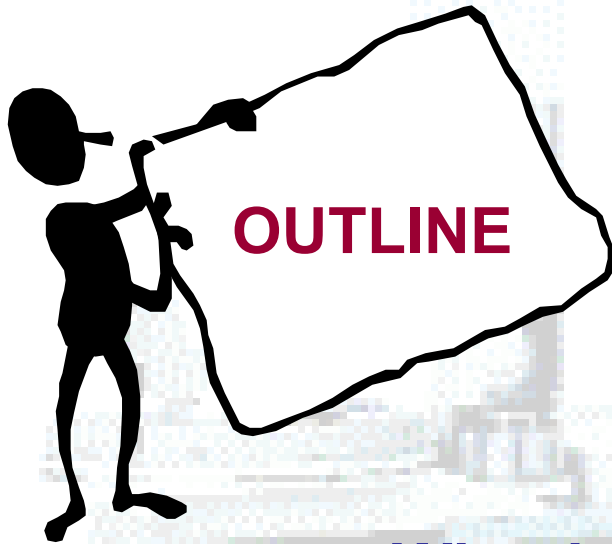


# MEASUREMENT UNCERTAINTY

.....and how it may impact on water testing and regulatory authorities

Graham Roberts  
National Association of Testing Authorities



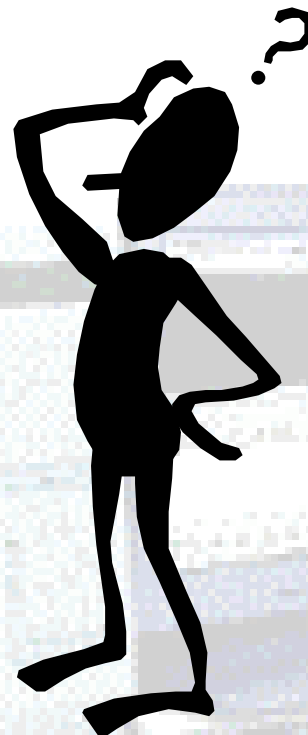
**What is measurement uncertainty?**

**Why do we need to estimate MU?**

**How do we estimate MU?**

**Regulatory decisions in the light of MU?**

**WHAT IS MU ?**



# How long is a piece of string?

**Individual measurements**

a range of results

uncertainty associated with each result

