Uncertainty from Sampling - *Evaluation and use in Validation*

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Overview

- Objectives
  + Role of new Eurachem/Eurolab/Citac/Nordtest Guide
- Sampling as part of the measurement process
- Methods for estimating uncertainty of measurements 'U' (including sampling)
  - *= key parameter for validation of whole measurement process*
  - Empirical (top down) approach
  - Modelling (bottom up) approach - *with examples*
- Validation versus QC of whole measurement process
  - Assessing fitness-for-purpose of measurements (inc sampling)
- Conclusions

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Objectives

- To make measurements more reliable (and the management decisions based upon them)
- Integrate sampling into the rest of the measurement process – take a more holistic approach
- Reconcile the two different approaches that have been taken to quality in sampling and chemical analysis
- Review research that has been undertaken on 'Estimating uncertainty of measurement arising from sampling'
- Provide European Guidance for users on:
  - Estimation of uncertainty in whole measurement process
    - Enable validation of whole measurement process
    - in new Eurachem/Eurolab/Citac/Nordtest Guide

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Traditional Approach to Sampling Quality

- Sampling traditionally considered separately from measurement.
- Design ‘correct’ sampling protocol to give a representative sample
- Train sampler to apply the protocol,
- Assume that is applied ‘correctly’
  - no quality control of sampling
- Assume that uncertainty of measurement arises only in the lab analysis

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Sampling as part of the measurement process

- Sampling really the first step in the measurement process
- *In situ* measurement techniques reveal this
  - Place the sensor→ make measurement = taking a sample
  - Uncertainty in sampling produces U in measurement
- Physical sample preparation (in field or lab)
  - e.g. filter, acidify, dry, store, sieve, grind, split
  - is also part of the measurement process
  - and potentially important source of U
  - include in the validation process

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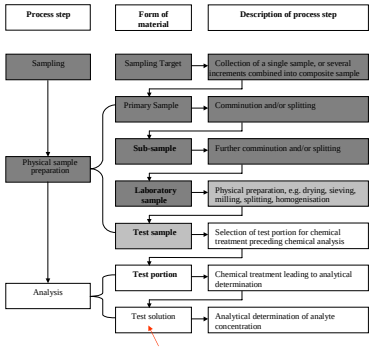
Sampling as part of the measurement process



More careful use of the word ‘sample’

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Sampling as part of the measurement process

- If the objective is to measure the true value
  - of the analyte concentration (or measurand)
  - in the sampling target *(e.g. batch of food)*
- Sampling is included in measurement process
- U from sampling part of measurement uncertainty*
  - method validation needs to include sampling
- If true value (or measurand) defined solely in terms of laboratory sample
  - sampling is not included
- Most user of analytical measurements assume x ± U apply to target, not just to lab sample
  - * Ramsey MH (2004) Accred Qual Assur., 9, 11-12, 727 - 728

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Methods for estimating uncertainty of measurement *(including sampling)*

- What are the options?
  - Empirical methods - 'Top down' approach
    - based on replicate measurements (within or between organisations)
    - *applicable to any system*
  - Modelling methods - 'Bottom up' approach
    - based on identifying, estimating and summing all of the components = 'Budget Approach'
      - (Kurfurst *et al*, 2004, Accred Qual Assur., 9, 64-75)
    - sometimes uses Sampling Theory (e.g. Gy's) to estimate components
      - (Minkkinen 2004, Chemometrics and Intelligent Lab. Systems, 74, 85-94)
      - *applicable to some particulate systems*

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Estimation of uncertainty – contributions in the empirical approach

Process	Effect class	
	Random *(precision)*	***Systematic*** *(bias)*
Analysis	*e.g. duplicate analyses*	*e.g. certified reference materials*
Sampling	*duplicate samples*	*Reference Sampling Target, Inter-Organisational Sampling Trial*

