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Osman Tiryaki
Dilan Baysoyu

Estimation of sample processing uncertainty for chlorpyrifos residue in cucumber

O. Tiryaki (✉) · D. Baysoyu
Turkish Atomic Energy Authority,
Saraköy Nuclear Research and
Training Center, Application
Department, Agriculture Unit,
Istanbul Road 30 km,
06983 Saray, Ankara, Turkey
e-mail: osmantiryaki@yahoo.com
Tel.: +90-312-8154300
Fax: +90-312-8154307

Abstract Sample processing is a very important component of uncertainty in analytical results. In order to have reliable results, the laboratory sample should be properly processed to obtain statistically homogenous matrix—before the representative test portions are withdrawn for analysis. The use of ^{14}C-labeled compound is preferable because the analyte can be quantified without cleanup. The method is based on surface treatment of cucumber with ^{14}C-chlorpyrifos, determination of ^{14}C-chlorpyrifos activity in the replicate test portions of different size, and determination of the uncertainty of sample processing.

Keywords Sample processing · Uncertainty · Sampling constant

Introduction

Recent developments in pesticide residue analysis have focused on extraction, cleanup, and detection techniques. The effect of sample processing on the variability of the result gained very little attention of analysts, despite the fact that the accuracy and precision of the analytical result can be affected more by the sample processing technique than by subsequent analytical steps [1].

To obtain accurate and precise results, preparation of well-mixed material from the whole laboratory sample is very important. The solid samples can rarely be thoroughly homogenized. Therefore, the expression of "homogeneous material" should be used with care. The portion of the sample that is to be analyzed depends on the purpose of the analysis. The uncertainty of residue results are influenced by the variation of sampling, sample processing, and analysis [2, 3].

Sample processing is a procedure, such as cutting, grinding, and mixing, which is used to make the analytical sample acceptably homogenous with respect to the analyte distribution to removal of the analytical portion. The efficiency and uncertainty of sample processing are general requirements of method validation and internal quality control of a laboratory. Inhomogeneity of analytical sample may be the source of substantial systematic and random errors, which

cannot be estimated. To overcome this problem, each laboratory must strictly follow the appropriate instructions [4].

One reason of the uncertainty of the sample processing is the possible losses of pesticide during comminution and mixing of samples. Loss of pesticides at the sample processing and/or subsequent analytical steps will result in underestimates of the residue levels with implications for both MRL compliance monitoring and consumer risk assessment. It is clearly desirable to develop and adopt sample processing procedures that eliminate or at least minimize residue losses [1, 5].

In 1967 Youden [6] defined the overall random error of an analysis (S_R) as a function of the random errors at each stage of the analysis by:

$$S_R = \sqrt{(S_S)^2 + (S_{SP})^2 + (S_A)^2} \tag{1}$$

Equation (1) gives the overall error in terms of the variances contributed by the sampling (S_S), sample processing (S_{SP}) and analysis (S_A). The expression can be modified to incorporate additional stages. A method was elaborated for the estimation of sampling uncertainty and studied the sampling component by adopting the concepts of sampling constant [7].

Sampling constant (K_S) is the weight of a single increment (W) that must be withdrawn from a well-mixed material to hold the relative sampling (withdrawing and pro-

cessing) uncertainty, to 1% with 68% level of confidence. K_S can be determined from the Eq. (2) [2].

$$K_S = W \times CV_{SP}^2 \qquad (2)$$

W is the weight of analytical portion, and CV_{SP} is the coefficient variation of sample processing.

