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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: PS2518

2. Project title:
   PS 2518. Uncertainty Developing a Low Cost Model for the Measurement of Uncertainty in Pesticide Residue Analysis

3. Contractor organisation(s):
   Central Science Laboratory
   Sand Hutton
   York

   YO41 1LZ

4. Total Defra project costs: £ 21,755

5. Project: start date ..... 01 April 2004

   end date ..... 31 May 2005

6. It is Defra's intention to publish this form.
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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

There is increasing interest, internationally, in making formal assessments of uncertainty associated with the analysis of pesticides in foods, not least because the information may be utilised to support, policy or enforcement, decision making. At present there is no harmonised approach to the assessment of uncertainty associated with the enforcement of pesticide maximum residue limits (MRLs) and different EU Member States can arrive at different decisions regarding the same analytical result.

A major challenge facing laboratories is to be able to produce realistic estimates of uncertainty at reasonable cost. A simple low cost model proposed by CSL [1] utilises routine analytical control data in a tiered approach, structured as follows:

Tier 1 (calibration data),
Tier 2 (recovery data),
Tier 3a (duplicate data),
Tier 3b (interlaboratory studies including proficiency test data)

A preliminary assessment of the model [1] included data for tier 1, tier 2, and tier 3a (within laboratory uncertainty), which are readily available or can be easily generated. No relevant data for tier 3b (between laboratory variation) were available and thus not included in the assessments of the model at that time. This represented a significant gap in the data set since tier 3b data are likely to be the most relevant but inevitably the least numerous compared with tiers 1 and 2. If it could be demonstrated that the use of tier 1, tier 2 and tier 3a data was still valid and that tier 3b data is not essential, then the CSL model would be more acceptable, more cost effective than other procedures and have much wider applicability.

In this latest study the use of log-transformed response against log-transformed concentration instead of standard least squares regression for calibration (tier 1 data) resulted in a reduction in the uncertainty associated with calibration. The assessment from the previous study [1] had concluded that a large proportion of the uncertainty at low pesticide concentrations was bias associated with calibration. An important advantage of the log-transformation method is that for

most purposes the value of uncertainty at tier one can be expressed as a single value (a relative standard uncertainty) rather than as a complicated bias function. This is because the value of the RMS (root mean square) of residuals was found to be constant across a much wider range of concentrations compared to standard linear regression.

Note: **Relative standard uncertainty**: standard uncertainty divided by measurement result.

The latest assessment also showed that uncertainty estimates based on data for tiers 1 to 3a was much smaller than estimates based on tier 3b data derived from the results of 8 laboratories that participated in an EU collaborative trial. This finding demonstrates that external information (collaborative trials, certified reference materials, proficiency test results etc.) must be included (in addition to within laboratory variation) in order to produce realistic estimates of uncertainty. Any model or procedure that does not take tier 3b into account will underestimate uncertainty. The paucity of tier 3b data means that it may not be possible to directly estimate the true level of uncertainty for the vast majority of pesticide-commodity combinations. A practical compromise; for example, the use of ± 50 or 60% proposed by a number of European pesticide residues chemists may have to be agreed even if the effects of the concentration of the analyte is largely ignored. Although limited in number, the results in this report, broadly supports the use of ± 50 or 60% for those pesticides where the method is under control. There will be exceptions, particularly for those pesticides susceptible to losses during sample processing.

Although the CSL model does take into account the concentration of the analyte, (by contrast to arbitrary limits), and does provide current, not historic, estimates of analytical performance, the dependence on higher tier data mean that it is unlikely to be perceived to provide any significant advantage over conventional procedures using the same data. Under these circumstances it may prove difficult to gain acceptance of a model, based on unconventional principles, in the wider scientific community.

Therefore, this report includes guidance for a general procedure for using non-bias corrected results for assessing the compliance of samples against legislative limits and bias corrected results for other purposes, including; estimating the range within which true concentration lies and assessing agreement of results.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the scientific objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

1.0 Introduction

There is increasing interest, internationally, in making formal assessments of uncertainty associated with the analysis of pesticides in foods, not least because the information may be utilised to support, policy or enforcement, decision making. At present there is no harmonised approach to the assessment of uncertainty associated with the enforcement of pesticide maximum residue limits (MRLs) and different EU Member States can arrive at different decisions regarding the same analytical result.

A major challenge facing laboratories is to be able to produce realistic estimates of uncertainty at reasonable cost. A simple low cost model proposed by CSL [1] utilises routine analytical control data in a tiered approach, structured as follows:

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Tier 2 (recovery data),
Tier 3a (duplicate data),
Tier 3b (interlaboratory studies including proficiency test data)

A preliminary assessment of the model [1] included data for tier 1, tier 2, and tier 3a (within laboratory uncertainty), which are readily available or can be easily generated. No relevant data for tier 3b (between laboratory variation) were available and thus not included in the assessments of the model at that time. This represented a significant gap in the data set since tier 3b data are likely to be the most relevant but inevitably the least numerous compared with tiers 1 and 2. If it could be demonstrated that the use of tier 1, tier 2 and tier 3a data was still valid and that tier 3b data is not essential, then the CSL model would be more acceptable, more cost effective than other procedures and have much wider applicability.

