

Measurement Uncertainty Associated with Sample Processing of Oranges and Tomatoes for Pesticide Residue Analysis

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The homogeneity of analytical samples and the stability of pesticides during the sample processing of oranges and tomatoes were evaluated. The mean concentrations of ¹⁴C-labeled chlorpyrifos in analytical portions (subsamples) after processing show that homogeneity is dependent on sample type as well as the processing procedure. The homogeneity of analytical samples of tomatoes processed cryogenically was much better than those processed at ambient temperature. For tomatoes, the minimum analytical portion masses required for between-analytical portion variation of <0.3 Ho were 110 and 5 g for processing at ambient and cryogenic temperatures, respectively. Results for orange showed that analytical portion sizes of 5 g provided sufficient homogeneity from both sample processing procedures. Assessments of pesticide stability demonstrated that most were relatively stable during processing at either ambient or cryogenic temperatures. However, some pesticides, including dichlofluanid, chlorothalonil, tolylfluanid, and dicloran, appeared to suffer much greater losses (>20%) during processing at ambient temperature. For these analytes, loss is interpreted as chemical degradation.

KEYWORDS: Homogeneity; oranges; pesticide stability; sample processing; tomatoes; uncertainty

INTRODUCTION

Control laboratories routinely monitor fruits and vegetables for pesticide residues to check for compliance with statutory maximum residue limits (MRLs) and to assess consumer exposure to pesticides. Fruit and vegetable samples for official monitoring programs are usually taken according to CODEX Sampling Guidelines (1); thus, the laboratory receives laboratory samples comprising a minimum of 5 or 10 units (individual fruit or vegetable units) with a minimum total mass of 2 or 1 kg, respectively. Laboratory samples comprising large crop units, e.g., lettuce, are randomly subdivided. The laboratory sample is then comminuted (chopped or blended) to form an analytical sample from which representative analytical portions (typically 10–50 g) are withdrawn for analysis.

Most laboratories comminute laboratory 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 (2–4). The extent of the loss is dependent on both the pesticide and the sample type and probably varies between different varieties and between different samples of the same variety.

Losses of pesticides at the sample processing stage and/or subsequent analytical steps will result in an underestimate of residue levels with possible implications on MRL compliance monitoring, consumer risk assessments, and measurement uncertainty. It is therefore desirable to develop and adopt analytical procedures that minimize pesticide losses and improve the reliability of results.

Data for the calculation of measurement uncertainty are mostly derived from in-house validation (5, 6) or proficiency test studies (7), involving the analysis of spiked homogenized analytical portions. Consequently, one of the largest sources of errors, sample processing, is not taken into account. Also, the quest for harmonization of European MRL controls across the different member states is further compromised by the fact that different laboratories inevitably process samples at different temperatures and using different types of processing equipment.

The general lack of attention to sample processing is probably due to the fact that such studies are technically challenging and costly to perform. However, the importance of such studies should not be underestimated, not least because comminution and thorough mixing of the laboratory sample are prerequisites to obtaining representative analytical portions prior to analysis. It is particularly relevant at a time when most laboratories are implementing methods with reduced analytical portion sizes in

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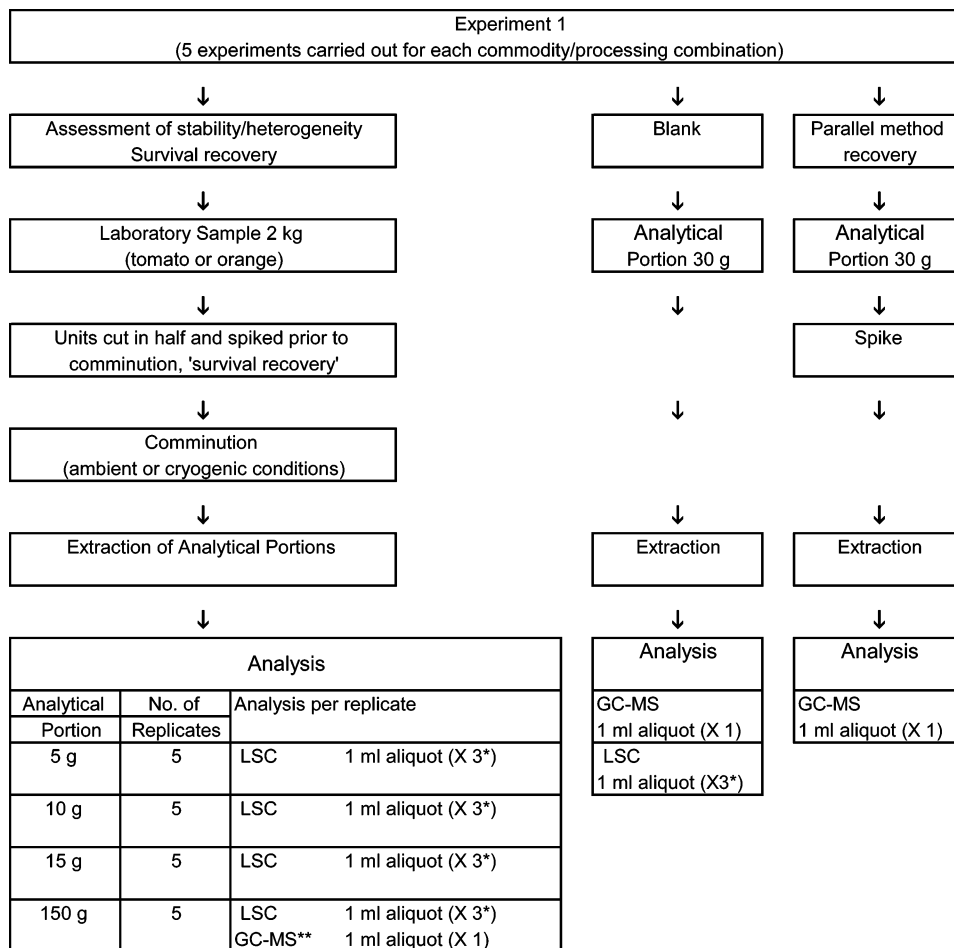


Figure 1. Overview of experimental protocol used to assess homogeneity of analytical samples and stability of pesticides during sample processing. *Each 1 mL aliquot was analyzed separately by LSC. **For 150 g analytical portion, a 1 mL aliquot was taken for GC-MS from three out of the five replicates.

order to reduce reagent and solvent usage etc. and at a time when there is increased interest in measurement uncertainty.

