When do we need the Uncertainty Factor?

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30 min + Questions

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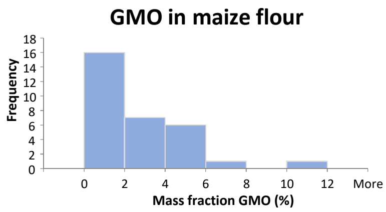
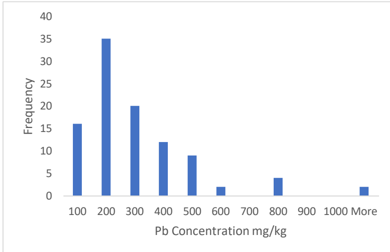
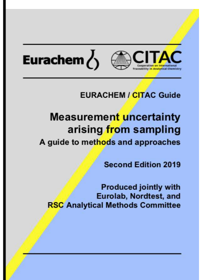




Overview

- Expressing Measurement Uncertainty (MU)
- What is the Uncertainty Factor (^{F}U) ? *Recent Eurachem Leaflet**
- How to calculate the Uncertainty Factor
- Worked examples applying the Uncertainty Factor
  - When analytical determination alone is source of MU
    - e.g. GMO in maize flour
    - other applications include microbiological, contaminants in river water & marine sediments
  - When sampling is dominant source of MU (includes UfS)
    - Requirement for including UfS in MU stated in ISO/IEC 17025:2017
- Using the Uncertainty Factor
  - advantages & overcoming challenges (by comparing ^{F}U and U')
- Conclusions



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* https://www.eurachem.org/index.php/publications/leaflets/uncertainty-factor

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Introduction

- Uncertainty of a measurement result (MU)
- MU often as important as measured quantity value itself
  - as it controls decisions made using that result (e.g. regulatory compliance)
- Appropriate expression of MU is crucial
  - especially when traditional, symmetric, expanded U interval is not accurate
- This is when concept of Uncertainty Factor (^{F}U) is useful
  - provides convenient and realistic uncertainty interval in particular circumstances

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Ways of expressing measurement uncertainty

- Most labs express measurement uncertainty (MU):-
  - as either expanded uncertainty (U)
  - or relative expanded uncertainty (U')
  - typically with coverage factor (k) of 2 (for ~ 95 % confidence)
- Measurement result expressed as $x \pm U$
  - where x is measurement quantity value, and ± is 'plus-minus'
- Range of values that contains value of measurand (i.e. true value of analyte concentration)
  - then between x - U and $x + U$ (for ~ 95 % confidence)

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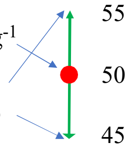
Ways of expressing measurement uncertainty

- **Example**: measurement result = 50 ± 5 mg kg^{-1}
- value of measurand believed to lie between Upper Confidence Limit (LCL) 55 (50 + 5)
  and Lower Confidence Limit (UCL) 45 (50 - 5)



- clearly an **<u>symmetric</u> confidence interval**
- Approach works well generally, unless:-
  - Value of MU is high (e.g. relative expanded uncertainty (U') is over 40 %)
    - ***Can give LCL value below zero*** *(see later GMO example)*
  - frequency distribution of repeated measurements is positively skewed
    - rather than Gaussian (i.e. Normal)

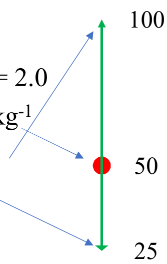
Another way of expressing measurement uncertainty

- When U' > 40% or frequency distribution of repeated measurements is ~ log-normal :-
- Expanded uncertainty factor (^FU) more accurate way to express MU (for k = 2)
- Measurement result expressed as

$$x \ {}^{x}/ \ {}^{F}U$$

  - where '^{x}/' is called 'times-over'
- In example - with much larger MU expressed as uncertainty factor of ^FU = 2.0

