Rome, 28 - 31 May 2002

Ministério da Agricultura, do Desenvolvimento Rural e das Pescas

DGPC
Direcção-Geral
de Protecção das Culturas

ROUTINE MEASUREMENT UNCERTAINTY QUANTIFICATION IN PESTICIDE RESIDUE ANALYSIS

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The result of a measurement without the involved uncertainty is only really useful for analysts familiarised with the analytical method, and even in this case, only when the result is far from the decision limit. The measurement uncertainty is essential for the objective evaluation of the collected information. However, the application of this concept to analytical chemistry in not straightforward, specially for complex and large scope analytical methodologies. The analysis of pesticide residues in foodstuffs, specially when a high number of pesticide/ matrix combinations are involved, represents a challenge to achieve uncertainty estimates.

This work presents a methodology for the quantification of worst case measurement uncertainty, which combines the Eurachem Guide (1) principles with the recommendations concerning recovery performance in the EU Guidelines for Pesticide Residues Analysis (2). This methodology involves very simple daily routine calculations and can be based on the detailed evaluation of the analytical method or on conventions to describe the more complex analytical steps. It involves the estimation of maximisation values for the different sources of uncertainty and requires the definition of limiting values for certain analytical parameters. The simplification of the instrumental quantification uncertainty estimation involves the use of the standard deviation obtained from control charts relating to the concentrations estimated, from the calibration curves, for control standards at the highest calibration level. The use of the linear unweighted model for heteroscedastic data is discussed. Three levels of simplification are suggested, in alternative to the detailed approach (3), which can be selected according to the proximity of the sample results to the decision limits. These approaches are applied to the determination of pesticide residues in apples (CEN, EN 12393), and the most simplified one showed a relative expanded uncertainty of 37.2% for a confidence level of approximately 95%.

1. EURACHEM Guide: Quantifying Uncertainty in Analytical Measurement, http://www.eurachem.bam.de/guides/quam2.pdf, 2nd Ed., 2000.
2. European Commission, Quality Control Procedures for Pesticides Residues Analysis – Guidelines for Residues Monitoring in the European Union, 2nd Edition, Document No. SANCO/3103/2000, Brussels, 2000.
3. R. J. N. B. Silva, M. J. Lino, J. R. Santos, M. F. G. F. C. Camões, Analyst, 125, 1459-1464, 2000.

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Table 1 Glossary of abbreviations and symbols.

C_{IP} – sample extract interpolated concentration;

Cmb – see equation in Table 4;

CSC – sample content corrected for analytical steps accuracy;

F_{MTS} and u_{MTS} – mass transfer steps accuracy correction factor and respective standard uncertainty;

FRu – final result relative standard uncertainty;

F_V – dilution factor;

G&V – combination of gravimetry with volumetries;

GPC – Gel permeation chromatography;

HCL – highest calibration level;

IP – instrumental interpolation;

IPRu – instrumental interpolation relative standard uncertainty;

LCL – lowest calibration level;

LuW – linear unweighted model;

LW – linear weighted model;

m – analytical portion mass;

max – maximum value;

MTS – mass transfer steps (combination of extraction, filtration, evaporations and clean-up procedure);

P – analyte standard purity;

Ru – relative standard uncertainty;

Simp. 1 to 3 – simplification level 1 to 3;

SC – sample content uncorrected for analytical steps accuracy;

STD – standard preparation and purity;