It is possible to determine with a high level of confidence whether a material is well mixed by analyzing two sets of increment of widely differing weight ($W_{Lg}/W_{Sm} \geq 10$), where W_{Lg} is the large portion size and W_{Sm} is the small portion size. If the laboratory sample matrix is well mixed, then K_S should be the same for small (W_{Sm}) and large (W_{Lg}) sample increments. Since the average residue concentration of the small and the large analytical portion is the same, the CV can be replaced with "S":

$$S_{Lg}^2 \times W_{Lg} = S_{Sm}^2 \times W_{Sm} \qquad (3)$$

or in terms of variance;

$$V_{SP\,Lg} \times W_{Lg} = V_{SP\,Sm} \times W_{Sm} \qquad (4)$$

For replicated analysis of small and large analytical portions, Eq. (4) can be applied to test if the homogenized laboratory sample is statistically well mixed. A two-tail F-test is used to determine whether $V_{SP\,Lg}$ and $V_{SP\,Sm}$ (W_{Sm}/W_{Lg}) differ significantly.

If the calculated ratio is smaller than the tabulated F-value, the material is well mixed and the K_S can be calculated with Eq. (2) from the CV and mass (W) of the large analytical portions, which are more precise than small ones.

For the estimation of uncertainty of sample processing, the use of ^{14}C-labeled compound is preferable because the analyte can be quickly quantified in the extract without cleanup. By eliminating the effects of the rest of the analytical procedure, the precision of the final results are significantly improved and the uncertainty of sample processing may be kept at $\leq 2\%$. For the same purpose, unlabeled pesticides can also be used, but their applications take much longer and the estimated uncertainty of sample processing may be less precise [7, 8].

The aim of this study was to determine the efficiency and the uncertainty of sample processing by using a radiolabeled compound. The methodology presented in this paper is taken from Ambrus et al. [2], who applied the sampling-constants concept for the analysis of pesticide residues.

Materials and methods

Chemicals

The standard chlorpyrifos-ethyl was obtained from Dr. Ehrenstorfer Laboratories GmbH, Germany, via the International Atomic Energy Agency (IAEA). ^{14}C-chlorpyrifos was also supplied by the IAEA. The cocktail used for liquid scintillation counting was dioxane basis scintillator (0.05 g
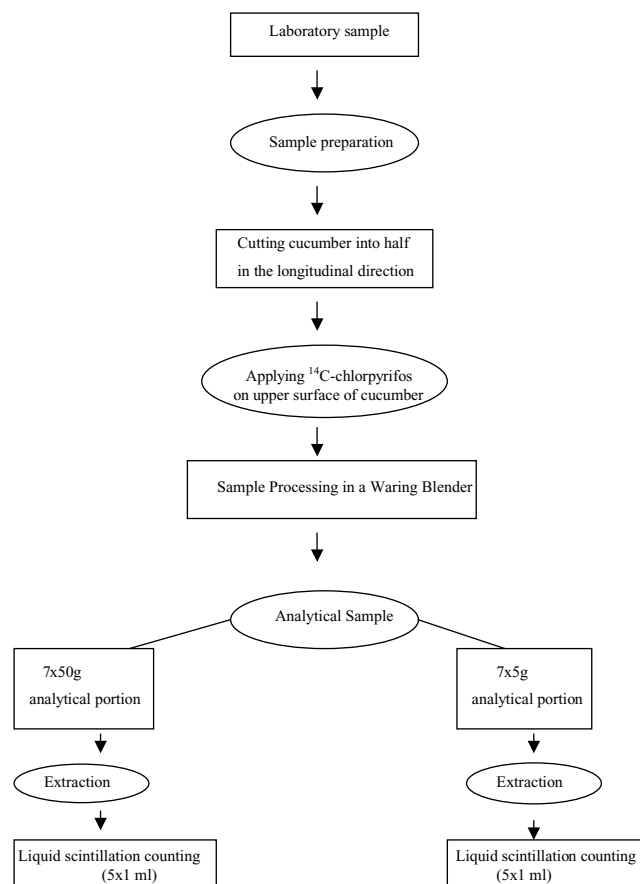
Fig. 1 Scheme of withdrawal of analytical portions from the processed analytical sample

POPOP+7 g PPO+100 g naphthalene in 1 L of dioxane) [9]. All other solvents and chemicals used were analytical grade from Merck.