  Uncertainty interval 50 ^{x}/ 2.0 mg kg^{-1}
  is from UCL = 100 (50 x 2)
  to LCL = 25 (i.e. 50/2)



clearly an **<u>asymmetric</u> confidence interval**

- ***and can't give a negative LCL***

How to calculate the Uncertainty Factor

- **Standard uncertainty factor (^{F}u)** calculated* as

  $$^{F}u = \exp(s_G) = e^{s_G}$$

  – where s_G is the standard deviation of the $\log_e$-transformed measurement values (x) *(see Example)*

  $$s_G = s(\ln(x)) = s(\log_e(x))$$

- **Expanded uncertainty factor (^{F}U)**, for 95% confidence, calculated as

  $$^{F}U = \exp(2s_G) = e^{2s_G}$$

- **Worked examples**
  1. Analysis only – GMO (Genetically Modified Organism) in Maize Flour
     - simple 'manual' calculation of ^{F}U in Excel
  2. Sampling and analysis - Pb-contaminated soil
     - Example A2 from Eurachem UfS Guide (2019) *- more details in following talk*
     - ^{F}U calculated automatically, by applying RANOVA2/3 to results of 'duplicate' method

*Ramsey M.H. Ellison S.L.R (2015) Uncertainty Factor: an alternative way to express measurement uncertainty in chemical measurement. Accreditation and Quality Assurance: Journal for Quality, Comparability and Reliability in Chemical Measurement. 20, 2,153-155. doi:10.1007/s00769-015-1115-6

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Calculation of ^{F}U for purely Analytical example
GMO in Maize Flour (% = mass fraction cg/g)

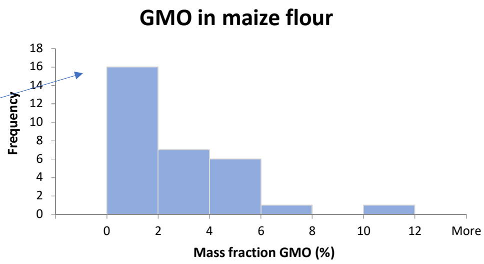
%GMO	loge %GMO
2.20	0.79
1.70	0.53
1.00	0.00
1.06	0.06
2.10	0.74
5.56	1.72
0.77	-0.26
1.10	0.10
5.59	1.72
5.33	1.67
4.50	1.50
1.14	0.13
1.86	0.62
0.80	-0.22
10.07	2.31
1.99	0.69
1.30	0.26
2.49	0.91
5.89	1.77
1.55	0.44
7.05	1.95
1.30	0.26
1.52	0.42
1.00	0.00
3.30	1.19
2.68	0.99
1.48	0.39
3.00	1.10
2.40	0.88
4.20	1.44
1.80	0.59
Mean	0.796
SD = sG	0.691
$^{F}u = \exp(s_G)$	2.00
$^{F}U = \exp(2s_G)$	3.98

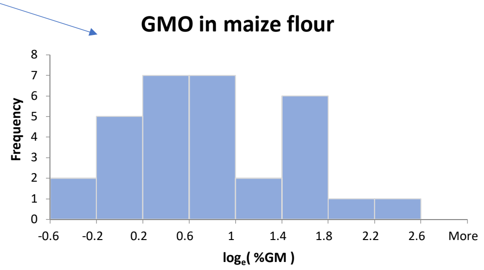
- Single PCR measurements from 31 labs in single PT round in 2004
  - on same test material
- Heavy positive skew
- Log_e transformation required to approach Normal distribution
  - command LN(x) in Excel
- Standard deviation of transformed measurement $s_G = 0.691$