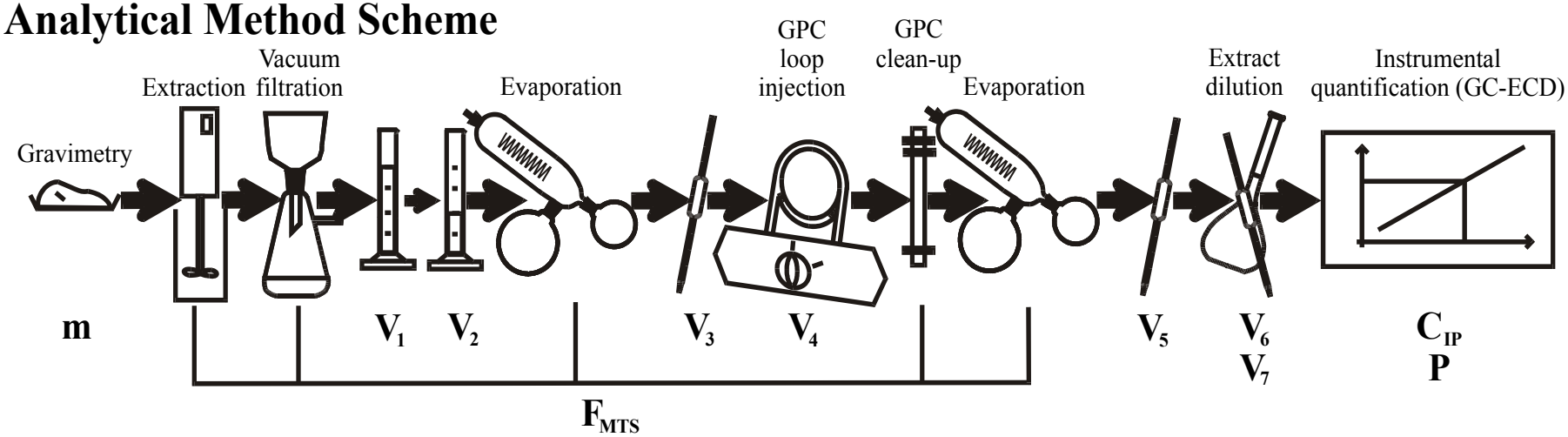
u_{CC} – standard uncertainty derived from control charts relating to the interpolated concentration, through the linear unweighted model, of control standards with a concentration equivalent to the HCL;

u_i – standard uncertainty associated to the parameter in subscript i;

u_{STD}/F_{STD} – combined relative standard uncertainty of the calibration standard preparation and purity;

V_1 to V_9 – analytical method volumetries.

Analytical Method Scheme



Equations

$$SC = \frac{V_1 \times V_3 \times V_5 \times V_7 \times C_{IP} \times P}{m \times [V_1 - V_2] \times V_4 \times V_6} \quad (1)$$

$$CSC = SC \times F_{MTS} \quad (2)$$

Table 2 Analyte dependent estimations involved at the quantification of measurement uncertainty.

	Detailed approach				Simplified approach					
	Standard preparation & purity, STD	Mass transfer steps, MTS			Instrumental interpolation, IP				Combination STD&MTS*	Combination STD&MTS&IP*
Analyte	(u_{STD}/ F_{STD})	F_{MTS}	u_{MTS}	Analytical range (µg kg^{-1})	u_{CC} pg µl^{-1}	(u_{CC}/LCL) %	(u_{CC}/HCL) %	Calibration range (pg µl^{-1})	($u_{STD\&MTS}$/ $F_{STD\&MTS}$)	($u_{STD\&MTS\&IP}$ $F_{STD\&MTS\&IP}$)
gamma-clordane	0.014	1.206	ns	5.6-120	0.114	11.4	2.9	1-4	0.014	0.12
deltamethrin	0.0098	1.257	ns	58.8-1260	1.38	13.1	3.3	10.5-42	0.0098	0.13
dicloran	**0.014**	1.278	0.082	4.2-90	0.0552	7.4	1.8	0.75-3	0.066	0.099
alpha-endosulfan	0.011	1.26	0.10	7-2000	0.208	**16.6**	**4.2**	1.25-5	0.080	**0.18**
beta-endosulfan	0.011	1.193	ns	8.4-2000	0.163	10.9	2.7	1.5-6	0.011	0.11
endosulfan sulfate	0.011	1.237	0.034	12.6-270	0.242	10.8	2.7	2.25-9	0.030	0.11
fenchlorphos	0.011	1.216	0.036	9.8-210	0.191	10.9	2.7	1.75-7	0.032	0.11
fenvalerate	0.0098	**1.139**	**0.094**	57.4-1230	0.997	9.7	2.4	10.25-41	**0.083**	0.13
iprodione	0.0097	1.326	0.053	88.2-1890	1.76	11.2	2.8	15.75-63	0.041	0.12
permethrin	0.0096	1.166	ns	130.2-2790	2.02	8.7	2.2	23.25-93	0.0096	0.087
tetradifon	0.011	1.208	ns	15.4-330	0.265	9.6	2.4	2.75-11	0.011	0.097
vinclozolin	0.011	1.320	0.078	9.8-2000	0.169	9.7	2.4	1.75-7	0.060	0.11
Ru (max)	**0.01427**	**0.08253**				**0.1661**	**0.04153**		**0.08310**	**0.1844**