Equipment

The following equipment was used to perform analysis: a Waring blender, 1-L stell containers (Waring Commercial Blender, USA), Ultra Turrax (T25 basic IKA-WERKE), centrifuge (Beckman Model TJ-6 Centrifuge), centrifuge tube up to 50 mL capacity, balance with the 0.0001 g digit, Packard 1550 Tri-Carb Liquid Scintillation Analyzer (LSC), polyethylene vials and other basic glassware and equipment such as measuring cylinders and Hamilton micro syringe.

Treatment and processing of sample

A 425-g sample of cucumber was weighed and cut into half in the longitudinal direction. The units with their cut surface were placed on a tray covered with clean aluminum foil. Then 1 mL of the ^{14}C-chlorpyrifos (2.5771×10^6 dpm mL^{-1} in ethyl acetate) standard solution was applied carefully on the upper surface of the cucumber to make sure that

Table 1 Recoveries determined in analytical portions of cucumber

Sample	R1	R2	R3	R4	R5	R (mean)	SR (SD)	SR² (Variance)	CV$_R$ (%)
5-g analytical portion									
1	0.87	0.86	0.85	0.86	0.86	0.859	7.298E-03	5.326E-05	0.850
2	0.99	0.99	0.99	1.00	0.99	0.998	3.151E-03	9.929E-05	0.316
3	0.94	0.94	0.94	0.94	0.94	0.94	2.495E-03	6.227E-06	0.265
4	1.03	1.03	1.02	1.03	1.03	1.031	1.444E-03	2.086E-06	0.140
5	1.08	1.09	1.09	1.08	1.08	1.084	3.993E-03	1.594E-05	0.368
6	0.92	0.92	0.92	0.92	0.92	0.921	1.991E-03	3.966E-06	0.216
7	0.88	0.88	0.88	0.88	0.88	0.880	2.532E-03	6.412E-06	0.287
50-g analytical portion									
1	0.97	0.97	0.97	0.98	0.97	0.972	3.612E-03	1.3E-05	0.371
2	0.86	0.86	0.86	0.86	0.86	0.861	1.382E-03	1.92E-06	0.161
3	0.87	0.88	0.88	0.87	0.87	0.874	3.760E-03	0.000014	0.429
4	0.83	0.84	0.83	0.83	0.84	0.834	3.724E-03	1.39E-05	0.446
5	0.89	0.89	0.89	0.88	0.89	0.890	4.159E-03	1.73E-05	0.467
6	0.87	0.88	0.88	0.87	0.89	0.883	9.841E-03	9.68E-05	1.114
7	0.90	0.90	0.90	0.89	0.89	0.895	5.409E-03	2.93E-05	0.604

the applied material did not run off from the surface. Sample materials were kept under a fume hood for 15 min to allow the pesticide to interact with the matrix and for some of the solvent to evaporate. Using sharp forceps, the units were placed in the bowl of the blender to avoid touching the treated part. To obtain homogenous material, the samples were processed with blender at ambient temperature and at low and high speeds for the determined times. Weighing a total of 425 g, four half longitudinal cucumbers was used for surface treatment. After subtracting the remaining radioactivity in the aluminum foil and syringe, applied specific activity for per g sample matrix was 23,968 dpm.

Extraction

Seven replicate analytical portions of 5-g and 50-g sample size were withdrawn from the blended sample; then 8.33 g and 0.83 g of NaHCO$_3$ were added to the 50-g and 5-g analytical portions, respectively, and mixed. Sodium sulphate and ethyl acetate also added to the sample at the ratio of 1:1 w/w and 2:1 v/w, respectively. The mixture was then extracted by using Ultra Turrax. Extractions were carried out at the same temperature and speed for all samples. The extracted material was centrifuged for 10 min, at 2,500 rpm.

The liquid part of the material in the tube was collected, and the volume and weight of extract recorded. Since the recovery was calculated based on weight, the mass of the sample matrix was recorded carefully, before and after each analytical step.

A schematic diagram of withdrawal of the analytical portions from the processed analytical sample is given in Fig. 1.