The most comprehensive tier 3b data set available for pesticides was generated by a EU interlaboratory study [2] co-ordinated by CSL. The trial provided replicate results for samples with spiked or incurred residues but with minimum calibration over a limited concentration range.

The main objectives of the current study were:

i) To undertake the additional pesticide residues analysis to fill the tier 3b gaps in the analytical data sets. I.e. to produce additional calibration data (over an extended concentration range and using the same experimental conditions employed in the trial) to allow the original trial results to be used to test the CSL Model for the estimation of uncertainty.

ii) To supplement the results of the initial evaluation of the model [1] with new results calculated using the EU trial data and to publish the findings in the scientific literature.

1.1 Method

CEN Method P1 (acetone extraction with ethyl acetate/cyclohexane partition) was re-established in the laboratory. Tomatoes known to be free of pesticide residues were extracted and cleaned-up using High Performance Gel Permeation Chromatography to provide sufficient volumes of blank extract for the preparation of matrix-matched calibration standards. A total of five calibration sets (approximately 16-point curves covering the range equivalent to 0.002145 – 1.425 mg/kg) were prepared on different days). Each calibration level was injected singly using the same GC-MS and identical GC-MS conditions employed in the EU trial. Each calibration standard included the same pesticides used for tomatoes in the original trial; bupirimate, chlorothalonil, dichlofluanid, alpha endosulfan, beta endosulfan, endosulfan sulfate and tetradifon.

The new calibration data and original trial results were used to evaluate three different aspects of the estimation of uncertainty for the measurement of pesticide residues:

a) The effect of different calibration functions on the uncertainty and bias associated with calibration (tier 1).

b) How the contribution made by different tiers of the analysis affect the estimation of uncertainty.

c) How to use and interpret uncertainty in a way that is consistent with definitions, standards and advice [3, 4, 5, 6, 7] applied to analytical chemistry in general and the need to compare results to legislative limits without correcting for bias.

Details of the statistical methods employed and the results obtained are outlined in the following text.

2.0 Estimation of Uncertainty at Tier 1 (Calibration)

2.1 Methods (calibration, tier 1)

The method previously described for the estimation of uncertainty at tier 1 was based on estimating the uncertainty associated with least squares linear regression, which is simple, but it leads to a large bias at low concentration and 'bias curves' that are difficult to estimate.

Alternative, slightly more complex regression methods were therefore evaluated in this latest study. The regression methods were selected such that the assumptions on which the they are based (measurement variability is proportional to concentration) more accurately reflected the calibration systems than the assumptions on which ordinary least squares linear regression are based (measurement variability is constant). The regression methods compared were: least squares linear regression [8]; weighted linear regression [8]; log-log transformed linear regression (least squares linear regression of log of response against log of concentration).

Results from five calibration series for seven pesticides (chlorothalonil, dichlofluanid, alpha-endosulfan, bupirimate, beta-endosulfan, endosulfan-sulphate and tetradifon) at up to 13 concentrations across at wide concentration range (0.001 to 5 μg/ml) were analysed.

The Akaike Information Criterion (AIC) [9] was used to assess the extent (expressed as an AIC weight) to which calibration methods were supported by the results. The effect of model selection on uncertainty estimates were made by comparing the root mean square relative residual for each calibration model.

2.2 Results (calibration, tier 1)

AIC weights for each calibration method showed that in general the log-log calibration model was the best model for describing the calibration data. In some cases (e.g set 2, α-endosulfan) the data was described equally well by log-log and weighted regression. In one case (set 5, tetradifon) weighted linear regression best described the data. Root mean square relative residuals were, in general, much lower for log-log calibration compared to ordinary linear regression (by a factor of 28 on average) and weighted linear regression (by a factor of 6 on average).

The superior performance of the log-log calibration was based on greatly reduced relative residuals for low concentrations as illustrated in Figures 1 & 2.

