93	1a, 1b
	lettuce	60.3	99.6	-39.4	-48.84	-29.89	3b
	orange	34.4	90.5	-56.1	-63.33	-48.85	1a, 3c
Heptenophos	apple	91.6	96.0	-4.4	-8.89	0.08	1a
	grapes	89.0	99.2	-10.2	-13.82	-6.57	1a
	lettuce	87.6	95.8	-8.2	-11.74	-4.75	1a
	orange	52.8	104.5	-51.7	-57.03	-46.35	3c
Malaoxon	apple	79.3	99.4	-20.1	-26.25	-14.01	1a
	grapes	101.3	102.5	-1.1	-6.13	3.87	1a
	lettuce	107.4	102.3	5.1	0.51	9.76	1a
	orange	101.2	101.7	-0.5	-5.08	4.17	1a
Phorate	orange	67.1	104.1	-37.0	-46.35	-27.61	4
Tebuconazole	apple	94.4	90.9	3.5	-2.67	9.75	1a, 2b
	grapes	96.5	117.1	-20.6	-25.86	-15.31	1a, 1b
	lettuce	106.9	115.3	-8.4	-13.31	-3.54	1a, 3a
	orange	97.0	102.0	-5.0	-11.91	1.91	1a
Tecnazene	apple	84.0	93.1	-9.1	-16.79	-1.45	1a
	grapes	63.3	85.9	-22.6	-26.86	-18.35	1a
	lettuce	60.0	94.8	-34.8	-38.90	-30.70	1a, 3a
	orange	60.6	94.8	-34.2	-38.27	-30.07	1a, 3c

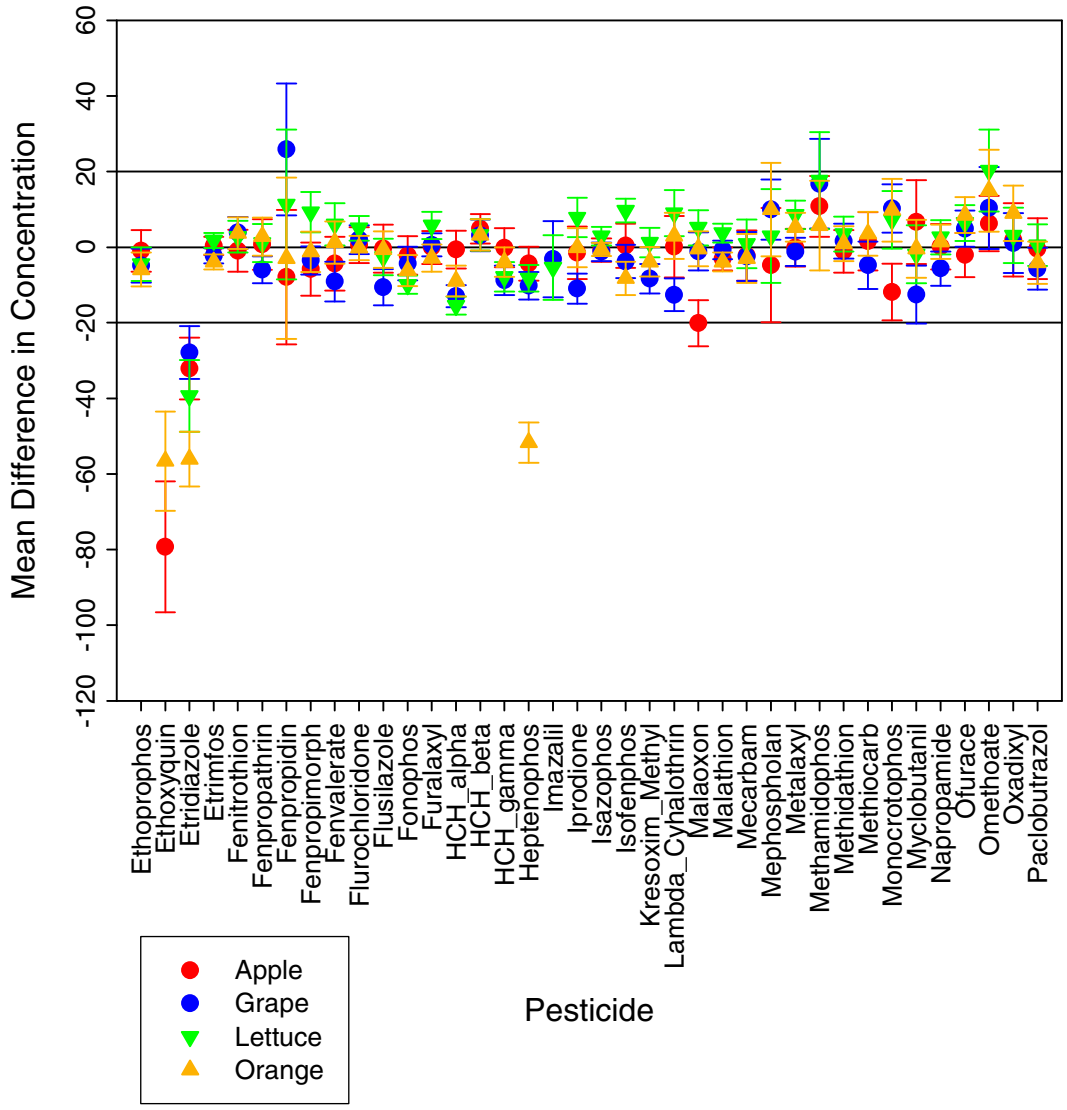
Pesticide	Commodity	Pre Mean	Post Mean	Mean Diff	LCI*	UCI**	Method Code
Trifluralin	apple	99.3	100.9	-1.5	-6.19	3.16	1a
	grapes	89.5	99.0	-9.5	-13.03	-5.96	1a
	lettuce	92.9	99.7	-6.8	-10.35	-3.28	1a
	orange	67.9	102.0	-34.0	-37.57	-30.50	1a, 3c