$F_{STD\&MTS\&IP}=F_{STD}\times F_{MTS}\times C_{IP}$; ns - not significant (combination of all the other relevant sources of uncertainty statistically equivalent to the observed dispersion of results); * - combined as relative standard deviations.

$$\frac{u_{STD\&MTS\&IP}}{F_{STD\&MTS\&IP}}=\sqrt{\left(\frac{u_{STD}}{F_{STD}}\right)^2+\left(\frac{u_{MTS}}{F_{MTS}}\right)^2+\left(\frac{u_{CC}}{LCL}\right)^2}$$

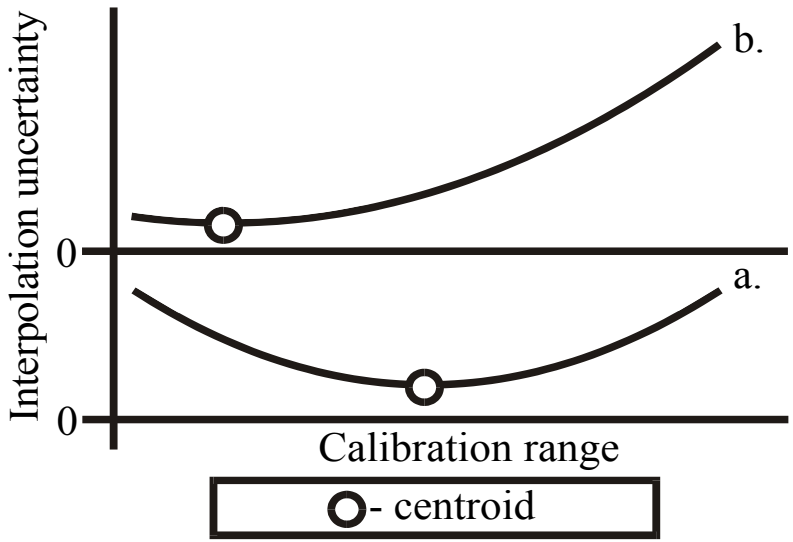


Fig. 1. a) Interpolation uncertainty over the concentration for the linear unweighted model. The minimum is in the middle of the calibration curve, centroid, and the maxima at the highest and lowest concentrations.
b) Interpolation uncertainty over the concentration for the linear weighted model (example with instrumental response with increasing variance with the concentration). The minimum is in the lower fraction of the calibration curve and the maximum at the highest concentration of the calibration range.