Figure 1. Calibration for bupirimate (linear least squares blue, weighted least squares black, log-log transformed least squares red)

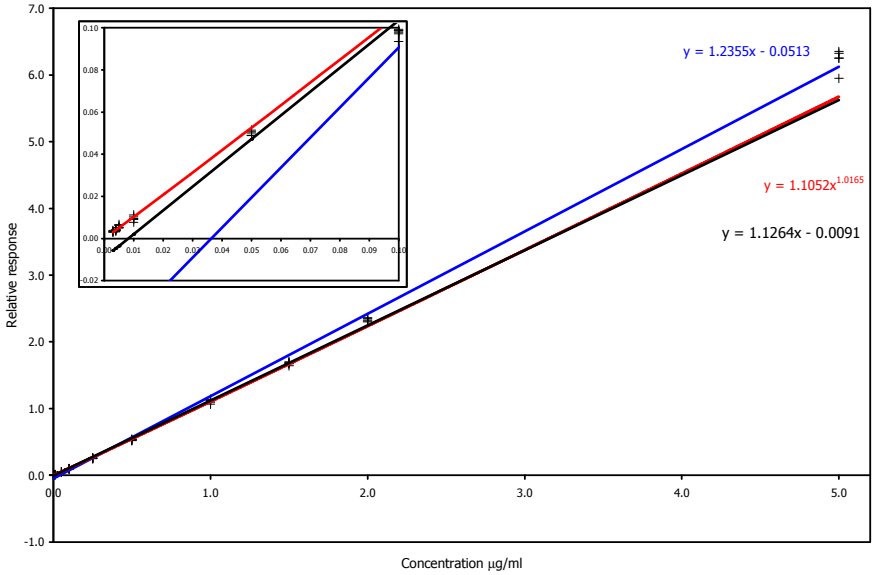
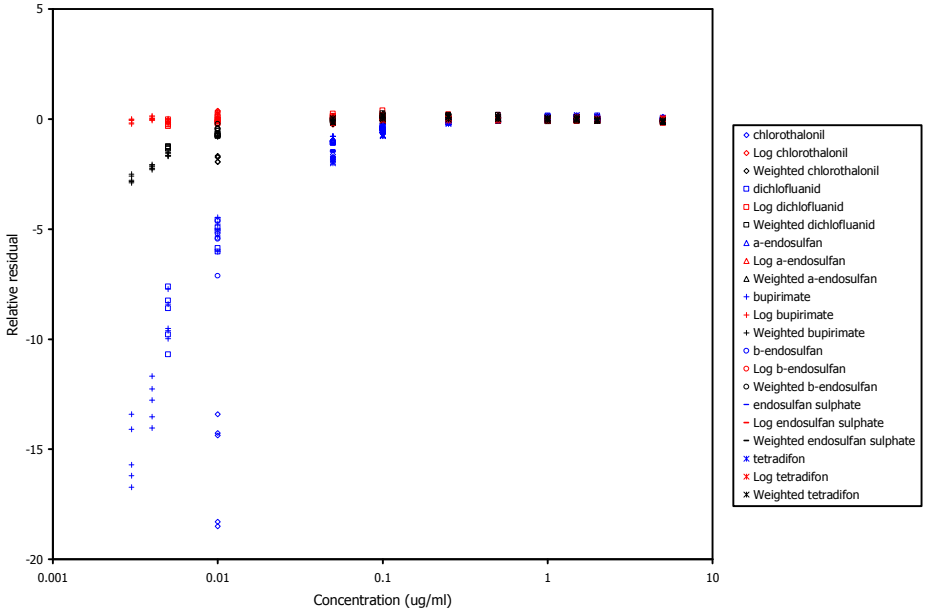
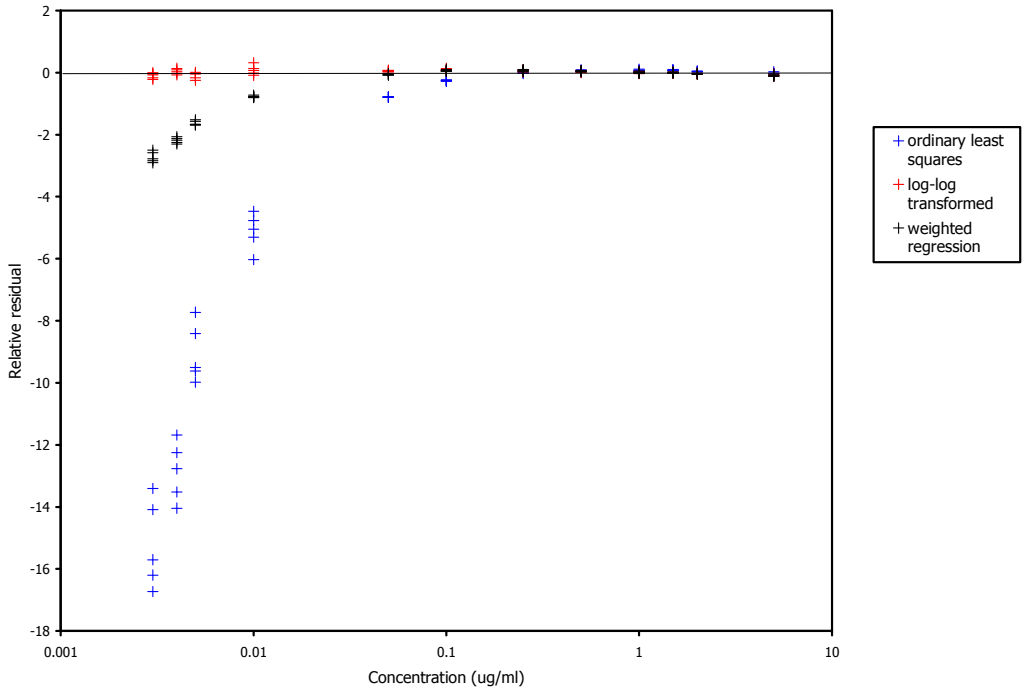