mean & standard deviation

**Mean result**

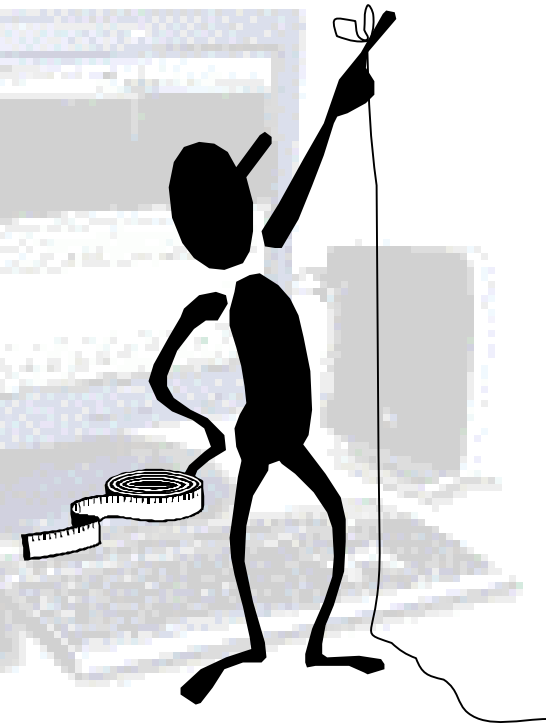
less uncertainty

**Standard method**

less uncertainty

**Different rulers**

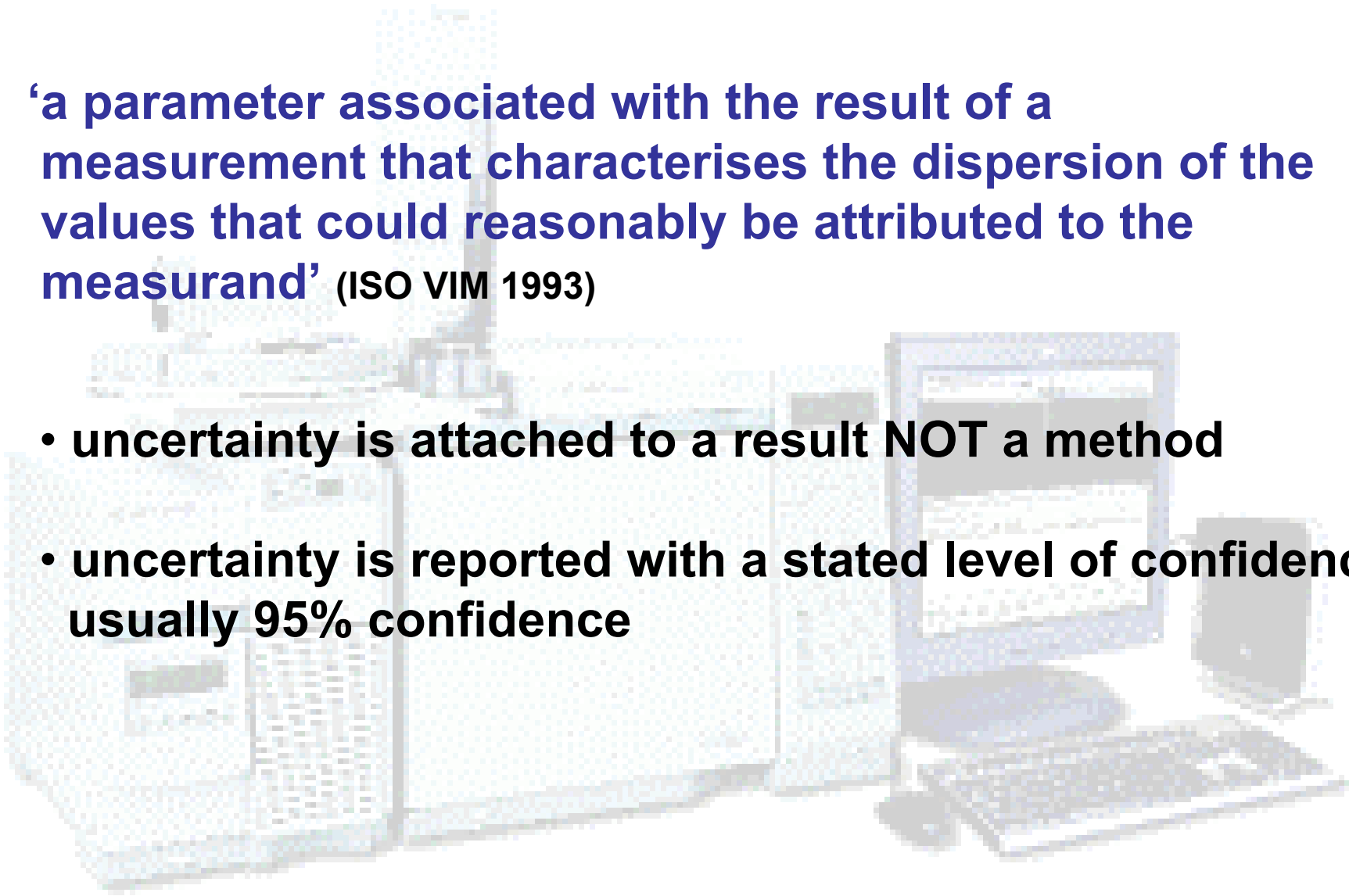
potential for instrumental bias



# Measurement Uncertainty

**‘a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand’ (ISO VIM 1993)**

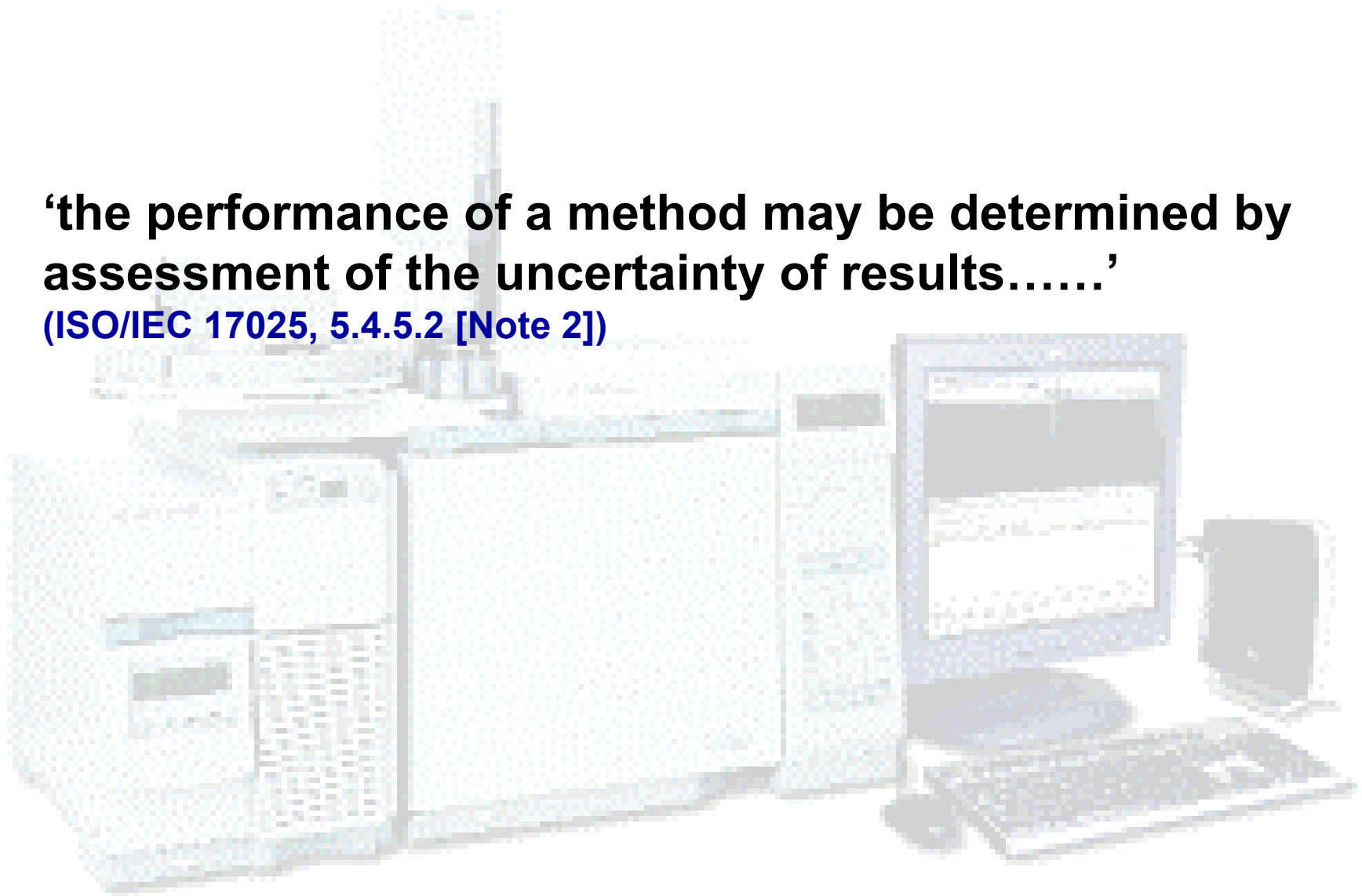
- uncertainty is attached to a result NOT a method**
- uncertainty is reported with a stated level of confidence usually 95% confidence**