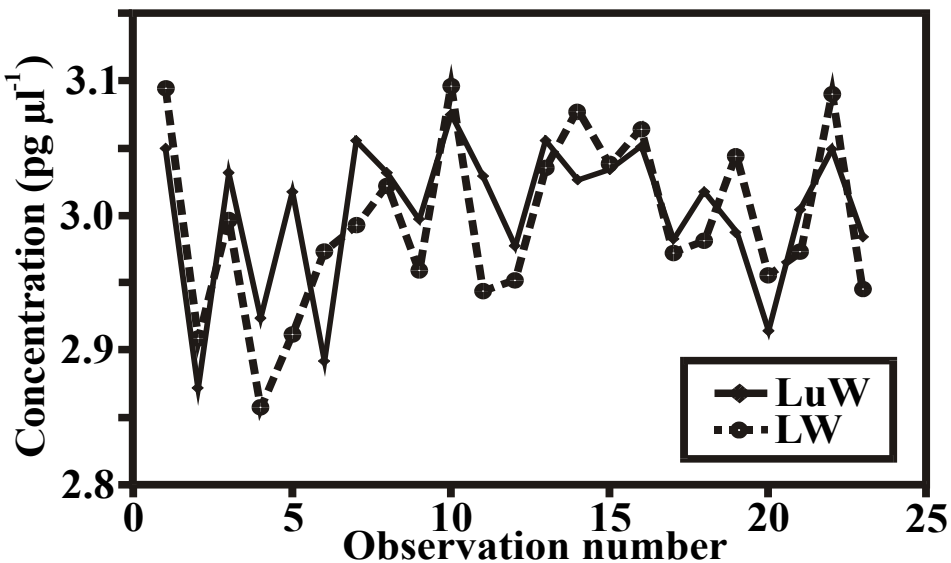


Fig. 2. Overlapping of two control charts of dicloran for the instrumental quantification involving the interpolation of a control standard with a concentration equivalent to the highest calibration level, HCL, considering the use of the linear unweighted model, LuW, and a calibration curve, CalC, described by two replicated readings of four concentration levels and considering the linear weighted model, LW, and a CalC described by four replicated readings of four concentration levels.

Table 3 Division of the analytical steps in types and procedures for estimating the value that maximises the involved sources of uncertainty.

Type of analytical step	Eqn. 1 and 2 parameters	Uncertainty sources description and maximisation	Maximisation/ Ru
Analyte dependent steps: STD, MTS and IP	F_{STD}, F_{MTS} and C_{IP}	(u_{STD}/F_{STD})*: function of STD purity and preparation; maximum: 0.01427 (dicloran); (u_{MTS}/F_{MTS})*: constant over the analytical range; function of the analyte; maximum: 0.08253 (fenvalerate); u_{IP}: complex function of concentration; maximised by u_{CC}; u_{CC}/LCL* maximum: 0.1661 (alpha-endosulfan); Combination of u_{STD}, u_{MTS} and u_{IP} $(u_{STD\&MTS\&IP}/F_{STD\&MTS\&IP})$*: maximised by alpha-endosulfan value.	$(u_{STD\&MTS\&IP}/F_{STD\&MTS\&IP})(max)$= =0.1844 or combination of $(u_{STD\&MTS}/F_{STD\&MTS})(max)$= =0.08310 with (u_{CC}/C_{IP}) or $[(HCL\times(u_{CC}/HCL)(max))/C_{IP}]$
Gravimetry	m	$m \geq 50g$ and performance of the balance.	$(u_m/m)(max)=0.0003024$
Volumetry	$V_{1;2}$= $=V_1/(V_1-V_2)=2$	$V_1 \geq 110ml$, $V_2=V_1/2$ and characteristics of the volumetric material.	$(u_{V1;2}/V_{1;2})(max)=0.006819$
	V_3, V_4 and V_5	Constant values.	$(u_{V3}/V_3)=0.002807$; $(u_{V4}/V_4)=0.01182$; $(u_{V5}/V_5)=0.003446$.
	single dilution $V_{6;7}=V_7/V_6$ or consecutive dilutions $V_7/V_6 \rightarrow V_9/V_8$ $V_{6..9}$= $=(V_7\times V_9)/(V_6\times V_8)$	Dilutions $[V_i/V_f]$ (initial volume, V_i, diluted in the final and independently measured volume, V_f) involving pipets of 0.5, 1, 2, 5 and 10ml and volumetric flasks of 5, 10, 25, 50 and 100ml: maximisation for a dilution of 0.5/5. Dilutions $[V_i/(V_i+V_f)]$(initial volume, V_i, diluted by the addition of the volume V_f - same solvent) involving pipets of 0.5, 1, 2, 5 and 10ml: maximisation for a dilution of (0.5/(10+0.5)).	single dilution $(u_{6;7}/V_{6;7})^f(max)=0.01201$ $(u_{6;7}/V_{6;7})^{i+f}(max)=0.01022$ or two consecutive equivalent dilutions $(u_{6..9}/V_{6..9})^f(max)=0.01698$ $(u_{6..9}/V_{6..9})^{i+f}(max)=0.01445$
	Combination of volumetric operations, F_V (dilution factor)	Combination considering one dilution $V_{6;7}$, (u_{FV}/F_V)(1dil), or two consecutive equivalent dilutions $V_{6..9}$, (u_{FV}/F_V)(2dil) (this ratio is function of the type of the involved dilutions – superscripts f or i+f).	$(u_{FV}/F_V)^f(1dil)(max)=0.01871$ $(u_{FV}/F_V)^{i+f}(1dil)(max)=0.01762$ $(u_{FV}/F_V)^f(2dil)(max)=0.02224$ $(u_{FV}/F_V)^{i+f}(2dil)(max)=0.02036$

* - see Table2.