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Statistical model
for empirical estimation of uncertainty

$$x = X_{true} + \varepsilon_{sampling} + \varepsilon_{analytical}$$

x = *measured* value of the analyte concentration in the sampling target

X_{true} = *true* value of the analyte concentration in the sampling target

$\varepsilon_{sampling} + \varepsilon_{analytical}$ = effects on measured concentration from sampling and analysis

variance of measurement = $s^2_{meas} = s^2_{sampling} + s^2_{analytical}$

- includes between-organisational effects (e.g. sampling & analytical bias)

standard uncertainty = $u = s_{meas}$

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Four empirical methods for estimating uncertainty
including that from sampling

Method #	Method description	Samplers (People)	Protocols	Component estimated			
				P_{samp}	B_{samp}	P_{anal}	B_{anal}
1	duplicates	single	single	Yes	No	Yes	No[1]
2	protocols	single	multiple	between protocols		Yes	No [1]
3	CTS	multiple	single	between samplers		Yes	Yes [2]
4	SPT	multiple	multiple	between protocols +between samplers		Yes	Yes [2]

P_{anal} = precision of analytical method, B_{samp} = bias of sampling method,

CTS = Collaborative Trial in Sampling , and SPT = Sampling Proficiency Test.

Simplest Empirical method is 'Duplicate Method' (#1)- *explained with case study*

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Case Study on Empirical Method #1
Nitrate Concentration in Lettuce

- Nitrate a potential risk to human health
- EU threshold 4500 mg/kg for batch concentration

- Current sampling protocol specifies taking 10 heads to make a single composite sample from each batch *(in 'W' or 'star' design)*
- Usual ambiguity in the protocol
  - *e.g. where to start and orientation*
- What is the uncertainty in measurements?
- Is method valid (suitable for routine use)?

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‘W’ Sampling Design for Lettuce

Duplicate is equally likely interpretation of ‘W’ design

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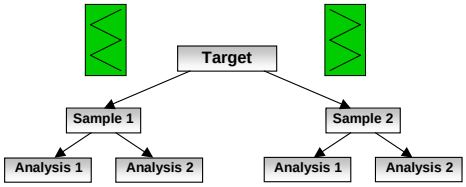
Estimating U with **Duplicate Method** using **Balanced Design**



At 10% of Sampling targets in whole survey $n \geq 8$

- aim to represents these targets in general

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Sampling of Lettuce for Nitrate

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Nitrate conc. in Duplicate Samples

S1A1	S1A2	S2A1	S2A2	mg/kg	
3898	4139	4466	4693		Most analytical duplicates agree well < x0.1 (approx)
3910	3993	4201	4126		Sampling duplicates agree only < x0.2 (approx)
5708	5903	4061	3782		
5028	4754	5450	5416	>4500?	Range of conc. between batches x1.6 (approx)
4640	4401	4248	4191		
5182	5023	4662	4839		Is level of Uncertainty OK?
3028	3224	3023	2901	<4500?	Reliable decisions whether batch is > 4500 mg/kg?
3966	4283	4131	3788		

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Uncertainty estimate for Lettuce

- Uncertainty = 361 mg/kg = s_{meas}
- = 16% *relative to concentration value (at 95% confidence)*
  - Calculated as $U' = 100 \times \frac{2s_{meas}}{x}$
    - from measurements on duplicates
  - Using Analysis of Variance (ANOVA)
    - Robust statistics to accommodate outlying values
  - U from analytical bias (from CRM/ or spike)
    - can be added – not detected in this case
- Does not include U from any sampling bias
  - Can be included using values from Sampling Proficiency Test (SPT) – with >8 organisations

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Validation of whole measurement procedure

- Includes one-time estimation of all uncertainty components
- Determined under conditions expected to be encountered in routine use of procedures
- May be done generically for the sampling method (initial validation) or
- Site-specifically for the method used “on site” to the selected target (on-site validation).

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Validation of whole measurement procedure

Initial validation

-used when sampling is done as a one-off campaign

-(spot sampling, e.g. contaminated site investigation)

-use initial estimation of U

-e.g. using duplicate method - requiring ≥32 measurements

-One target/site validation may need repeating at intervals

-i.e. repeated sampling, (e.g. time or flow- proportional sampling of waste water).

Validation demonstrates what can be achieved and,

-if that conforms to fitness-for-purpose requirement,

-then procedures deemed suitable for routine use.