* LCI – lower confidence interval

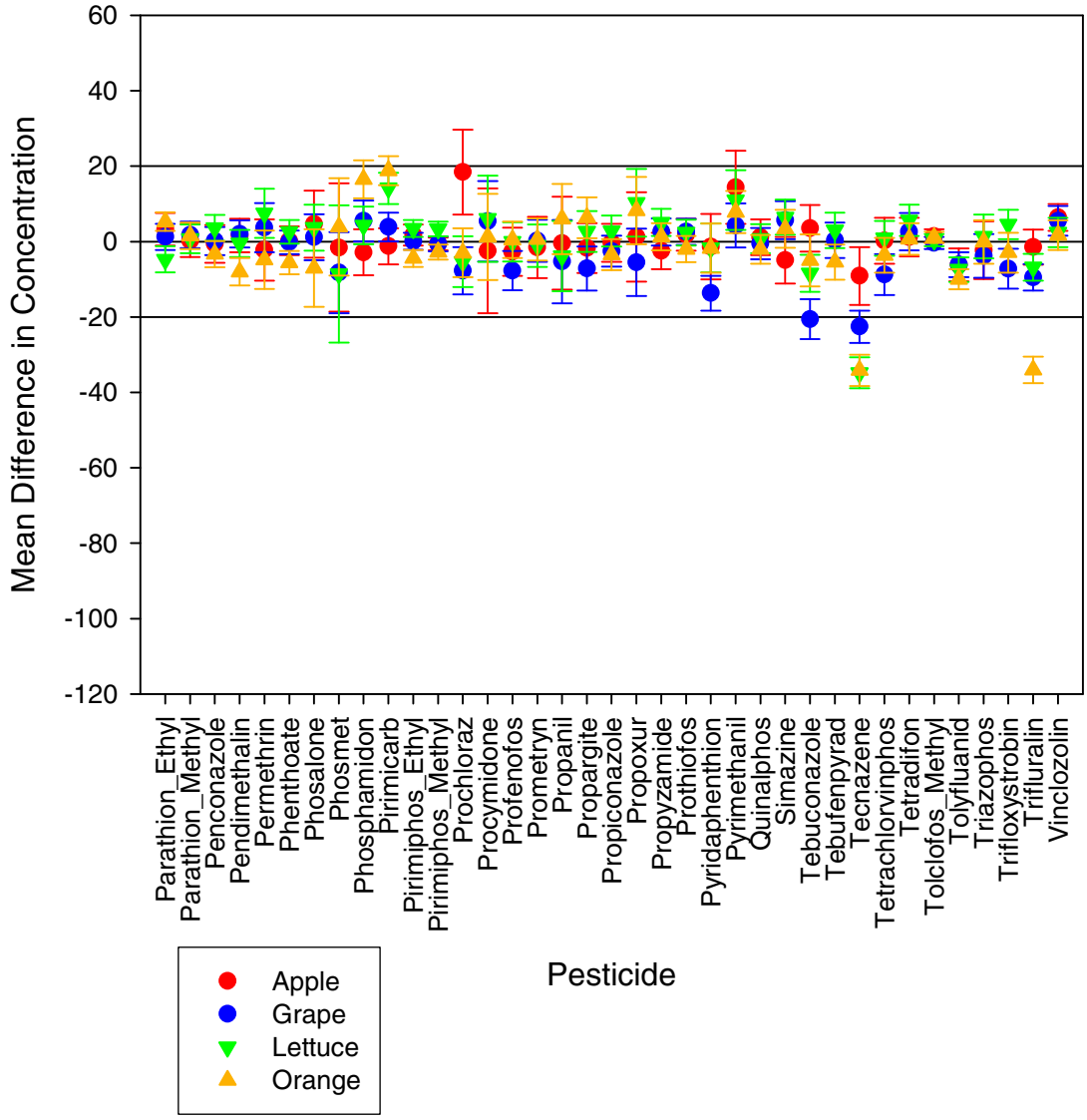
** UCI – upper confidence interval


Mean Difference in Concentration
60
40
20
0
-20
-40
-60
-80
-100
-120
Acephate
Azinphos_Methyl
Azoxystrobin
Bendiocarb
Bifenthrin
Biphenyl
Bitertanol
Bromopropylate
Bupirimate
Buprofezin
Cadusafos
Captan
Carbaryl
Carbofuran
Chlorfenvinphos
Chlorothalonil
Chlozolinate
Cyfluthrin
Cypermethrin
DDD_pp
DDE_pp
DDT_op
DDT_pp
Deltamethrin
Diazinon
Dichlofluanid
Dicloran
Dichlorvos
Dicofol
Dicofol_bd
Dimethoate
Diphenylamine
EPN
Endosulfan_I
Endosulfan_II
Endosulfan_Sulphate
Ethion
Ethofumesate
Apple
Grape
Lettuce
Orange
Pesticide