Relatively few studies have been reported in the literature. Ambrus et al. (8) proposed the use of sampling constants for estimating the uncertainty associated with the ambient sample processing of apples and head cabbages containing incurred pesticide residues. A minimum of 64 g of cabbage and 440 g of apples was required to hold the uncertainty of sample preparation to <5%. These analytical portion masses are much higher than those currently employed (10–50 g) for many residue methods. Maestroni et al. (9) described a method involving the use of ^{14}C -labeled chlorpyrifos with liquid scintillation counting (LSC) of extracts to estimate uncertainty associated with processing fruit and vegetables. It should be noted that ^{14}C -labeled chlorpyrifos provides information on total loss and not analyte stability. The method was quick, robust, and precise, because LSC allowed direct analysis of crude extracts eliminating the uncertainty contribution associated with cleanup. Using a top-down approach, Lynn et al. (3) concluded that the standard measurement uncertainty associated with physical sample preparation can be high and dominate the overall measurement uncertainty for some pesticide–commodity combinations. Bettencourt de Silva et al. (10) proposed a differential method for the estimation of sample processing and subsampling performance, based on a comparison of the dispersion of results for the global method with the uncertainty estimated from developed models for the individual analytical steps (bottom-up approach).

There is evidence to suggest that sample processing at low temperatures (cryogenic processing) can minimize the extent of the reported losses and thus produce more reliable results (2, 11). Cryogenic processing requires the laboratory sample to be frozen, usually at $-20\text{ }^\circ\text{C}$, 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 laboratory 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 losses of pesticides can be minimized. Despite acknowledging these losses, most laboratories continue to carry out extensive validation of the extraction, cleanup, and detection stages of methods but not the sample processing stage.

The aim of this study was to assess the impact of two different sample-processing conditions (comminution at ambient and at cryogenic temperatures) on measurement uncertainty of pesticide residues analysis. Two sources of uncertainty associated with sample processing were considered as follows: the stability of pesticides during comminution of two representative commodities, oranges and tomatoes, and second, the heterogeneity of “homogenized” analytical portions. The work was undertaken as a contribution to a Coordinated Research Project (CRP) on Testing the Efficiency and Uncertainty of Sample Processing for Analysis of Food Contaminants, coordinated by the International Atomic Energy Agency (IAEA).

MATERIALS AND METHODS

Experimental Design. The stability of pesticides during sample processing (comminution) was assessed by comparing the mean concentration of pesticides in laboratory samples (2 kg) spiked before comminution (mean “survival recovery”) with the mean concentration of pesticides in samples (30 g) spiked immediately after comminution (mean “method recovery”) at either ambient or cryogenic temperatures. These recovery experiments were repeated five times at both ambient and cryogenic temperatures, with each experiment being conducted on a separate occasion. The pesticide concentrations in solvent extracts of comminuted analytical portions were measured by LSC and gas chromatography with mass spectrometry detection (GC-MS) as outlined in the experimental protocol (Figure 1).

An internal deposition standard (IDS), chlorpyrifos (known to be stable under conditions employed), was included in the spiking standard to allow the method recovery and survival recovery results to be corrected for physical losses and volumetric errors. Therefore, chlorpyrifos (IDS) deposited on the surface of the crop units was subjected to the same conditions as the pesticides being assessed.

Preparation of Spiked Laboratory Samples. For each experiment, on each occasion, individual units of tomatoes or oranges comprising a 2 kg laboratory sample were cut vertically into two approximately equal halves and the halved units were placed cut side downward on an aluminum foil-covered tray. An aliquot (4.0 mL) of a mixed pesticide standard in ethyl acetate solution [containing 50 $\mu\text{g}/\text{mL}$ of each individual pesticide including 45 $\mu\text{g}/\text{mL}$ “cold” unlabeled chlorpyrifos and 5 $\mu\text{g}/\text{mL}$ ^{14}C -labeled chlorpyrifos (approximately 750000 dpm/ μg)] was applied dropwise, using a microsyringe, to the outer skin of each of the individual half units to give a spiking level of approximately 0.1 mg/kg for each individual pesticide. The pesticides contained in the spiking mix are listed in Tables 1 and 2 for tomatoes and oranges, respectively. The spiked sample was then left for 15 min at ambient temperature to allow sufficient time for the solvent to evaporate. A total of 20 spiked laboratory (2 kg) samples (five samples of each of oranges and tomatoes for cryogenic processing and five of each for ambient processing) were prepared. Laboratory samples for ambient processing were comminuted as soon as possible, but laboratory samples for cryogenic processing were placed in a freezer ($-20\text{ }^{\circ}\text{C}$) for a minimum of 16 h.

Laboratory Sample Processing. For ambient processing, the fresh spiked units were placed in a Stephan Mill (model UMC12, Stephan AG, Germany) and comminuted at room temperature for 3 min to produce a homogenized analytical sample. Five replicate analytical portions at each mass (150, 15, 10, and 5 g) were withdrawn for immediate analysis by LSC.

For cryogenic processing, the frozen spiked units were transferred to the same mill (precooled with dry ice) and comminuted for 3 min in the presence of dry ice (approximately 1.5–2 kg). As much as possible of the comminuted analytical sample was recovered and immediately transferred to an unsealed polythene bag, which was stored in a freezer ($-20\text{ }^{\circ}\text{C}$) for a minimum of 16 h, to allow the carbon dioxide to dissipate, and a maximum of 24 h before analysis.

Laboratory samples of oranges and tomatoes, labeled as organically produced and shown to be free of pesticide residues, were comminuted by cryogenic sample processing to provide blank material for blank control samples, “method validation” and “method recovery” spikes, and for the preparation of matrixed-matched calibration standards.

Extraction. Five replicate analytical portions each of 150, 15, 10, and 5 g were taken randomly from the comminuted analytical samples. Analytical portions of analytical samples, comminuted at ambient temperature, were withdrawn without delay and with continuous mixing, to avoid separation of the liquid and solid phases. Analytical portions of analytical samples, comminuted at cryogenic temperature, were withdrawn quickly to prevent thawing of the cryogenically milled material.

Ethyl acetate (2 mL/g), sodium hydrogen carbonate (0.167 g/g), and then anhydrous sodium sulfate (1 g/g) were added to the analytical portions. The ratio of reagent, solvent, and analytical portion amounts was kept constant for all of the analytical portion sizes. The temperature of the mixture was equilibrated to $30\text{ }^{\circ}\text{C}$ (water bath set at $30 \pm 1\text{ }^{\circ}\text{C}$)