Figure 2. Residuals for the calibration of response to pesticides (linear least squares: blue, weighted least squares: black, log-log transformed least squares: red)



The log-log calibration function also produced unbiased (in the context of variability) estimates of concentration (see.Figure 3).

Figure 3. Residuals for the calibration of response to bupirimate (linear least squares: blue, weighted least squares: black, log-log transformed least squares: red)



The relative standard uncertainty associated with calibration $(u(x)/x)$ estimated from the back transformed standard error associated with the linear regression of the log transformed calibration data is shown in Table 1.

Estimates were between 0.020 and 0.16 with the exception of one estimate of 0.45 (set 1, dichlofluanid). None of the models used in the study adequately described the measurement results for dichlofluanid in set 1. Using a narrower range of concentrations should also reduce the uncertainty associated with calibration.

Table 1. Estimates of relative standard uncertainty at tier 1 using the log-log calibration model

Data set	chl	dic	aen	bup	ben	ens	tet
1	0.037	0.45*	0.020	0.11	0.030	0.031	0.039
2	0.097	0.15	0.083	0.093	0.080	0.045	0.057
3	0.046	0.12	0.049	0.036	0.020	0.023	0.041
4	0.043	0.16	0.069	0.096	0.11	0.079	0.052
5	0.054	0.11	0.080	0.057	0.046	0.043	0.075

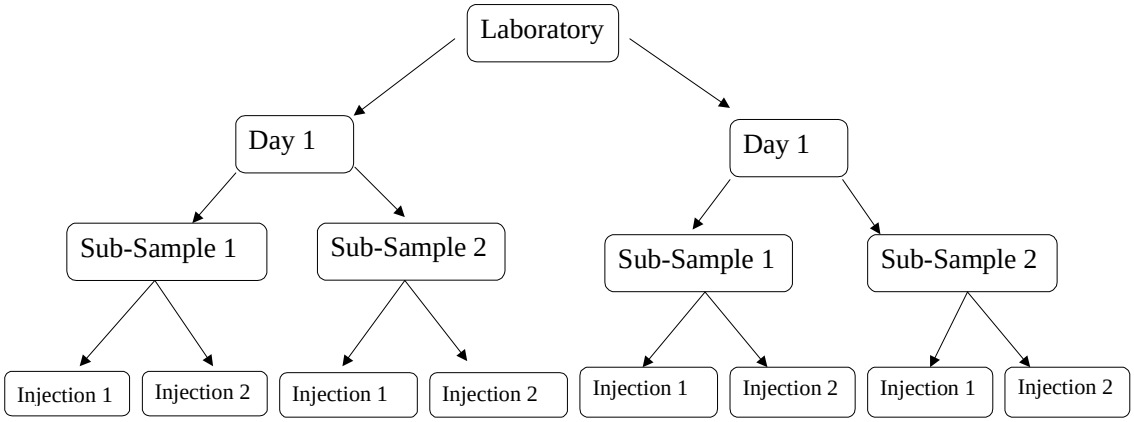
chl=chlorothalonil, dic=dichlofluanid, aen=α-endosulfan, bup=bupirimate, ben=β-endosulfan, ens=endosulfan sulphate, tet=tetradifon

* none of the calibration models yielded good fits to the data for dichlofluanid in data set 1. A different model, or a narrower concentration range should be used.

3.0 Contribution from variation at tiers 2 to 3b to measurement uncertainty

The data-set consisted of the results of the measurement of the same seven pesticides studied at tier 1 (chlorothalonil, dichlofluanid, alpha-endosulfan, bupirimate, beta-endosulfan, endosulfan-sulphate and tetradifon) in two bulks of tomatoes (incurred between 0.1mg/kg and 0.3 mg/kg; and spiked between 0.2 and 3 mg/kg) measured in a collaborative trial by eight laboratories. Each laboratory carried out measurements on two days. On each day, pesticides in single extracts, produced from each of two sub-samples were measured by duplicate injection using GC-MS (Figure 4). Each Laboratory produced eight results for each pesticide in each bulk sample.