  $^{F}u = \exp(s_G) = \exp(0.69) = 2.00$

  $^{F}U = \exp(2s_G) = \exp(2 * 0.69)$

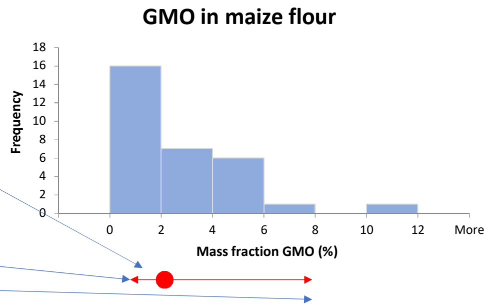
  $^{F}U = 3.98 = 4$





Interpretation of ${}^{F}U$ for **GMO in Maize Flour**

- MU on typical single measurement value of 2 % GMO on this one material
- Measurement result is x ×/ ${}^{F}U$
- Measurement result is 2 ×/ 4 %GMO
- LCL = 2 / 4 = 0.5 %
- UCL = 2 x 4 = 8 %



- Reflects MU of each PT result overall
- Better match to real distribution here
  - *But not all GMO distributions are approx. log-normal*
- ***No risk of negative LCL***

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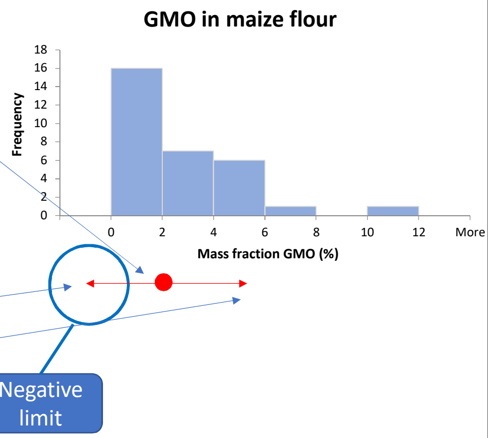
Interpretation for **GMO in Maize Flour using *U′***

	%GMO
	2.20
	1.70
	1.00
	1.06
	2.10
	5.56
	0.77
	1.10
	5.59
	5.33
	4.50
	1.14
	1.86
	0.80
	10.07
	1.99
	1.30
	2.49
	5.89
	1.55
	7.05
	1.30
	1.52
	1.00
	3.30
	2.68
	1.48
	3.00
	2.40
	4.20
	1.80
mean	2.83
SD	2.20
$U'=200s/\bar{x}$	155

- MU on typical single measurement value of 2 % GMO on this one material
  - ***without transformation***
- U' = 155%, U = 3.1% GMO
- Measurement result is

  2 +/- 3.1 % GMO
- LCL = 2 – 3.1 = **-1.1 %**
- UCL = 2 + 3.1 = 5.1 %.



- Use of ${}^{F}U$ is more realistic expression of MU

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Other published Analytical applications of ^{F}U

- Microbial contamination (e.g. of pharmaceutical products)[1]
  $^{F}U_{anal} = 1.2 - 3.0$
- Rapid microbiological methods[2]
  - need ^{F}U due to lognormal distribution of potency values
  $^{F}U_{anal} = 1.08 - 1.13$
- Contaminants in marine sediments (e.g. 29 polycyclic aromatic hydrocarbons)[3]
  – U' often exceeds 30% and appears log-normally distributed
- Analytical ^{F}U within MU of 25 contaminants in river water[4]
  - $^{F}U_{anal} = 1.1 - 2.1$ (13 contaminants > 1.4)

1. Francielle Regina Silva Dias, Felipe Rebelo Lourenço (2020). Top-down evaluation of the matrix effects in microbial enumeration test uncertainty. Journal of Microbiological Methods, 171, 105864, ISSN 0167-7012, https://doi.org/10.1016/j.mimet.2020.105864.
2. Alessandro Morais Saviano, Ricardo J.N. Bettencourt da Silva, Felipe Rebello Lourenço (2019) Measurement uncertainty for the potency estimation by rapid microbiological methods (RMMs) with correlated data. Regulatory Toxicology and Pharmacology, 102, 117-124, ISSN 0273-2300, https://doi.org/10.1016/j.yrtph.2019.01.023
3. Shaw DG, Blanchard AL (2020), Estimation of measurement uncertainty including surrogate recoveries in the study of polycyclic aromatic hydrocarbons in marine sediments. Marine Pollution Bulletin, 158, September 2020, http://dx.doi.org/10.1016/j.marpolbul.2020.111407
4. Nathalie Guigues, Bénédicte Lepot, Michèle Desenfant, Jacky Durocher. (2020) Estimation of the measurement uncertainty, including the contribution arising from sampling, of water quality parameters in surface waters of the Loire-Bretagne river basin, France. Accreditation and Quality Assurance 25:281–292 https://doi.org/10.1007/s00769-020-01436-6