Interpolation in the calibration curve

- **Maximum interpolation uncertainty at the highest calibration level, HCL; (when instrumental response variance increases or is constant with concentration).**
- **Estimated independently from day-to-day performance variation from control charts involving a control standard equivalent to the HCL (regressions based on unweighted models independently from the homogeneity of variances).**

Table 4 Relative standard uncertainty estimated for the final result, FRu, considering the level of simplification of the uncertainty estimations (Simp. level) and several types of dilutions of the sample extract.

Simp. level	Estimation of the source of uncertainty, Ru			Extract dilution	FRu u_{CSC}/CSC or u_{SC}/SC
	STD&MTS	IP	G&V		
1					$\sqrt{Cmb+\left(\frac{u_{CC}}{C_{IP}}\right)^2}$
	$(u_{STD\&MTS}/F_{STD\&MTS})(max)=0.08310$	(u_{CC}/C_{IP})	0.01872	1dil (f)	Cmb=0.007257
	"	"	0.01762	1dil (i+f)	=0.007217
	"	"	0.02224	2dil (f)	=0.007401
	"	"	0.02037	2dil (i+f)	=0.007321
2					$\sqrt{Cmb+\left(\frac{HCL\times 0.04153}{C_{IP}}\right)^2}$
	"	$[(HCL\times(u_{CC}/HCL)(max))/C_{IP}]$		1dil (f)	Cmb=0.007257
	"	"		1dil (i+f)	=0.007217
	"	"	"	2dil (f)	=0.007401
	"	"		2dil (i+f)	=0.007321
3	$(u_{STD\&MTS\&IP}/F_{STD\&MTS\&IP})(max)=0.1844$			1dil (f)	0.1854
	"			1dil (i+f)	0.1853
	"		"	2dil (f)	0.1858
	"			2dil (i+f)	0.1856

G&V - combination of the sources of uncertainty relating to gravimetric and volumetric operations; 1dil - one dilution of the sample extract; 2dil - two consecutive dilutions of the sample extract; f - dilution type (V_i/V_f); i+f - dilution type $(V_i/(V_i+V_f))$;$(u_{CC}/HCL)(max)=0.04153$.

$$Cmb=\left(\frac{u_m}{m}\right)^2+\left(\frac{u_{FV}}{F_V}\right)^2+\left(\left(\frac{u_{MTS\&STD}}{F_{MTS\&STD}}\right)(max)\right)^2$$

- **Simp. 3: All analyte dependent sources (MTS&STD&IP) are combined and maximised (Final result relative standard uncertainty, FRu, of 0.18);**
- **Simp. 2: Maximisation of the combination of MTS and STD (MTS&STD) and u_{IP} is estimated from the maximum relative interpolation uncertainty of the analytes;**
- **Simp. 1: Maximisation of the combination of MTS and STD (MTS&STD) and u_{IP} is estimated from the maximum relative interpolation uncertainty of each analyte.**

