Evaluation of the sampling procedure adopted by the EU pesticide residue control programme to assess consumer exposure to high acute toxicity pesticides – methamidophos in sweet peppers

Abstract
Food chemical control, particularly control involving the analysis of pesticide residues in fruits and vegetables, is routinely performed on composite samples. This sample design is adequate for the control of the majority of pesticides which have only long-term effects. However, from the viewpoint of consumer risk assessment, this sample design is inadequate for pesticides with relevant acute toxicity, when their residues occur in large food items the consumption of which may represent an important fraction of a single meal or an important fraction of the consumption in 24 h. This work presents the results of the Portuguese official pesticide residue control authority concerning the variability of methamidophos concentration in sweet pepper units, collected from the market, in the framework of the EU 1999 co-ordinated programme for the official control of pesticides in products of vegetable origin. The results were expressed with uncertainty and the conclusion was that the observed dispersion cannot be allocated to the method precision. The studied samples presented a variability factor (ratio between the maximum and the mean concentration of the units) ranging from 1.8 to 3.5 supporting the need to consider a variability factor representing the potential unit-to-unit variation in residues concentration for the purpose of consumer risk assessment.

Keywords Sampling · Sample heterogeneity · Uncertainty · Pesticides · Residues · Vegetables

Introduction
Food safety regarding pesticide residues in products of vegetable origin and compliance with legal limits have been controlled by governmental authorities through the detailed evaluation of phytosanitary practices and by the regular conduction of inspection programmes. Such programmes can only be effective if all the important combinations commodities/analytes are covered, if the extension and frequency of action is adjusted to the consumption and, last but not least, if the sample is representative and adequate to the assessment of residues exposure through food intake.

Normally, due to the fact that the great majority of pesticides only present potential long-term effects, laboratory samples (composite samples) are designed in such a way as to establish the risk of long-term exposure, i. e. of chronic toxicity. When the residues have a relevant acute toxicity, the established laboratory sampling procedure can be unsatisfactory. This problem increases with the heterogeneity of lots and special consumption behaviour. The consumption of single large units or large portions of the food product, representing a large part of the meal weight, reflects the more critical situation. The routine analysis of individual food items is however too demanding and not practicable.

The EU 1999 co-ordinated programme for the official control of pesticides in products of vegetable origin [1] included the unit-to-unit analysis of methamidophos content in sweet peppers, every time a composite parallel
sample, previously analysed, revealed the presence of residues of this insecticide. This evaluation aimed at the establishment of a variability factor capable of estimating the heterogeneity of residues in individual items in market lots, therefore providing a safer basis for the estimation of consumer exposure when high acute toxicity pesticides are involved.

This work presents the results on the heterogeneity of methamidophos content in sweet peppers obtained by the official control authority in Portugal. The sample results were presented with uncertainty in order to objectively distinguish the sample heterogeneity from the analytical method precision. The uncertainty estimation was based on a “bottom-up” [2] approach which took into account the knowledge of the performance of all analytical steps, including the sample processing and the mass transfer steps.

Methamidophos is a highly polar insecticide and when analysed by a multi-residue method (MRM) which includes a large number of non-polar and moderately polar compounds, its performance parameters (recovery and precision) can be significantly different from the average. Therefore, the expression of results with uncertainty is even more relevant in this borderline case.

A MRM included in a CEN standard [3] was used for the determination of methamidophos in sweet peppers. The instrumental quantification was performed by GC-FPD.

Methodology for the expression of results with uncertainty

Description of the analytical method

The sample (a single unit or a composite sample of ten sweet peppers) was minced in a food cutter (Hobart, Troy, Ohio, USA) and homogenised (sample processing, SP). An analytical portion was weighted, m, (50.0±0.1 g) and extracted with 100 ml ethyl acetate and 60 g of sodium sulphate in a macerator (IKA, Ultra-Turrax T25; Janke and Kunkel, Staufen, Germany). The raw extract was filtered under reduced pressure and the filtrate measured, V1. A fraction of this volume, V1-V2, where V2=V1/2 is the remaining volume, was evaporated on a rotary evaporator (Büchi, Flawil, Switzerland) and the dry residue redissolved in 10 ml, V3, of a mixture of cyclohexane/ethyl acetate (1+1, V/V). A 5 ml, V4, aliquot of this solution was purified by gel permeation chromatography (GPC) (Latek, Eppelheim, Germany) column (580 mm x 25 mm id; Bio-Beads S-X3, Bio-Rad, Hercules, Cal., USA) and the eluted fraction was evaporated to just dryness in a rotary evaporator; the remaining was dissolved in 5 ml, V5, of ethyl acetate before the quantification by GC-FPD (Gas-chromatography with flame photometric detector) (Varian, Palo Alto, Cal., USA) 3400 chromatograph with a phenyl (5%) methylsiloxane column (30m x 320µm id, 0.50µm film thickness) aiming at the estimation of the concentration Cinter of the final solution (Fig. 1).

Hence, the concentration of the sample content (SC) uncorrected for the accuracy of the involved analytical steps was estimated by Eq. (1):

\[
SC(\text{mg kg}^{-1}) = \frac{C_{\text{int}}(\text{ng µl}^{-1}) \times V_1(\text{ml}) \times V_3(\text{ml}) \times V_5(\text{ml})}{m(\text{g}) \times [V_1 - V_2](\text{ml}) \times V_4(\text{ml})}
\]  

The concentration of the sample content corrected for the analytical steps accuracy (CSC) was estimated by Eq. (2):

\[
CSC(\text{mg kg}^{-1}) = SC(\text{mg kg}^{-1}) \times F_{SP} \times F_{MTS}
\]

where FSP is the sample processing (SP) correction factor and FMTS is the combined mass transfer step (MTS) (extraction, filtration, evaporation and clean-up procedure) correction factor; FSP and FMTS represent the values capable of correcting the sample content for the SP and MTS accuracy, respectively (inverse of the respective mean recoveries).

Identification of the sources of uncertainty

The analytical method is divided in steps which involve sources of uncertainty described by well-established models (WM) (gravimetry, volumetries and instrumental quantification steps, after a careful validation of the model used to describe the calibration curve) [4] and steps involving sources of uncertainty that lack describing models (LM) (SP and MTS) (Fig. 1).

Quantification of sources of uncertainty described by WM

Gravimetric step

The gravimetric step uncertainty, u_m, includes the uncertainty associated with the calibration of the balance and its repeatability [2, 5, 6].

Volumetric steps

The volumetric step uncertainty results from the combination of the uncertainty associated with the volumetric material manufacture, the repeatability of the manipulation by the operator and the uncertainty associated with the effect of temperature [2, 5, 6]. The last component is not significant when consecutive volumetries at the same temperature are involved (namely for volumetries V1 and...