Table 1. Difference between Concentration of Pesticides in Ambiently and Cryogenically Processed Tomato Analytical Portions (Adjusted for IDS Concentration)^a

pesticide	$\mu\text{g}/\text{kg}$			uncertainty associated with difference
	ambient	cryogenic	difference	
<i>captan</i>	9.0	76.1	67.1	15.7
<i>folpet</i>	43.7	100.0	56.4	24.2
<i>dichlofluanid</i>	45.0	96.6	51.6	6.3
<i>tolyfluanid</i>	55.5	97.3	41.8	7.2
<i>chlorothalonil</i>	37.6	75.8	38.2	10.6
<i>imazalil</i>	57.1	88.1	31.0	6.5
<i>chlozolinate</i>	74.6	103.1	28.5	5.3
<i>dimethoate</i>	91.2	110.6	19.4	3.5
<i>azinphos-methyl</i>	73.3	92.1	18.8	6.4
<i>methidathion</i>	84.7	98.9	14.2	2.5
<i>pirimicarb</i>	97.8	110.8	12.9	2.9
<i>phosalone</i>	85.9	97.6	11.7	5.0
<i>pyrimethanil</i>	99.8	109.9	10.1	3.5
<i>tebuconazole</i>	81.7	90.8	9.1	4.8
<i>ethoxyquin</i>	74.2	82.9	8.7	9.6
<i>ethoprophos</i>	93.3	102.0	8.6	3.4
<i>diazinon</i>	93.4	101.8	8.4	4.2
<i>dicloran</i>	100.8	109.1	8.3	4.7
<i>parathion-methyl</i>	93.8	101.7	8.0	4.2
<i>heptenophos</i>	89.3	97.1	7.8	4.6
<i>isofenphos</i>	97.2	104.8	7.6	4.5
<i>myclobutanil</i>	90.6	97.7	7.1	3.6
<i>pirimiphos-methyl</i>	96.8	103.6	6.7	2.4
<i>chlorpyrifos-methyl</i>	99.0	105.6	6.6	4.6
<i>procymidone</i>	99.2	105.0	5.8	2.5
<i>kresoxim-methyl</i>	90.4	95.8	5.4	3.7
<i>ethion</i>	95.3	100.5	5.3	4.5
<i>bitertanol</i>	86.8	91.3	4.5	6.4
<i>metalaxyl</i>	104.4	108.6	4.2	3.2
<i>parathion-ethyl</i>	96.8	100.2	3.4	4.4
<i>tolclofos-methyl</i>	101.1	104.2	3.2	3.7
<i>vinclzolin</i>	98.1	100.2	2.1	3.6
<i>permethrin</i>	97.8	99.0	1.2	8.4
<i>iprodione^b</i>	95.9	97.0	1.1	24.3
<i>propargite</i>	92.1	93.0	0.9	3.9
<i>cypermethrin</i>	93.8	94.0	0.2	5.8
<i>deltamethrin</i>	97.0	94.9	-2.1	15.5
<i>carbaryl^b</i>	103.5	85.5	-18.0	24.9

^a Italic results indicate statistically significant differences between processing methods. Bold results indicate practically important (>11%) differences between processing methods. ^b Subject to poor analytical precision.

and then homogenized for 30 s (1.5 min for 150 g portions) using an Ultra-Turrax Model T25 Homogenizer (IKA, Germany). Five replicate analytical portions (of each of the various masses) were extracted in a single batch on each day for each processing experiment. A method recovery (30 g) and a blank control sample (30 g) were also extracted in the experiments for the GC-MS assessment of stability of pesticides. The blank control samples were not spiked with IDS.

Measurement of Radioactivity in Extracts. Homogeneity was assessed using LSC to determine the ^{14}C chlorpyrifos content of all of the different size analytical portions. Three individual aliquots (1.0 mL) were taken from each extract of each analytical portion (i.e., each of the five replicate analytical portions of 150, 15, 10, and 5 g) into individual scintillation vials and LSC cocktail (19 mL) added to each vial. The vials were closed, shaken to mix the contents, and then transferred to a liquid scintillation counter (Beckman LS 6000TA Liquid Scintillation Counter, Beckman). The level of ^{14}C -labeled chlorpyrifos was quantified by calibration against standards prepared in blank sample matrix. Residual ^{14}C -labeled chlorpyrifos remaining in the vial (which had contained the standard solution used for spiking), on the foil (runoff from spiking) and in the syringe, was recovered and measured using LSC. Each of the five analytical portions at each mass were analyzed in triplicate on each of five occasions to give a total of 300 individual LSC measurements for each of tomatoes and oranges at each of ambient and cryogenic processing conditions (see Figure 1).

Table 2. Difference between Concentration of Pesticides in Ambiently and Cryogenically Processed Orange Analytical Portions (Adjusted for IDS Concentration)^a

pesticide	$\mu\text{g}/\text{kg}$			uncertainty associated with difference
	ambient	cryogenic	difference	
<i>dichlofluanid</i>	46.1	89.1	43.0	4.2
<i>chlorothalonil</i>	26.2	67.2	41.0	14.2
<i>tolyfluanid</i>	68.8	95.3	26.4	4.4
<i>dicloran</i>	77.2	102.9	25.7	4.1
<i>dichlorvos</i>	19.5	36.2	16.7	9.0
<i>bitertanol</i>	91.8	105.5	13.8	12.9
<i>azinphos-methyl</i>	78.5	91.9	13.4	7.0
<i>deltamethrin</i>	91.7	104.0	12.3	6.4
<i>biphenyl</i>	32.1	41.1	8.9	3.7
<i>etridiazole</i>	42.2	50.5	8.3	4.6
<i>imazalil</i>	91.8	99.0	7.2	5.5
<i>heptenophos</i>	89.5	96.5	6.9	4.1
<i>tecnazene</i>	75.6	82.5	6.9	4.1
<i>pirimicarb</i>	100.4	107.3	6.9	4.9
<i>myclobutanil</i>	93.9	100.8	6.9	3.8
<i>tolclofos-methyl</i>	97.7	104.1	6.4	5.1
<i>trifluralin</i>	92.7	98.6	5.8	2.8
<i>pyrimethanil</i>	99.7	105.5	5.8	3.9
<i>metalaxyl</i>	101.1	105.6	4.6	4.7
<i>tebuconazole</i>	92.7	97.1	4.4	4.6
<i>iprodione</i> ^b	91.4	95.8	4.3	11.5
<i>carbaryl</i> ^b	110.8	113.7	2.9	28.7
<i>ethion</i>	101.9	104.7	2.7	3.5
<i>procymidone</i>	99.6	102.0	2.4	5.2
<i>chlorpyrifos-methyl</i>	99.6	102.0	2.4	4.3
<i>cadusafos</i>	96.2	98.5	2.3	4.1
<i>chlozolinate</i>	99.3	101.5	2.2	4.1
<i>ethoprophos</i>	98.8	100.9	2.2	5.8
<i>pirimiphos-methyl</i>	99.9	102.0	2.1	3.7
<i>kresoxim-methyl</i>	100.0	101.6	1.7	3.2
<i>methidathion</i>	100.7	101.7	1.0	4.2
<i>dimethoate</i>	104.9	105.7	0.8	6.4
<i>vinclozolin</i>	103.4	104.0	0.7	4.8
<i>parathion-ethyl</i>	99.2	99.7	0.5	5.0
<i>permethrin</i>	102.0	102.3	0.3	4.3
<i>phosalone</i>	100.3	100.6	0.3	3.8
<i>isofenphos</i>	103.0	102.2	-0.8	5.2
<i>propargite</i>	98.9	97.9	-1.0	5.5
<i>parathion-methyl</i>	102.7	100.7	-2.0	7.9
<i>diazinon</i>	98.9	95.0	-3.8	2.6

^a Italic results indicate statistically significant differences between processing methods. Bold results indicate practically important differences between processing methods. ^b Subject to poor analytical precision.