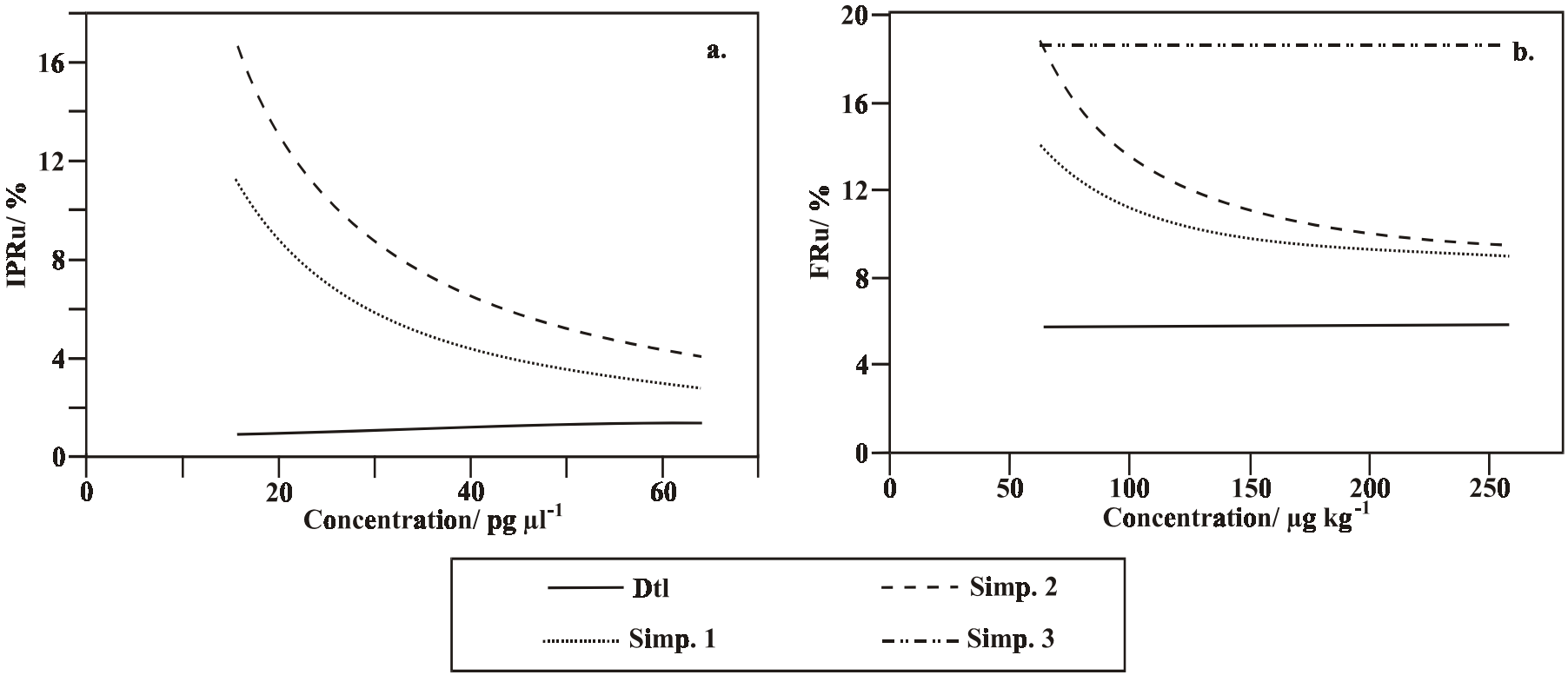


Fig. 3 Comparison of the uncertainty estimation performed by the detailed (Dtl) and simplified approaches (Simp.1, 2 and 3) for the determination of iprodione in apples - dilution of the sample extract 1/10ml. a) Instrumental interpolation relative standard uncertainty, IPRu, over the calibration range; b) Final result relative standard uncertainty, FRu, over an analytical range correspondent to the calibration range (extract dilution 2dil (f)(Table 4)).

Conclusions - For the presented example (pesticide residues in apples) and developed uncertainty budget, the approach with highest simplification level, Simp.3, can be applied to sample results differing by more than 37.2% from the decision limits producing conclusive comparisons. Fortunately, this is the most common situation in the enforcement of pesticide residues in foodstuffs. Each time this situation is not met, another simplified approach, or the detailed one can be used from the collected data. According to the obtained results, the difference between Simp.1 and Simp.2 is not significant enough to justify the use of the most laborious Simp.1 approach (the estimated (uCC/HCL) values (Table 2) are very similar).

All the presented simplified approaches involve very simple routine calculations and are therefore well fitted to the daily work, and can be either based on the detailed description of the analytical method performance or on the definition of conventional values for certain sources of uncertainty in agreement with simpler quality control criteria.

The presented methodology can be applicable to other analytical methods, including or not, an instrumental quantification step, when the estimated uncertainty allows conclusive comparison between sample results and decision limits.

Acknowledgements - The authors are very grateful to Mrs. Lina Teixeira for the professionalism and dedication demonstrated during collaboration in this work, also supported by Mrs. Isabel Paula, Joaquina Ribeiro and Regina Santos.