Figure 4 . Design of the Study.



3.1 Methods (tiers 2 to 3b)

A linear mixed model was used to extract the components of variation for each level of the experimental design. Variance estimates were derived for: between laboratory, between days, between sub-samples and between injections. The 'non-linear mixed effect' module within R (a statistical software package) was used to compute these estimates. Variance at tier 3a was estimated from the sum of between days, between sub-sample and between injection variance for results generated by 'incurred samples'. Variance at tier 3b was estimated from the sum of between laboratory variance, for incurred samples and variance at tier 3a. Variance at tier 2 was estimated from the sum of between day, between sub-sample and between injection variances for results generated by 'spiked samples'. Estimates were converted to relative standard uncertainties by division by the mean (across laboratories) result.

3.2 Results (tiers 2 to 3b)

The most important result was that between laboratory variations makes the largest contribution to measurement variability for incurred residues (Table 2) and laboratory spiked residues (Table 3).

Table 2. Components of variation expressed as relative standard deviations (incurred pesticides in tomatoes)

	Lab	Day	Sub-sample	Injection
aen	0.19	0.0023	0.11	0.032
ben	0.15	0.0027	0.093	0.033
bup	0.14	0.0043	0.057	0.020
chl	0.38	0.089	0.083	0.025
dic	0.43	0.0057	0.045	0.049
ens	0.27	0.052	0.088	0.12
tet	NE	NE	NE	NE

chl=chlorothalonil, dic=dichlofluanid, aen=α-endosulfan, bup=bupirimate, ben=β-endosulfan, ens=endosulfan sulphate, tet=tetradifon

NE = not estimated

Note; **Relative standard deviation** = standard deviation divided by the mean of a set of observations

Table 3. Components of variation expressed as relative standard deviations (spiked pesticides in tomatoes)

	Lab	Day	Sub-sample	Injection
aen	0.067	0.0011	0.033	0.043
ben	0.061	0.036	0.030	0.054
bup	0.088	0.066	0.031	0.029
chl	0.46	0.054	0.048	0.035
dic	0.31	0.039	0.092	0.048
ens	0.076	0.050	0.033	0.066
tet	NE	NE	NE	NE

chl=chlorothalonil, dic=dichlofluanid, aen=α-endosulfan, bup=bupirimate, ben=β-endosulfan, ens=endosulfan sulphate, tet=tetradifon

The total within laboratory variation associated with the measurement of each pesticide, expressed as a relative standard deviation, lay between 0.15 and 0.5 of the total between laboratory variations (*Table 4*).

Uncertainty at Tier 1, expressed as a relative standard uncertainty lay between 0.014 and 0.52 times the value of total between laboratory variations. Estimates of variation at Tier 2 (spiked samples) were of similar size to estimates at Tier 3a. However, there were too few results to formally test the relationship between the variation of results given by the analysis of spiked and incurred samples.

Table 4: Estimated variation at tiers 1, 2, 3a and 3b expressed as relative standard deviations

	3b	3a (between-run)	2 (between-run)	1*
aen	0.22	0.11	0.054	0.065
ben	0.18	0.099	0.071	0.066
bup	0.16	0.060	0.078	0.083
chl	0.41	0.12	0.085	0.059
dic	0.44	0.067	0.11	0.14
ens	0.31	0.16	0.089	0.048
tet	NE	NE	NE	0.054

*pooled estimate from five calibration series

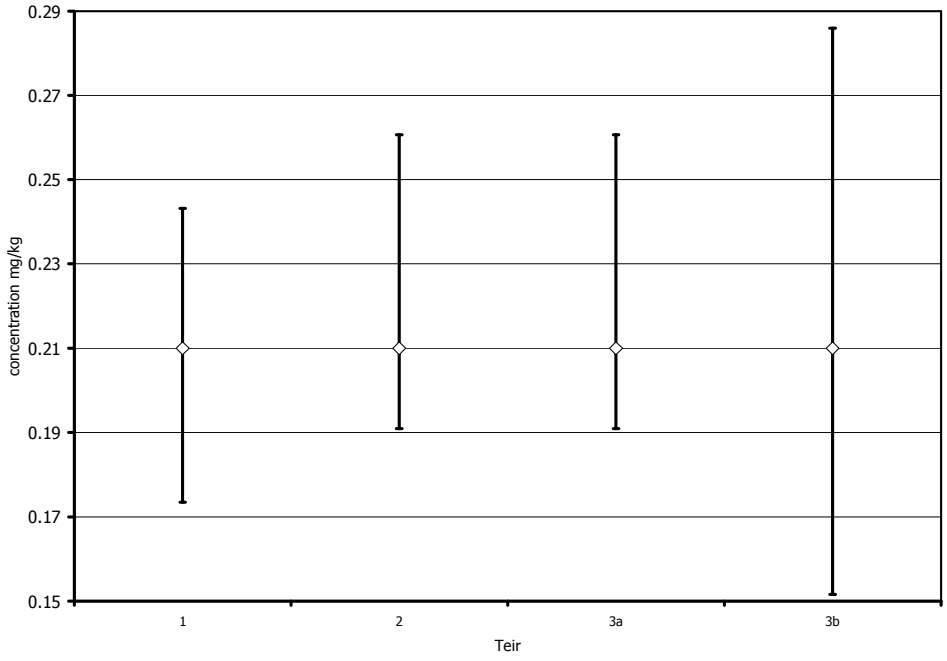
Table 4 shows that at a concentration of 1 mg/kg the range under repeatability conditions will be 0.87 - 1.13 at a value of 0.065 and 0.56 – 1.44 at 0.22, etc., etc.