GC-MS Measurements. Extracts of three of the 150 g analytical portions were analyzed singly on each of 5 days to provide 15 results for each pesticide—commodity combination for the ambient processing and cryogenic processing experiments.

After addition of a “syringe” standard (tetraphenylethylene) to correct volumetric errors associated with GC injection, the pesticide concentrations in the crude extracts were determined using capillary GC-MS detection. The mass selective detector was operated in selected ion monitoring mode, and GC injection was splitless (2 μL) at 250 °C using a glass liner fitted with a carbofrit insert. Quantification was carried out using multilevel, matrix-matched calibration standards (prepared fresh each day) bracketing the sample extracts. Parallel method recovery and blank control extracts were processed in an identical manner.

Accuracy of the Analytical Methods. Prior to the experiments on sample processing, the accuracy of the GC-MS method was determined by analyzing nine 30 g sample replicates spiked with pesticides at 100 $\mu\text{g}/\text{kg}$. The use of ¹⁴C-labeled chlorpyrifos restricted the choice of equipment for extraction and cleanup. A simple, low cost, rapid inline GC clean up procedure using a carbofrit insert in the GC liner provided a satisfactory solution. The method, which permitted the direct analysis of crude solvent extracts, minimized the possibility of time-dependent matrix-induced degradation of certain pesticides. All determinations

were made using multipoint, matrix-matched calibration standards, which bracketed the validation recovery extracts. The LSC measurement of ¹⁴C-labeled chlorpyrifos was validated in the same manner.

Assessment of Inhomogeneity between Analytical Portions; Statistical Methods. Estimates of between-sample (analytical portion) variation and analytical measurement variation (between-replicate) were gained by the analysis of variance (ANOVA) of results produced for each analytical portion mass on each day. Estimates were pooled across the 5 days to give estimates of between-sample variation for each analytical portion mass.

The between-sample variance of the analyte concentration may be expected to be inversely proportional to the analytical portion mass, which is equivalent to the standard deviation associated with between-analytical portion variation being inversely proportional to the square root of the analytical portion mass. Hence, a linear regression of between-analytical portion standard deviation and the reciprocal of the square root of the analytical portion mass were used to examine the relationship between analytical portion size and between-analytical portion variation.

Estimates of the minimum fit-for-purpose analytical portion size were gained by finding the analytical portion mass for which the between-analytical portion relative standard deviation was equal to $0.3 \times H_0$, where H_0 is the modified Horwitz RSD (I_2) for the concentrations of analyte present in the analytical sample (for concentrations less than 120 $\mu\text{g}/\text{kg}$, $H_0 = 22\%$). A value of $0.3 \times H_0$ was chosen because if the analytical relative uncertainty is fit for purpose (equivalent to the Horwitz RSD) then the effect of between analytical portion variation will be small (sampling variation expressed as a RSD combined with analytical uncertainty = $1.04 \times H_0$).

Uncertainty Associated with Analyte Stability; Statistical Methods. For each commodity (orange and tomato), the data set consisted of results of the measurement of pesticides spiked (each at an equivalent concentration of 100 $\mu\text{g}/\text{kg}$) onto the surface of commodity units of the laboratory sample prior to homogenization using ambient or cryogenic conditions. For each processing condition, three replicate GC-MS determinations (randomly selected from the extracts of the 5×150 g analytical portions) were carried out on each of five occasions. On each occasion, an estimate of the method recovery (proportion of pesticide recovered from a blank analytical portion spiked with a solution containing the same pesticides) was also produced. Results were also calculated using chlorpyrifos as an IDS.

ANOVA provided estimates of the size of variation associated with measurement and extraction; hence, using eq 1, the uncertainty associated with the difference between the mean of results produced by each analytical portion mass.

$$U = 2 \times \sqrt{\text{SE}(\text{ambient})^2 + \text{SE}(\text{cryogenic})^2} \quad (1)$$

where U is the uncertainty associated with the difference between mean results expressed as a 95% confidence interval, $\text{SE}(\text{ambient})$ is the standard error associated with the mean of ambient results, and $\text{SE}(\text{cryogenic})$ is the standard error associated with the mean of cryogenic results (the factor 2 converts the resulting standard error associated with the difference into a 95% confidence interval).

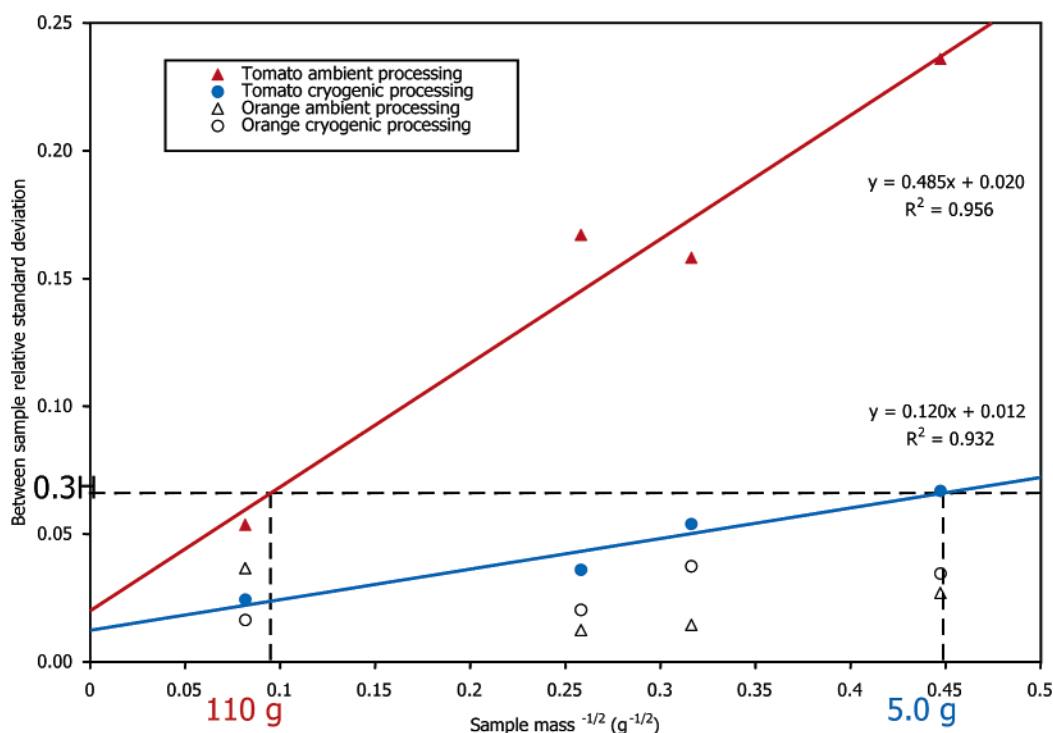
Results from parallel method recovery measurements were used to give an estimate of the effect of analytical bias on measurement results; hence, results corrected for this method recovery were generated and analyzed by ANOVA to give an estimate of the effect of sample processing that was unaffected by analytical bias.