Measurement of radioactivity

Five 1-mL aliquot was pipetted to the LSC vial from each extract and the mass of the extract was recorded to an accuracy of 0.0001 g. By adding liquid scintillation cocktail, they subjected liquid scintillation counting for determination radioactivity. The recovery is calculated as the ratio of the measured and applied radioactivity.

Results and discussion

The recoveries of ^{14}C-chlorpyrifos from the analytical portions of surface treated cucumber are shown in Table 1. The recovery, average recovery, standard deviation (SR), variance (SR²), and coefficient variation (CV) of five repli-

Table 2 Testing well mixed conditions of processed cucumber samples and summary of calculations

Statistical parameter		Analytical portion (g)	
Symbol	Formula/explanation	50	5
R %	Mean recovery	0.887	0.959
V_T	S_L^2	1.649E-03	5.950E-03
V_A	S_A^2	2.662E-05	1.397E-05
CV$_A$ %	$100[(V_A)^{0.5}]/R$	0.581	0.389
$F_{calculated}$*	V_T/V_A	61.92	425.77
V_{SP}	V_T-V_A	1.622E-03	5.936E-03
CV$_{SP}$ %	$100[(V_{SP})^{0.5}]/R$	4.539	8.033
$F_{calculated}$**	$(V_{SP\ 50}/V_{SP\ 5})\ 10$	2.73	
$K_{S,\ 50}$	$W(CV_{sp})^2$	1.03***	

*$F_{tab(0.05;\ 34/28)}=1.846$

**$F_{tab(0.1;\ 6/6)}=4.28$

***kg

cate LSC measurements based on each analytical portion's replications is included in Table 1 for the 5 and 50-g analytical portions.

The calculated CV_A of analysis was less than 2% for both analytical portions (Table 2), which shows that handling of the analytical portions, including extraction and liquid scintillation counting, was carried out properly.

Table 2 also contains a summary of the calculations. V_T is the variance of all recoveries by taking all data as a single sample with mean R. V_A is the variance of analysis as the average of the variances of each analytical portion.

The tabulated F-values for checking the differences of the variations of V_T [variance of all recoveries with the $(7\times5)-1=34$ degrees of freedom] and V_A [variance of analysis with the $7\times(5-1)=28$ degrees of freedom], and large and small samples were $F_{(0.05, 34/28)}=1.846$, and $F_{(0.1; 6/6)}=4.28$, respectively [10].

A one-tailed F-test was applied at 95% confidence level, as it is shown in Table 2. Calculated F-values, i.e., the ratio of V_T/V_A, were 425.77 and 61.92 for the small and large portions, respectively. Since the $F_{calculated} > F_{tabulated}$ for both analytical portions, V_T is larger than V_A. If $V_T > V_A$ then

$$V_{SP} = V_T - V_A \tag{5}$$

Applying Eq. (5), the estimated variance of sample processing (V_{SP}) for small and large portion size are given in Table 2. A two-tail F-test at 90% confidence level was used to check that $V_{SP\ Lg}$ and $V_{SP\ Sm}$ (W_{Sm}/W_{Lg}) were not significantly different. The calculated F-value was 2.73, which was less than the tabulated value of 4.28. It means the analytical sample was well mixed and sampling constant (K_s) was calculated from the CV and mass (W) of the large analytical portions with the Eq. (2) as 1.03 kg (Table 2).

The determined K_S-value for the Waring blender is in agreement with the reported experimental K_s ranges (0.1–1.3 kg), which is indicated by Meastroni [11].

The value of K_s can be used to select the test portion size that assures a target level of CV_{SP}, which fits the purpose of the analysis. Similarly, the uncertainty of sample processing (CV_{SP}) can be estimated for any analytical portion size (W), from the K_s-value. The K_S can also be used to select the test portion size that assures a target level of CV_{SP}, which fits the purpose of the analysis.

The efficiency of sample processing depends on the equipment used and the type of processed matrix. It may also depend on the variety and maturity of the commodity. Each laboratory should check the homogeneity and the efficiency of sample processing, which cannot be derived from the literature or from other laboratories. Eventually, the efficiency of sample processing should become a routine internal quality control check [8].

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