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Relationship between validation and quality contro

of whole measurement procedure

Quality control of sampling (and analysis) SAQC

- to ensure that conditions prevailing at validation
- and therefore the expected uncertainty attached to the results)
- are still applicable every time those sampling/analytical procedures executed.
- i.e. routine measurements are still fit-for-purpose

Differences between sampling and analytical validation/QC

- Some sampling targets (like analysis?) quite consistent between batches (e.g. water in butter)
- Many targets are very variable between 'batches' (e.g. contaminated land – hetero)
- Estimates of U, and FFP criteria (if site specific), may have varied since time of validation
- May need more elaborate SAQC – or repeated validation, at each target/batch/site

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Judging fitness-for-purpose in validation

- How can you judge if you have too much uncertainty?
- One option -use the optimised uncertainty (OU) method*
- Balance the cost of measurement
  - against the cost of making incorrect decisions
- Knowing sampling and analytical components
- judge whether either is not FFP
- therefore where improvements/ increased expenditure required

* Lyn, J.A., Ramsey, M.H., and Wood, R. (2002) Analyst, 127, 1252 – 1260
based upon Thompson, M. and Fearn, T (1996), Analyst, 121, 275

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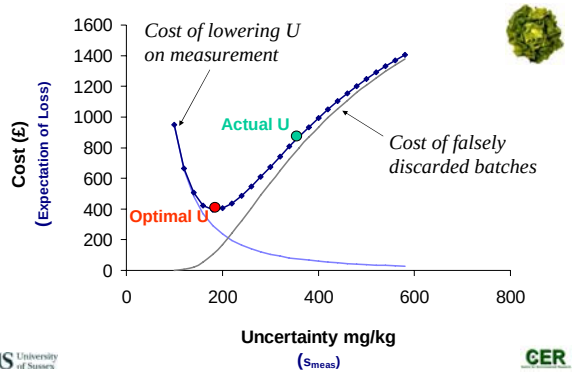
Acceptable level of Uncertainty?



Achieving FFP at Optimal Uncertainty

- Graph shows that U is too high – need to reduce it
- Need to know source of U
  - from sampling or from chemical analysis?
  - Duplicate Method + ANOVA - tells us sampling 78% of U
- We need to reduce the U by a factor of 2 (360→180)
- Sampling theory predicts (e.g. Gy's) increasing sample mass by factor of 4 (= 2^2)
- Reduction in U was achieved in practise → FFP
  - By taking composite sample with 40 heads instead of 10
  - Make whole method valid (i.e. suitable for routine use)

Budget Modelling Approach

to estimating U

Summation of all individual components of uncertainty (e.g. Kurfurst, 2004)

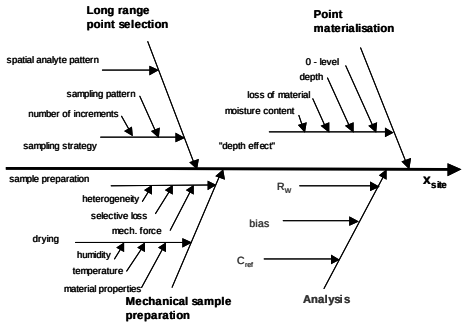
-e.g. applied to concentration of Cd and P in field of arable top soils

$$\bar{x}_{site} = \bar{x}_{anal} \times f_{b-loc} \times f_{strat} \times f_{depth} \times f_{prep} \times f_{dry}$$

- $\bar{x}_{site}$ = measurement result
- $\bar{x}_{anal}$ = mean from the analysis of test samples
- $f_{b\text{-}loc}$ = correction factor for deviation "between locations"
- f_{strat} = correction factor for bias due to sampling strategy
- f_{depth} = correction factor for the "depth effect"
- f_{prep} = correction factor for errors during mechanical sample preparation
- f_{dry} = correction factor for deviation of moisture content

$$u_{site} = \sqrt{u_{anly}^2 + u_{b\text{-}loc}^2 + u_{strat}^2 + u_{depth}^2 + u_{prep}^2 + u_{dry}^2}$$

Cause & effect diagram
for Budget Modelling (soil sampling)