Uncertainty Associated with Analyte Stability; Statistical Approach. A measure of the importance of measurement bias, introduced by factors such as analyte stability, can be made by estimating its effect on the confidence that the “true value” of a measurement result is within a certain interval. For example, for an unbiased measurement method that just meets the modified Horwitz fitness-for-purpose criterion, we can say that 95% of results lie within $\pm 44\%$ of true concentration. In other words, if, given a set of results of 100 $\mu\text{g}/\text{kg}$, we assert that the true value lies between 56 and 144 $\mu\text{g}/\text{kg}$, we will be right 95% of the time and wrong 5% of the time. One measure that we can use to estimate the importance of bias is the effect that it has on the number of times that we are wrong. For this study, bias was deemed to be

Table 3. Mean "Survival" Recovery and Variation Associated with the LSC Measurement of ^{14}C -Labeled Chlorpyrifos in Analytical Portions Taken from Comminuted Bulk Samples^a

mass (g)	ambient processing			cryogenic processing		
	average recovery (%)	between-analytical portion RSD ($n = 5$)	analytical (replicates) RSD ($n = 5$)	average recovery (%)	between-analytical portion RSD ($n = 5$)	analytical (replicates) RSD ($n = 5$)
			tomato samples			
5	98	0.236	0.059	79	0.027	0.016
10	101	0.158	0.016	77	0.014	0.013
15	97	0.167	0.026	79	0.012	0.012
150	100	0.054	0.013	77	0.037	0.011
			orange samples			
5	93	0.027	0.016	78	0.034	0.058
10	91	0.014	0.013	79	0.037	0.015
15	92	0.012	0.012	79	0.020	0.012
150	84	0.037	0.011	80	0.016	0.012

^a RSD, standard deviation/mean result.

**Figure 2.** Relation between sample (analytical portion) mass and between-analytical portion variation for 0.1 mg/kg ^{14}C -labeled chlorpyrifos in tomatoes and oranges.

important if it would lead to an additional 5% probability that the true value of a measured quantity was outside of the 95% confidence interval of an unbiased measurement. For conclusions based on a single measurement (using a method that just meets the modified Horwitz fitness for purpose criterion), a bias of 11% leads to an additional 5% probability of a "wrong conclusion". Hence, in this study, statistically significant differences that are also greater than 11% between processing methods are labeled as "differences"; smaller differences were not considered practically important and were ignored.

RESULTS AND DISCUSSION

Assessment of Inhomogeneity between Analytical Portions of Comminuted Tomatoes. Measurement results (^{14}C chlorpyrifos expressed as percentage survival recovery) and estimates of the variance associated with analysis and sampling for each analytical portion size are summarized in **Table 3**. The between-analytical portion RSDs were larger than the analytical RSD (replicate measurements) for tomatoes comminuted at ambient

temperature but were similar for tomatoes processed cryogenically. The relationship between analytical portion size and between-analytical portion relative standard deviation for the survival recovery of ^{14}C chlorpyrifos is illustrated in **Figure 2**. A linear regression analysis showed that between-sample standard deviation for analytical portions varied significantly with analytical portion mass tomatoes comminuted at both ambient ($p = 0.011$) and cryogenic ($p = 0.017$) temperatures, where p is the probability of observing this gradient "by chance" when there is no relationship between analytical portion variation and sample mass. The gradients of the regression lines were significantly different (ambient = $0.485 \text{ g}^{1/2}$, cryogenic = $0.120 \text{ g}^{1/2}$, $p = 0.021$) indicating that laboratory samples of tomatoes comminuted at cryogenic temperatures were more homogeneous than the laboratory samples of tomatoes processed at ambient temperatures. The estimates of minimum fit-for-purpose analytical portion size are 110 g for laboratory samples comminuted at ambient temperature and 5 g for laboratory samples com-

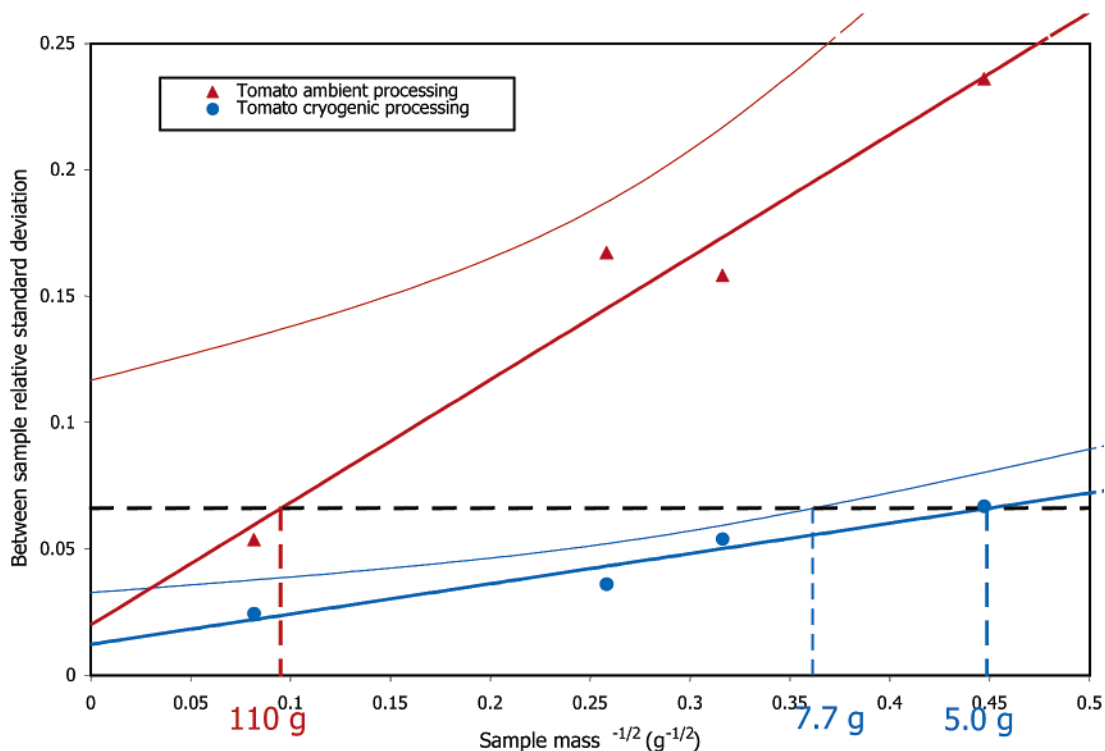


Figure 3. Uncertainty associated with fit-for-purpose sample (analytical portion) mass of tomatoes. Thick line, linear regression; thin line, upper 95% confidence of regression.

minuted cryogenically. Thus, conventional analytical portion sizes of 10–50 g, commonly employed, are not sufficient for tomatoes processed at ambient temperature.