Confidence intervals (95%) were calculated using tier 1 only, tier 1+2; tier 1+2+3a, and tier 1+2+3b data for bupirimate (measurement result of 0.21 mg/kg), using the method previously described [1] . The uncertainty estimates produced using this method do not include uncertainty associated with bias. Variation at tier 3b made the largest contribution to uncertainty as shown in Table 5 and Figure 5.

Table 5. Estimation of confidence intervals

	Tier 1	Tier 2	Tier 3a	Tier 3b
precision	0.083	0.078	0.060	0.16
bias	1.008	0.93	Not estimated	0.96
Result (mg/kg)	0.21	0.21	0.21	0.21
Lower limit (mg/kg)	0.17	0.19	0.19	0.15
Upper limit (mg/kg)	0.24	0.26	0.26	0.29

Figure 5. Estimates of uncertainty based on tier 1, tier 2, tier 3a and tier 3b



4.0 CSL Model for estimating uncertainty; summary of findings

The main findings were;

i) tier 3b data is essential for the calculation of realistic estimates of the uncertainty associated with pesticide residues analysis

ii) It is very difficult to include bias associated with results that have not been corrected for recovery. The use of recovery corrected results would overcome this problem but correction of results for recovery is not acceptable in the official control of pesticide residues in foods.

Taking these facts into account the following procedures are recommended for the calculation of uncertainty in pesticide residues analysis.

5.0 Recommended procedures

5.1 To estimate combined uncertainty

The observation that between laboratory variation was a factor of 2 to 6 times larger than within laboratory variation shows that some information that is external to the testing laboratory and/or method needs to be used to provide a realistic estimate of uncertainty. External sources include, collaborative trials, certified reference materials and proficiency testing schemes [10]. However, in the absence of these sources of information an in-house reference material with an assigned 'true value' can act as a source of external information for the purposes of estimating bias and its uncertainty.

There is a considerable body of guidance on the estimation of uncertainty in analytical chemistry [3, 4, 5, 6, 7,10,11,12] that, in many situations, can be applied *directly* to the estimation of uncertainty associated with the results of the measurement of pesticide concentrations in food; and the related task of comparing measurement results. The only exception is where uncertainty is estimated to determine the extent to which a measurement result shows that a sample complies with a legislative limit. For that purpose a slightly modified procedure should be used.

5.2 To compare measurement results against limits

In order to compare results, and their uncertainty, against legislative limits it is necessary to 'de-correct' the measurement result for bias in order to comply with the practice of 'not correcting results for bias'. However, it is recommended here that the uncertainty associated with bias be retained with the 'de-corrected' result. One reason for retaining the uncertainty associated with bias (even though a result has been de-corrected) is that uncertainty about bias is reflected in the values given by measurement results (even though a bias correction is not applied).

For concentrations much greater than the limit of detection the relative standard uncertainty $u_{y_{corr}}$ associated with a bias corrected result y_{corr} can be expressed in terms of the relative variability of results RSD_y (estimated at the highest tier possible) and the relative uncertainty associated with measurement bias $u_{bias}/bias$.

$$\frac{u_{y_{corr}}}{y_{corr}} = \sqrt{RSD_y^2 + \frac{u_{bias}^2}{bias^2}} \qquad \text{Equation 1}$$

In the most simple case [11] *bias* is estimated as:

$$bias = \frac{\bar{y}_{ref}}{ref} \text{ and } y_{corr} = \frac{y}{bias} \qquad \text{Equation 2}$$

Where

$\bar{y}_{ref}$ = long term mean result of measurement of material with an assigned true concentration (ideally a certified reference material or in-house reference material.