^{F}U Estimation (including effects of sampling & analysis)

using Duplicate Method & ANOVA

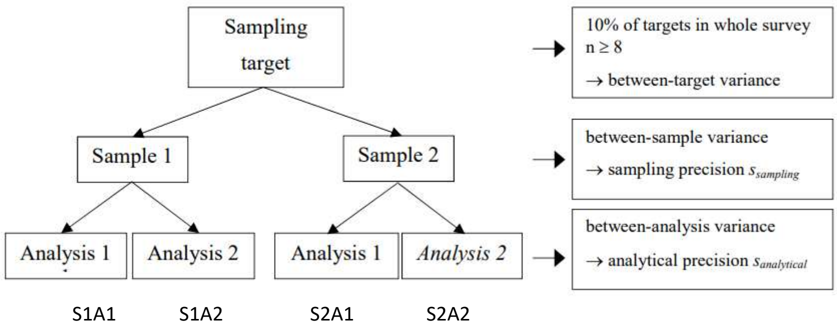
Scenario:
- Contaminated land investigation
- Former landfill, in West London
- 9 hectare = 90 000 m^2
- Potential housing development
- measurand → [Pb] in each sampling target

Area of investigation:
- 300 m x 300 m area → depth of 0.15 m
- 100 sampling targets in a regular grid (10 x 10)
- 100 primary samples (taken with soil auger)
  - each intended to represent a 30 m x 30 m target

Example A2 : From Eurachem UfS Guide (2019), http://www.eurachem.org – *more detail in next talk*

Application of Duplicate Method to estimate MU inc. UfS

Figure 2: A balanced design



- Duplicate samples taken at 10/100 sampling targets (i.e. 10%)
  - randomly selected
  - Duplicate sampling point 3 m from the original sampling point
    - within the sampling location
    - in a random direction - within the sampling target

Application of Duplicate method to estimate UfS

Aims of design of duplicate taking to reflect:-

- ambiguity in the sampling protocol
  - how differently could it be interpreted by a different samplers?
- uncertainty in locating sampling location within sampling target
  - e.g. survey error by using tape and compass
- effect of small-scale heterogeneity within each sampling target on measured concentration
  - e.g. at 10% of grid spacing distance, 3m for 30m

Sample prep and analysis in the lab

- Soil samples dried, sieved (<2 mm), ground (<100 µm)
- Test portions of 0.25g digested in nitric/perchloric acid
- [Pb] measured with ICP-AES, under full AQC
- 6 soil CRMs measured to estimate analytical bias
  - over range of concentration
  - found to be negligible (- 3.4% ± 1.3%) – *discussed further in UfS Guide (Example A2)*
- corrected for reagent blank concentrations
  - where statistically different to zero
- Raw measurements for use for estimation of uncertainty were:
  - **untruncated** – e.g. 0.0124 mg/kg, not < 0.1 or < detection limit
  - **unrounded** – e.g. 2.64862 mg/kg, not 3 mg/kg

Duplicated Measurements for MU estimation (including UfS)

Sampling target

Sample 1 | Sample 2

Analysis 1 | Analysis 2 | Analysis 1 | Analysis 2

- Large differences between some **sample duplicates** (e.g. D9) = high level of UfS
- Good agreement between **analytical duplicates** (< 10 % difference)