The difference between the techniques is due to fact that the skins of the tomatoes separate from the pulp but, because of their elasticity, do not disintegrate during sample processing at ambient temperatures. This results in phase separation of the water layer and heterogeneous pulp and peel fraction. It is critical that the homogenized analytical sample is continually mixed during the withdrawal of analytical portions. In the cryogenic procedure, the laboratory sample is frozen before processing and dry ice is added. The addition of dry ice keeps the temperature during processing low, making the skins brittle and easier to disintegrate. The result is the production of a flowable powder and thus a more homogeneous analytical sample.

Figure 3 illustrates the uncertainty associated with the linear regression of between-sample standard deviation against the reciprocal of the square root of analytical portion mass.

The plot shows that the best estimate of a fit-for-purpose analytical portion mass for tomatoes processed cryogenically is 5 g. However, there is some uncertainty associated with the value of the regression parameters (gradient and intercept), which means that the true value of the minimum fit-for-purpose analytical portion mass may be higher. Hence, calculation of one-tailed upper 95% confidence intervals for the regression shows that, for tomatoes, an analytical portion of mass 7.7 g taken from a 2 kg analytical sample homogenized at cryogenic temperatures is fit-for-purpose. It was not possible to estimate the upper limit of the equivalent fit-for-purpose analytical portion size from a laboratory sample of tomatoes processed at ambient temperature because of the large uncertainty associated with the regression parameters.

These results are in good agreement with Maestroni et al. (13) who evaluated a variety of sample processing techniques

and reported that lettuce, carrot, and orange generally showed very good reproducibility as compared to tomatoes. It was suggested that the variety and ripening stage may be key factors affecting the uncertainty of sample processing, especially comminution at ambient temperature. In the case of tomatoes, the use of a double processing procedure (further homogenization of the homogenized analytical sample at ambient temperature) reduced the variability by a factor of 2–3 but did not take into account the likely increased uncertainty due to the instability of certain pesticides. The addition of dry ice during the processing of the fresh laboratory sample also reduced the variability by 2–3 times. The addition of dry ice to the frozen laboratory sample further reduced the uncertainty but to a lesser extent (a further 1.3-fold improvement as compared to the addition of dry ice to the fresh laboratory samples).

Assessment of the Inhomogeneity between Analytical Portions of Comminuted Oranges.

Measurement results (expressed as percentage survival recovery) and estimates of the variance associated with analysis and sampling for each analytical mass are shown in **Table 3**. The between-analytical portion RSDs (^{14}C chlorpyrifos) were similar to the analytical RSDs (replicate measurements) for oranges comminuted at both ambient and cryogenic temperatures. The relationship between analytical portion size and between-analytical portion relative standard deviation for the survival recovery of ^{14}C chlorpyrifos is illustrated in **Figure 2**. There was no statistically significant relationship between analytical portion mass and between-analytical portion standard deviation for ^{14}C chlorpyrifos ($p > 0.05$) in oranges for analytical portions taken from analytical samples comminuted either at ambient or at cryogenic temperatures. Between analytical portion variation was not statistically significantly different for analytical samples produced at ambient or cryogenic (ambient = 0.023, cryogenic = 0.027, $p > 0.05$) temperatures. The between-analytical portion variation was

below the fitness-for-purpose criterion of 0.3 Ho for all analytical portion masses (5, 10, 15, and 150 g).

Thus, comminution using either ambient or cryogenic conditions produced sufficiently homogeneous analytical samples, for the minimum analytical portion size (5 g) tested. Maestroni et al. (13) reported that the uncertainty of sample processing for oranges was normally very low and included in the 2% reproducibility of the analysis.

Accuracy of the GC-MS Analytical Method for the Determination of Pesticides in Tomatoes and Oranges. Calibrations for all analytes were generally linear over the range of interest, 0.02–0.25 $\mu\text{g mL}^{-1}$ (40–500 $\mu\text{g kg}^{-1}$ equivalent), with correlation coefficients > 0.980. Except for chlorothalonil in tomatoes, mean method validation recoveries (not corrected using IDS) were within the range 71–94% with coefficients of variation (% CVs) mostly below 8%. The mean validation recovery of chlorothalonil in tomatoes was 63% with an associated CV of 13%. The CVs for captan, dichlofluanid, and tolylfluanid in tomatoes were 15, 13, and 10%, respectively. The % CVs for azinphos-methyl, chlorothalonil, and folpet in oranges were 12, 11, and 10%, respectively.

Assessment of the Stability of Pesticides. The mean survival recovery results (corrected for chlorpyrifos IDS concentration) for the measurement of pesticides in ambiently and cryogenically processed tomatoes and oranges (nominally spiked with pesticides at 100 $\mu\text{g/kg}$) are summarized in **Tables 1** and **2**, respectively. The tables also include the mean differences between concentration of pesticides in the two sample processing procedures and the uncertainty associated with the mean difference value. In cases where the difference between the processing methods is statistically significant, the results are presented in italics. Where the differences are considered to be of practical importance (>11%), the results are presented in bold italics. Results from method recovery measurements (pesticides added to homogenized samples known not to contain pesticide residues) are not shown, but uncorrected mean method recoveries were generally between 90 and 110% with associated % CVs generally <10.

Stability of Pesticides during Comminution of Tomatoes. The IDS corrected results (survival recovery) shown in **Table 1** were much higher for cryogenically processed laboratory samples as compared to ambiently processed laboratory samples for a number of pesticides including captan, dichlofluanid, folpet, chlorothalonil, and tolylfluanid, which are unstable when processed at ambient temperature. However, the survival recovery of captan, chlorothalonil, imazalil, and ethoxyquin was significantly less than 100% for both sample processing conditions indicating that some degradation also occurred for these pesticides during cryogenic sample processing. By contrast, the results for dimethoate and pirimicarb were significantly greater than 100% suggesting that the differences between ambient and cryogenic processing are a result of imprecision of the method rather than real differences. For cryogenic processing, the results (survival recovery) for carbaryl were much lower (18 $\mu\text{g/kg}$) as compared to ambient processing, but the associated uncertainty of the analytical method was high, indicating that this difference was not practically significant.