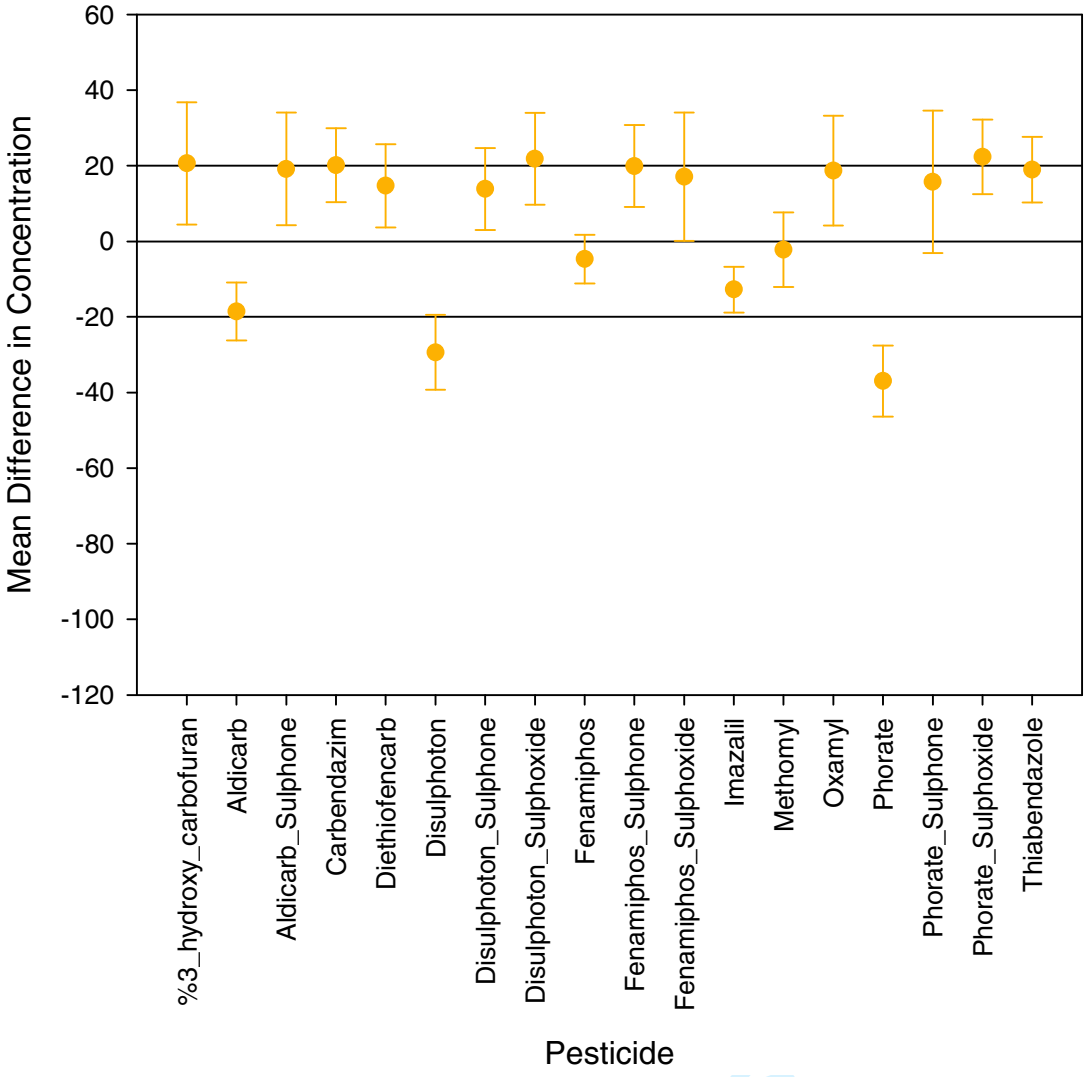
[Figure 1]



[Figure 2]


Mean Difference in Concentration
60
40
20
0
-20
-40
-60
-80
-100
-120
Parathion_Ethyl
Parathion_Methyl
Penconazole
Pendimethalin
Permethrin
Phenthoate
Phosalone
Phosmet
Phosphamidon
Pirimicarb
Pirimiphos_Ethyl
Pirimiphos_Methyl
Prochloraz
Procymidone
Profenofos
Prometryn
Propanil
Propargite
Propiconazole
Propoxur
Propyzamide
Prothiofos
Pyridaphenthion
Pyrimethanil
Quinalphos
Simazine
Tebuconazole
Tebufenpyrad
Tecnazene
Tetrachlorvinphos
Tetradifon
Tolclofos_Methyl
Tolyfluanid
Triazophos
Trifloxystrobin
Trifluralin
Vinclozolin
Pesticide
Apple
Grape
Lettuce
Orange

[Figure 3]



[Figure 4]