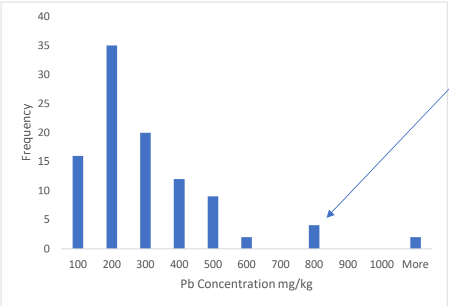
Target #	S1A1	S1A2	S2A1	S2A2
A4	787	769	811	780
B7	338	327	651	563
C1	289	297	211	204
D9	662	702	238	246
E8	229	215	208	218
F7	346	374	525	520
G7	324	321	77	73
H5	56	61	116	120
I9	189	189	176	168
J5	61	61	91	119

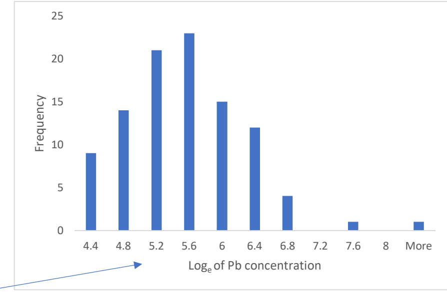
mg kg^{-1}

- Needs inspection of frequency distribution to select the best approach to UfS estimation

Estimating the Uncertainty as ^{F}U - Histograms

- Frequency distribution of [Pb] across the site = between-targets = long range heterogeneity
- Distribution of Pb measurements on 100 sampling targets is positively skewed = approximately log-normal
- Log-transformation necessary to remove skew





- Distribution closer to Normal after log$_e$ transformation

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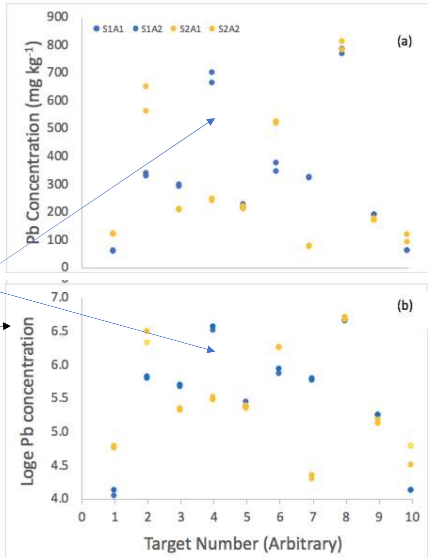
Estimating the Uncertainty as ^{F}U - Scatter Plots

Frequency distribution of [Pb] between each sample duplicate (= within-target)
= UfS part of MU - mainly due to within-target (short range) heterogeneity
Pb concentration values made on duplicated samples (10 of 100 targets) in either:-

(a) original concentration units
Duplicate samples (S1 , S2) generally differ by more than the duplicate analyses (A1 and A2 in same colour) - as seen in Table
(a) Four targets (2, 4, 6 and 7) have particularly large difference between duplicate samples, suggesting a positively skewed distribution for sampling uncertainty,
- like that between the targets (in previous Histogram).

e.g. D9

(b) log$_e$ transformed
Values show generally much smaller differences, more similar across range of concentration.
Distribution made closer to normal by log transformation (like Histogram #2).



Analytical Methods Committee (2019). **Why do we need the uncertainty factor?** Technical Brief 88, 27. DOI: http://dx.doi.org/10.1039/c9ay90050k Anal. Methods, 2019, 11, 2105–2107
https://pubs.rsc.org/en/content/articlelanding/2019/ay/c9ay90050k#!divAbstract

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Need for log-transformation?

- Classical analysis of variance (ANOVA) assumes approximately normal distributions
- Robust ANOVA can accommodate up to 10% outlying values,
  - but not more, and not heavy skew as in this case
- Use of log-transformation (where there is a log-normal distribution), can:
  1. Avoid negative LCL (lower confidence limit) - clearly impossible,
     i.e. when a normal distribution is assumed erroneously
  2. Compensates for any approximate proportional change of U with increasing concentration
  3. Enables justified use of Classical ANOVA (if log-transform produces a near normal distribution)
- However…

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Need for log-transformation?