R_w is within-laboratory reproducibility

U Estimates from Budget Modelling

Effect	Relative Standard Uncertainty(%)	
	Cd	P
Variation "between locations"	5.4	2.9
Sampling strategy	1.0	0.5
Depth	3.5	3.7
Splitting	3.7	3.3
Drying	0.6	0.6
Analysis	5.2	9.7
Combined Uncertainty	**9.1**	**11.3**

Modelling using Sampling Theory

Several sampling theories,

e.g. Gy, 1979 – defines 8 sampling errors (see Fig)

- includes 'fundamental sampling error' described by:-

$$\sigma_r^2 = Cd^3\left(\frac{1}{M_S} - \frac{1}{M_L}\right)$$

$\sigma_r = \frac{\sigma_a}{a_L}$ = Relative standard deviation of the fundamental sampling error

σ_a= absolute standard deviation (in concentration units)

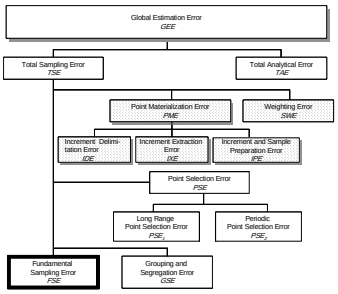
a_L = average concentration of the lot

d = characteristic particle size = 95 % upper limit of the size distribution

M_S = Sample size

M_L = Lot size

Sampling Theory of Gy



$$GEE = TSE + TAE$$

$$TSE = (PSE + FSE + GSE) + (IDE + IXE + IPE) + SWE$$

Modelling using Sampling Theory

C = fgβc= sampling constant (depends on the properties of the material sampled)

f = shape factor

g = size distribution factor (g =0.25 for wide size distribution, g =1 for uniform)

β= liberation factor, β = 1 for materials where particles are completely liberated,

c = constitution factor and can be estimated if the necessary material properties are available by using:

$$c = \frac{\left(1-\frac{a_L}{\alpha}\right)^2}{\frac{a_L}{\alpha}}\rho_c + \left(1-\frac{a_L}{\alpha}\right)\rho_m$$

a_L = average concentration of the lot =concentration of analyte in critical particles,

ρ_c = density of the critical particles

ρ_m = density of the matrix or diluent particles.

Input values for Sampling Theory

Applied to determination of **Enzyme in Chicken Feed**

Primary Sample	Secondary Sample	Comment
M_1 = 500 g	M_2 = 2.0 g	Sample sizes
M_{L1}= 25000 g	M_{L2} = 500 g	Lot (sampling target) sizes
d_1 = 0.1 cm	d_2 = 0.05 cm	Particle sizes
g_1 = 0.5	g_2 = 0.25	Estimated size distribution factors
Both Samples		
a_L = 0.05 % m/m		Mean concentration of enzyme in the lot
α = 100 % m/m		Enzyme concentration in enzyme particles
ρ_c = 1.08 g cm^{-3}		Density of enzyme particles
ρ_m = 0.67 g cm^{-3}		Density of matrix particles
f = 0.5		Default shape factor for spheroidal particles
β = 1		Liberation factor for liberated particles

U estimates from Sampling Theory

s_{r1} = 0.033 = 3.3 % …. Primary sample

s_{r2} = 0.13 = 13 % …. Secondary sample

s_{r3} = 0.05 = 5 % …. Analytical determination

Total relative standard deviation (combined uncertainty) by applying the rule of propagation of errors:

$$s_t = \sqrt{\sum s_{ri}^2} = 0.143 = 14.3\%$$

U' = 28.6%

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Conclusions

- Sampling needs to be considered as first step in measurement process
- Validation of measurement process requires estimation of the uncertainty caused by all steps – *including sampling*
- Several approaches to estimating uncertainty
  - -Each approach has particular strengths and weakness – different costs/feasibility
  - -Select the approach best suited to measurement system under study
- Estimates of U have their own UonU – estimation is area of research
- Values of U can be used to judge FFP of measurements and
  - -Validity of whole method
- Value of U from validation might not be applicable to subsequent batches
- Sampling (and analytical) QC needed to monitor possible changes in U

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