Stability of Pesticides during Comminution of Oranges. The IDS corrected results (survival recovery) in **Table 2** show that there was no difference in the stability of most pesticides in oranges comminuted at either ambient or cryogenic temperatures. However, the survival recovery of dichlofluanid, chlorothalonil, tolylfluanid, dicloran, dichlorvos, bitertanol, azinphos-methyl, and deltamethrin were higher in laboratory samples

processed at cryogenic temperatures as compared to laboratory samples processed at ambient temperature. The survival recovery of biphenyl, chlorothalonil, dichlorvos, dichlofluanid, and etridiazole was significantly less than 100% for both sample processing conditions, indicating that some losses also occurred for these pesticides during cryogenic sample processing. The losses of biphenyl, dichlorvos, and etridiazole, irrespective of the sample processing conditions employed, suggest that an accurate determination of residues levels for the relatively volatile pesticides may not be possible until sample processing procedures to prevent volatilization can be devised.

Generally, the size of the difference between processing methods for results corrected for method recovery (spiked after comminution) was similar to the size of difference between processing methods for nonrecovery-corrected results. This indicates that differences are due to sample processing and not the determination steps.

Overall the survival recoveries (uncorrected for IDS) for the majority of pesticides were around 20% lower for tomato samples and 10% lower for orange samples processed cryogenically as compared to samples processed at ambient temperature as shown in **Figures 4** and **5**, respectively. The reason for the lower values could not be determined. Only a small percentage (approximately 2–3%) of ^{14}C -labeled chlorpyrifos remained on the tray and in the syringe after spiking so losses at this stage are unlikely. The CO_2 effluent during processing only contained low levels (0.5%) of the total amount of ^{14}C chlorpyrifos spiked. Gravimetric errors due to incomplete volatilization of CO_2 were discounted by checking the mass of sample after dissipation of CO_2 . The condensation of water on the cold surface (14) is unlikely to account for the apparent losses observed. The most likely explanation is that losses occurred during the evaporation of spiking solvent or during the freezing process, an unavoidable experimental artifact. It is unlikely that the pesticides applied in solvent (an artificial model) behave in the same way as field-incurred residues formed from a film or layer following application of pesticide formulations. Therefore, application of an organic solvent spiked with pesticides could possibly result in higher losses, due to volatilization, as compared to the use of formulated products, which have been developed for optimum surface adherence or penetration. Schmidt et al. (15) conducted experiments using radiolabeled pesticides and reported that losses up to 20% occurred during the spiking and freezing process, but no significant losses were observed during the cryogenic homogenization step.

The first conclusion to be drawn from this study is that the treatment of samples prior to extraction can have a large effect on both the variability of results and the bias associated with results. This uncertainty is often neglected in method validation studies.

Losses of a number of pesticides that occurred during ambient processing were not observed during cryogenic processing. If MRLs are set using results based on a cryogenic processing method and then enforced using results generated using ambient temperature processing, then the wrong conclusion could possibly be made with respect to the concentration of the pesticide residue in a sample as compared to the MRL. This is particularly the case for pesticides as compared to the monitoring of other contaminants because neither the results used to set pesticide MRLs nor the measurement results used to enforce them are corrected for recovery. Losses of the relatively volatile pesticides such as biphenyl and dichlorvos during both ambient and cryogenic processing procedures suggest that enforcement of MRLs for these pesticides is at least questionable and may

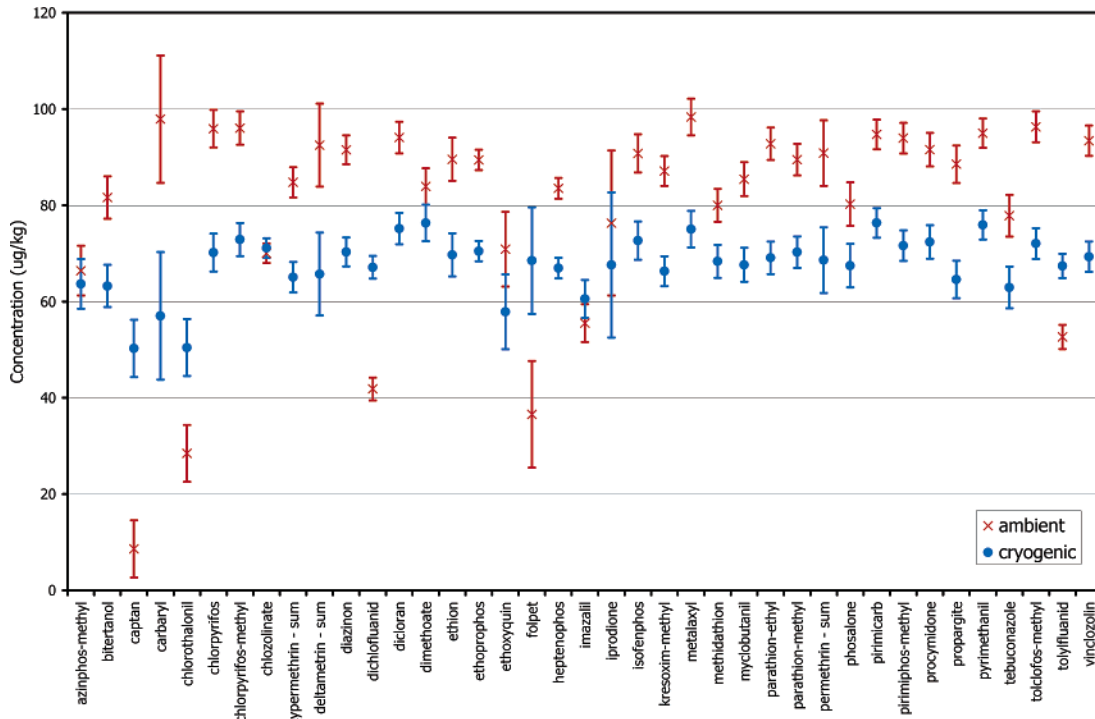


Figure 4. Differences between concentration [not corrected for IDS (chlorpyrifos)] of pesticides in analytical samples of tomato comminuted at ambient and cryogenic temperatures.