Y = measurement result not corrected for bias

The relative uncertainty associated with bias is given by the combination of the uncertainty associated with the mean measurement result and the uncertainty associated with the reference value:

$$u_{bias} = \sqrt{\frac{s_{y_{ref}}^2}{n\bar{y}_{ref}^2} + \frac{u_{ref}^2}{ref^2}} \qquad \text{Equation 3}$$

Where

$s_{y_{ref}}$ = the standard deviation displayed by results of measurements of the reference material

n = the number of measurements

u_{ref}= uncertainty associated with reference value (including uncertainty associated with applicability of reference value [12]).

Hence, given an uncorrected measurement result, *y*, the best estimate of true concentration of pesticide and its uncertainty is reported as, "$y_{corr} \pm k \times u_{y_{corr}}$" where k is a coverage factor used to convert the standard uncertainty in to a confidence interval. By convention k = 2 to give a confidence interval of approximately 95%. $y_{corr} \pm k \times u_{y_{corr}}$ can be used as an estimate of the interval within which the true concentration of pesticide in a sample lies. Also, if

$$\left|y_{1corr} - y_{2corr}\right| \leq k\sqrt{u_{y_{1corr}}^2 + u_{y_{2corr}}^2} \qquad \text{Equation 4}$$

then measurement 1 and measurement 2 are in agreement (strictly, the difference between measurements is not sufficiently large to demonstrate that they disagree).

Note: **Coverage factor** = factor used to convert a standard uncertainty in to a measurement uncertainty. By convention a coverage factor of 2 is usually used measurement uncertainty equivalent to a confidence interval of 95%.

For testing against limits the following tests should be applied:

If $y - 2 \times bias \times u_{y_{corr}} > limit$, then the measurement result shows that the sample contains pesticide at a concentration above the legislative limit

If $y + 2 \times bias \times u_{y_{corr}} < limit$, then the measurement result shows that the sample contains pesticide at a concentration below the legislative limit.

If neither condition above is satisfied then the sample may contain pesticide at a concentration above or below the limit.

5.3 To estimate the uncertainty associated with the mean of replicate results

Where the relative standard uncertainty associated with the mean of *n* replicates (RSU_n) is given by

$$RSU_n = \sqrt{RSU^2 - \frac{RSD_{rep}^2 (n-1)}{n}} \qquad \text{Equation 5}$$

Where *RSU* is the relative standard uncertainty associated with a single measurement result and RSD_{rep} is the relative standard deviation describing between-replicate variation.

For example, duplicate analysis of bupirimate was undertaken in separate analytical runs. The results were 0.190 mg/kg and 0.224 mg/kg. Hence a mean result of 0.207 mg/kg was reported. The relative standard uncertainty associated with a single result was 0.16 (Table 4). Between run-variation was estimated to be equal to 0.066 (Table 4). Hence, using Equation 5, the uncertainty associated with the mean result was given by

$$RSU_n = \sqrt{0.16^2 - \frac{0.066^2}{2}} = 0.15$$

This does not appear to represent a worthwhile improvement compared the extra effort required to undertake a second analysis, but additional measurements do increase confidence that the measurements are in control (at least not out of control). In cases where the results are close to the LOD then replication becomes increasingly valuable.

5.4 To Estimate between-replicate variation

In order to estimate the size of the uncertainty associated with the mean replicate analyses an estimate of between-replicate standard deviation (RSD_{rep} in Equation 5) is required. One way of gaining an estimate is to perform a number of replicate analyses of a single sample (at least six [7]) using the level of replication under study and calculate the standard deviation associated with the result.

A better method for estimating the value of between-replicate relative standard deviation [9] is to perform duplicate analyses within separate runs to produce an estimate of between-replicate standard deviation that is continually updated by new results rather than relying on a single estimate that will eventually become outdated.

5.5 To Estimate uncertainty associated with measurement of multi-component pesticides (e.g. endosulfan)

Where the concentration *c* of a pesticide is estimated from the sum of the concentrations (c_1, c_2 etc) of a number of individual components (e.g. endosulfan) then the relative standard uncertainty *RSU* associated with the concentration of the pesticide can be estimated from the relative standard uncertainties associated (RSU_1, RSU_2 etc) with the concentration of each component using Equation 6 if measurement results are assumed to be independent.

$$RSU = \frac{\sqrt{(RSU_1 \times c_1)^2 + (RSU_2 \times c_2)^2 + (RSU_3 \times c_3)^2}}{c_1 + c_2 + c_3} \qquad \text{Equation 6}$$

For example, for the measurement of the endosulfan components the following results were recorded: α-endosulfan 0.194 mg/kg (RSU 0.19), β-endosulfan 0.219 mg/kg (RSU 0.15) and endosulfan sulphate 0.141 mg/kg (RSU 0.27). Hence the relative standard uncertainty associated with the sum of the endosulfan components is given by:

$$RSU = \frac{\sqrt{(0.19 \times 0.194)^2 + (0.15 \times 0.219)^2 + (0.27 \times 0.141)^2}}{0.194 + 0.219 + 0.141} = 0.11$$