- However, transformed measurement values (and ANOVA results) -
- <u>are no longer given in input units</u> of concentration
  - e.g. mass fraction, mg kg $^{-1}$

Measurement values of Pb concentration

In mg kg^{-1}

Target #	S1A1	S1A2	S2A1	S2A2
A4	787	769	811	780
B7	338	327	651	563
C1	289	297	211	204
D9	662	702	238	246
E8	229	215	208	218
F7	346	374	525	520
G7	324	321	77	73
H5	56	61	116	120
I9	189	189	176	168
J5	61	61	91	119

$\log_e$-transformed

Target #	S1A1	S1A2	S2A1	S2A2
A4	6.67	6.65	6.70	6.66
B7	5.82	5.79	6.48	6.33
C1	5.67	5.69	5.35	5.32
D9	6.50	6.55	5.47	5.51
E8	5.43	5.37	5.34	5.38
F7	5.85	5.92	6.26	6.25
G7	5.78	5.77	4.34	4.29
H5	4.03	4.11	4.75	4.79
I9	5.24	5.24	5.17	5.12
J5	4.11	4.11	4.51	4.78

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RANOVA2 output for Soil Example

Classical ANOVA

Mean	*317.8*		No. Targets	*10*	
Total Sdev	*240.19*				
	Btn Target	Sampling	Analysis	Measure	
Standard deviation	*197.55*	*135.43*	*17.99*	*136.62*	
% of total variance	*67.65*	*31.79*	*0.56*	*32.35*	
Expanded relative uncertainty (95%)		*85.23*	*11.32*	*85.98*	
Uncertainty Factor (95%)		*2.6032*	*1.12*	*2.6207*	

- Software RANOVA2* (in Excel) performs Classical
- Classical ANOVA output gives poor estimate of ***U'* = 86%** (due to heavily skewed distribution)
- but also estimate of ***^{F}U* as 2.62** (after automatic $\log_e$-transformation)
- Transformation can be either to base 'e' or to base 10
  - Get same ^{F}U, but $\log_e$ has some advantages, and is recommended
- RANOVA2, does $\log_e$ transformation internally and **calculates *^{F}U* directly**
  - Also performs Robust ANOVA – not applicable in this case (> 10% outliers)

- https://www.rsc.org/membership-and-community/connect-with-others/through-interests/divisions/analytical/amc/software/

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Uncertainty Factors of components of MU

Classical ANOVA

Mean	*317.8*		No. Targets	*10*	
Total Sdev	*240.19*				
	Btn Target	Sampling	Analysis	Measure	
Standard deviation	*197.55*	*135.43*	*17.99*	*136.62*	
% of total variance	*67.65*	*31.79*	*0.56*	*32.35*	
Expanded relative uncertainty (95%)		*85.23*	*11.32*	*85.98*	
Uncertainty Factor (95%)		*2.6032*	*1.12*	*2.6207*	

- Classical ANOVA on raw data using 'RANOVA2' gives:
- $^{F}U_{sampling}$ = 2.60 = expanded uncertainty factor of the **sampling**
- $^{F}U_{analysis}$ = 1.12 = expanded uncertainty factor of the **analysis** – *really analytical repeatability (U'~12%)*
- $^{F}U_{meas}$ = 2.62 = expanded uncertainty factor of the **measurement**

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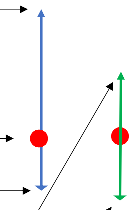
Confidence Limits on Measurement Value

- ***For*** ^{F}U **= 2.62, for a typical Pb measurement value of 300 mg kg^{-1}**

Upper confidence limit (UCL) = 784 mg kg^{-1} (300 x 2.62)

Measurement value of 300 mg kg^{-1}

Lower confidence limit (LCL) = 115 mg kg^{-1} (300 / 2.62)