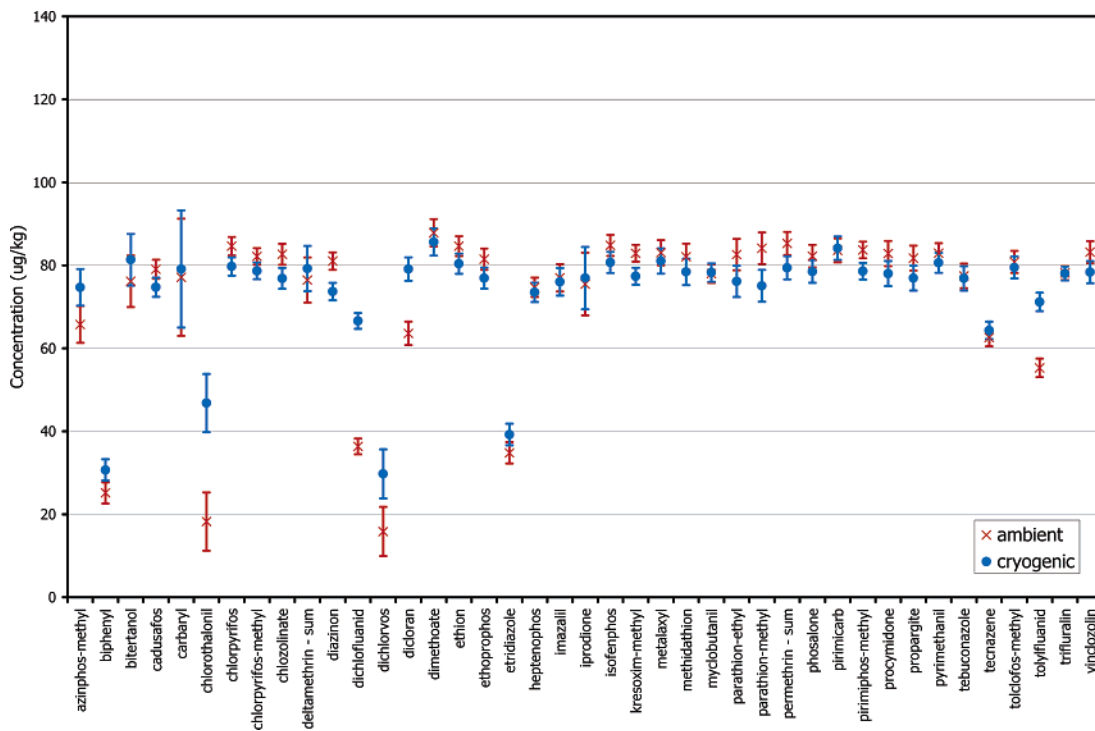


Figure 5. Differences between concentration [not corrected for IDS (chlorpyrifos)] of pesticides in analytical samples of oranges comminuted at ambient and cryogenic temperatures.

not be possible. To facilitate the correct interpretation of the results, all laboratory reports should indicate the processing procedure employed when reporting pesticide residue results.

Cryogenic processing provides improved homogeneity of analytical portions as compared to ambient processing, especially for commodities such as tomatoes. Sufficient homogeneity is critical for the successful implementation of smaller scale (≤ 10 g) extraction methods, which are becoming increasingly popular in pesticide residues analysis. Therefore, cryogenic processing

should be considered as the preferred method when using small-scale extraction methods.

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LITERATURE CITED

- (1) Codex Alimentarius Commission (CAC). *Recommended Methods of Sampling for the Determination of Pesticide Residues*; CAC, FAO: Rome, 1993; Vol. 2, pp 369–386.
- (2) Hill, A. R. C.; Harris, C. A.; Warburton, A. G. Effects of sample processing on pesticide residues in fruits and vegetables. In *Principles and Practices of Method Validation*; Fajgelj, A., Ambrus, Á., Eds.; Royal Society of Chemistry: Cambridge, United Kingdom, 2000; pp 41–48.
- (3) Lyn, J. A.; Ramsey, M. H.; Fussell, R. J.; Wood, R. Measurement uncertainty from physical sample preparation: Estimation including systematic error. *Analyst* **2003**, *128*, 1391–1398.
- (4) El-Biadoui, M.; Jarju, O. P.; Maestroni, B.; Phakaeiw Y.; Ambrus, Á. Testing the effect of sample processing and storage on the stability of residues. In *Principles and Practices of Method Validation*; Fajgelj, A., Ambrus, Á., Eds.; Royal Society of Chemistry: Cambridge, United Kingdom, 2000; pp 75–88.
- (5) Cuadros-Rodríguez, L.; Hernández Torres, M. E.; Almansa López, E.; Egea González, F. J.; Arrebola Liébanas, F. J.; Martínez Vidal, J. L. Assessment of uncertainty in pesticide multiresidue analytical methods: Main sources and estimation. *Anal. Chim. Acta* **2002**, *454*, 297–314.
- (6) Christensen, H. B.; Poulson, M. E.; Pedersen, M. Estimation of the uncertainty in a multiresidue method for the determination of pesticide residues in fruit and vegetables. *Food Addit. Contam.* **2003**, *20*, 764–775.
- (7) Alder, L.; Korth, W.; Patey, A.; Van Der Schee, H.; Schoene-weiss, S. Estimation of measurement uncertainty in pesticide residue analysis. *J. Assoc. Anal. Chem. Int.* **2001**, *84*, 1569–1578.
- (8) Ambrus, Á.; Solymosné, E. M.; Korsós, I. Estimation of uncertainty of sample preparation for the analysis of pesticide residues. *J. Environ. Sci. Health* **1996**, *B31*, 443–450.
- (9) Maestroni, B.; Ghods, A.; El-Bidaoui, M.; Rathor, N.; Ton T.; Ambrus, Á. Testing the efficiency and uncertainty of sample processing using ¹⁴C-labelled chlorpyrifos. Part I. description of the methodology. In *Principles and Practices of Method Validation*; Fajgelj, A., Ambrus, Á., Eds.; Royal Society of Chemistry: Cambridge, United Kingdom, 2000; pp 49–58.
- (10) Bettencourt de Silva, R. J. N.; Figueiredo, H.; Santos, J. R.; Filomena, M.; Camões, G. F. C. Evaluation of the analytical method performance for incurred samples. *Anal. Chim. Acta* **2003**, *485*, 241–252.
- (11) Fussell, R. J.; Addie, K. J.; Reynolds, S. L.; Wilson, M. F. Assessment of the stability of pesticides during cryogenic sample processing. 1. Apples. *J. Agric. Food Chem.* **2002**, *50*, 441–448.
- (12) Thompson, M. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. *Analyst* **2000**, *125*, 385–386.
- (13) Maestroni, B.; Ghods, A.; El-Bidaoui, M.; Rathor, N.; Jarju, O. P.; Ton, T.; Ambrus, Á. Testing the efficiency and uncertainty of sample processing using ¹⁴C-labelled chlorpyrifos: Part II. In *Principles and Practices of Method Validation*; Fajgelj, A., Ambrus, Á., Eds.; Royal Society of Chemistry: Cambridge, United Kingdom, 2000; pp 59–74.
- (14) Lichon, M. J.; James, K. W. Sample preparation for chromatographic analysis of food. *J. Assoc. Anal. Chem. Int.* **1990**, *73*, 820–825.
- (15) Schmidt, E.; Preu, M.; Sur, R.; Zimmer, D. Evaluation of losses during cryogenic sample homogenization with liquid nitrogen and dry ice. Poster presentation, 6th European Pesticide Residues Workshop, Corfu, Greece. 2006.

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