6.0 Conclusion

The uncertainty estimates based on tiers 1 to 3a was much smaller than estimates based on tier 3b data derived from the results of 8 laboratories that participated in a EU collaborative trial. This finding demonstrates that external information (collaborative trials, certified reference materials, proficiency test results etc.) must be included (in addition to within laboratory variation) in order to produce realistic estimates of uncertainty. Any model or procedure that does not take tier 3b into account will underestimate uncertainty. The paucity of tier 3b data means that it may not be possible to directly estimate the true level of uncertainty for the vast majority of pesticide-commodity combinations. A practical compromise; for example, the use of ± 50 or 60% proposed by a number of European pesticide residues chemists may have to be agreed even if the effects due to the concentration of the analyte are largely ignored. Although limited in number, the results in this report, broadly supports the use of ± 50 or 60% for those pesticides where the method is under control. There will be exceptions, particularly for those pesticides susceptible to losses during sample processing.

Although the CSL model does take into account the concentration of the analyte, (by contrast to arbitrary limits), and does provide current, rather than historic, estimates of analytical performance, the dependence on higher tier data mean that it is unlikely to be perceived to provide any significant advantage over conventional procedures using the same data. Under these circumstances it may prove difficult to gain acceptance of a model, based on unconventional principles, in the wider scientific community.

Therefore, alternative procedures for using non-bias corrected results for assessing the compliance of samples against legislative limits have been outlined in this report.

The results of this latest assessment of procedures for the measurement of uncertainty and the previous assessment [1] are currently will be collated in the form of a paper for submission to a peer-reviewed scientific journal.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

REFERENCES

1. Hill, ARC, Deppe C, Murray AWA, Macarthur R, Fussell, 2004, Estimation of uncertainty in pesticide residues analysis, CSL PVME Report PS2506 S010308
2. S. L. Reynolds, R. J. Fussell, M. Caldow, S. Nawaz, C. Ebden, S.Lovell and H. Diserens (1999), Intercomparison Study of Two Multi-Residue Methods for the Enforcement of MRLs for Pesticides in Fruit, Vegetables and Grain, Phase III: Intercomparison Study of test Materials Containing Incurred Residues - *EU Report EUR 19306 EN, ISBN 92-828-8309-4.*
3. Eurachem, 2000, Quantifying Uncertainty in Analytical Measurement, second edition, available from http://www.measurmentuncertainty.org
4. ISO, 1993, International Vocabulary of basic and general standard terms in Metrology., Geneva, Switzerland, ISBN 92-67-10175-1
5. ISO, 1995, Guide to the expression of uncertainty in measurement, Geneva Switzerland, ISBN 92-67-10188-9
6. Thompson M, Ellison SLR, Wood R, 2002, Harmonized guidelines for single-laboratory validation of methods of analysis, *Pure and Applied Chemistry*, 74(5), 835 – 855
7. European Commission, 2005, Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of EU Food and Feed legislation (updated 01/03/2005),available from http://europa.eu.int/ comm/ food/ food/ chemicalsafety/ contaminants/ sampling_en.htm
8. Draper NR, Smith H, 1998, Applied Regression Analysis, Wiley-Interscience, ISBN: 0471170828
9. Burnham, K. P., and D. R. Anderson, 2002, Model selection and multimodel inference: a practical information-theoretic approach, Springer-Verlag, New York, NY, ISBN 0-387-95364-7.
10. Castle L, Macarthur R, Mead EM, Read WA, 2004, Measurement uncertainty associated with overall migration testing, *Food Additives and Contaminants*, **21 (3)**, 256-264
11. Thompson M, Ellison SLR, Fajgeli A, Willetts P, Wood R, 1999, Harmonised guidelines for the use of recovery information in analytical measurement, *Pure and Applied Chemistry*, **71(2),** 337-348
12. Barwick VJ, Ellison SLR, 1999, Measurement uncertainty: approaches to the evaluation of uncertainties associated with recovery, *Analyst*, **124,** 981-990

Glossary

Standard deviation: measure of the variation displayed by a set of observations.
Relative standard deviation: standard deviation divided by the mean of a set of observations
Measurement uncertainty: Defined by ISO [1993 in references] as “parameter associated with the result of a measurement that characterises the dispersion of values that could reasonably be attributed to the measurand”. For analytical chemistry this means the range of concentrations within which the true concentration of analyte lies given a measurement result.
Standard uncertainty: measure of uncertainty associated with a measurement result, mathematically similar to a standard deviation.
Relative standard uncertainty: standard uncertainty divided by measurement result
Coverage factor: factor used to convert a standard uncertainty in to a measurement uncertainty. By convention a coverage factor of 2 is usually used measurement uncertainty equivalent to a confidence interval of 95%.