- Asymmetric confidence limits around the measured value
- -185 and +484 mg kg^{-1} (away from 300)
- Reflects skew in frequency distribution of the uncertainty as seen in scatter plot & histograms
- Not seen in symmetrical confidence limits from classical U' = 86% = 258 (300 * 0.86) = 300 +/- 258 mg kg^{-1}
  UCL = 558 (300 + 258)
  LCL = 42 (300 - 258)
  - calculated without log-transformation
  - doesn't reflect actual (skewed) distribution (misses non-compliant targets)

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Understanding Uncertainty Factor by comparison with U'

Get appreciation for meaning of ^{F}U

From rough approximation. $U' \approx {}^{F}U - 1$

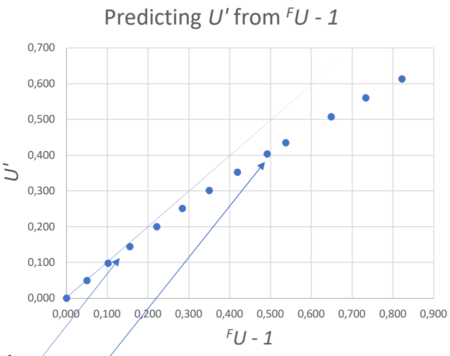
e.g.

^{F}U = 1.05 is roughly equivalent to U' = 5% - really ~4.9%

^{F}U = 1.10 " U' = 10% - really ~9.5%

^{F}U = 1.15 " U' = 15% - really ~14%

^{F}U = 1.20 " U' = 20% - really ~18%



- i.e. 10% overestimate at 1.2 - but a useful rough guide at low levels
- Breaks down at high levels: 20% overestimate at ^{F}U = 1.50, really 40%
- Gives instant intuitive appreciation of ^{F}U values
  - Calculations based upon a better approximation $u' = \sqrt{\exp(s_G^2) - 1}$

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Conclusions

- Uncertainty Factor (^FU) is a useful alternative way to express measurement uncertainty when:
  - Uncertainty values are high (U' > 40%)
  - Frequency distribution (of MU) is visibly log-normal (e.g. highly positively skewed)
- ^FU applicable to purely analytical sources, when U' is high (> 40%)
  - e.g. contaminant in water/sediment, microbiology, and some cases of GMO by PCR
  - Where there is an inherent expectation of log-normal distributions (e.g. PCR)
- When sampling materials with substantial heterogeneity of analyte concentration (within or between-target) – for estimation of UfS and MU
- Also allows for possible variation of U, with U proportional to concentration
- Never gives a negative Lower Confidence Limit
- ^FU *can* give more accurate Confidence Limits (e.g. UCL) for make assessments of compliance
- ^FU is harder to explain, but can made more accessible through recent Eurachem Leaflet on ^FU
  - and through approximations e.g. $^FU = 1.20 \sim U'$ *of 20%*

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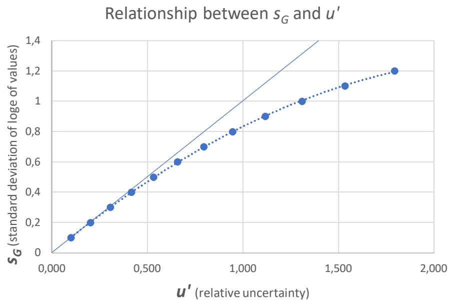
Relative U & Uncertainty Factor

– advantage of using natural logarithms (not base 10)

- Relative uncertainty u', expressed as a fraction, can be calculated from Approximation*

$$u' = \sqrt{\exp(s_G^2) - 1}$$

- E.g. $s_G = 0.20$, $u' = 0.20$ (=20% RSD)
- Approximation inaccurate if $s_G > 0.5$



s_G	u'
0	0.000
0.1	0.100
0.2	0.202
0.3	0.307
0.4	0.417
0.5	0.533

* known feature of log-normal distribution, e.g.

Johnson, N. L., Kotz, S. and Balakrishnan, N. (1995) _Continuous Univariate Distributions_, volume 1, chapter 14. Wiley, New York.
https://en.wikipedia.org/wiki/Log-normal_distribution

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