# Measurement Uncertainty

**‘the performance of a method may be determined by assessment of the uncertainty of results.....’**

**(ISO/IEC 17025, 5.4.5.2 [Note 2])**



**WHY DO WE NEED  
TO ESTIMATE MU?**

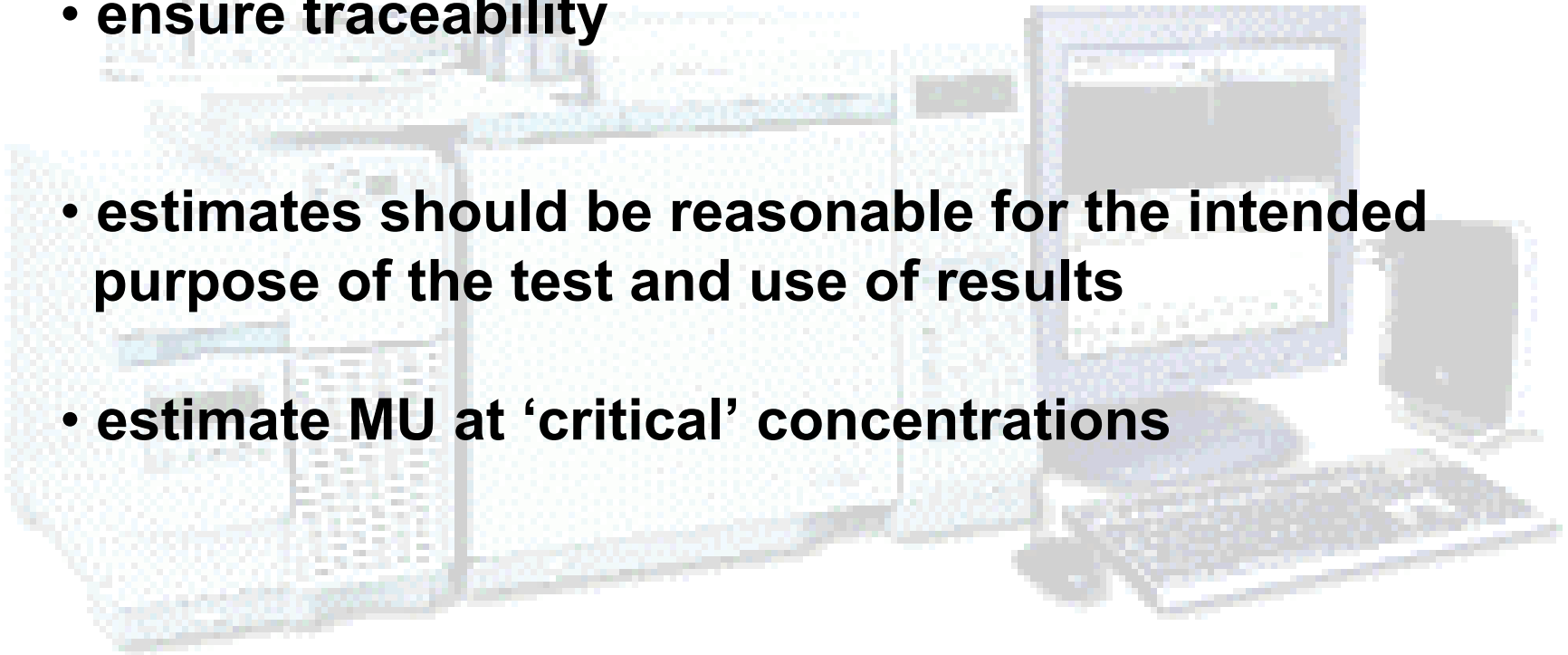


**Because ISO/IEC 17025 says so.**

# Better reasons.....

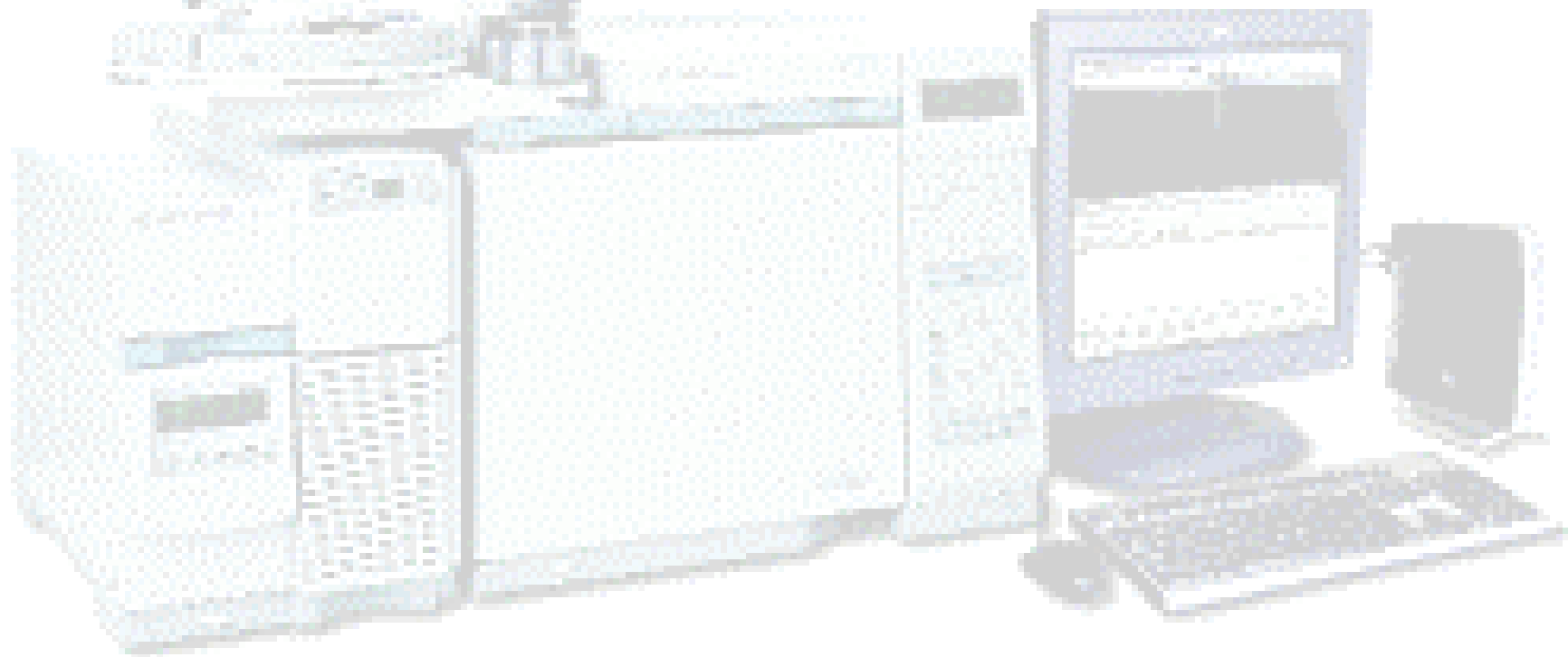
**knowledge of MU is necessary to:**

- **ensure results are 'fit for purpose'**
  - **compare results**
  - **judge compliance with legal or regulatory limits**
  - **ensure traceability**
- 
- **estimates should be reasonable for the intended purpose of the test and use of results**
  - **estimate MU at 'critical' concentrations**



# Traceability

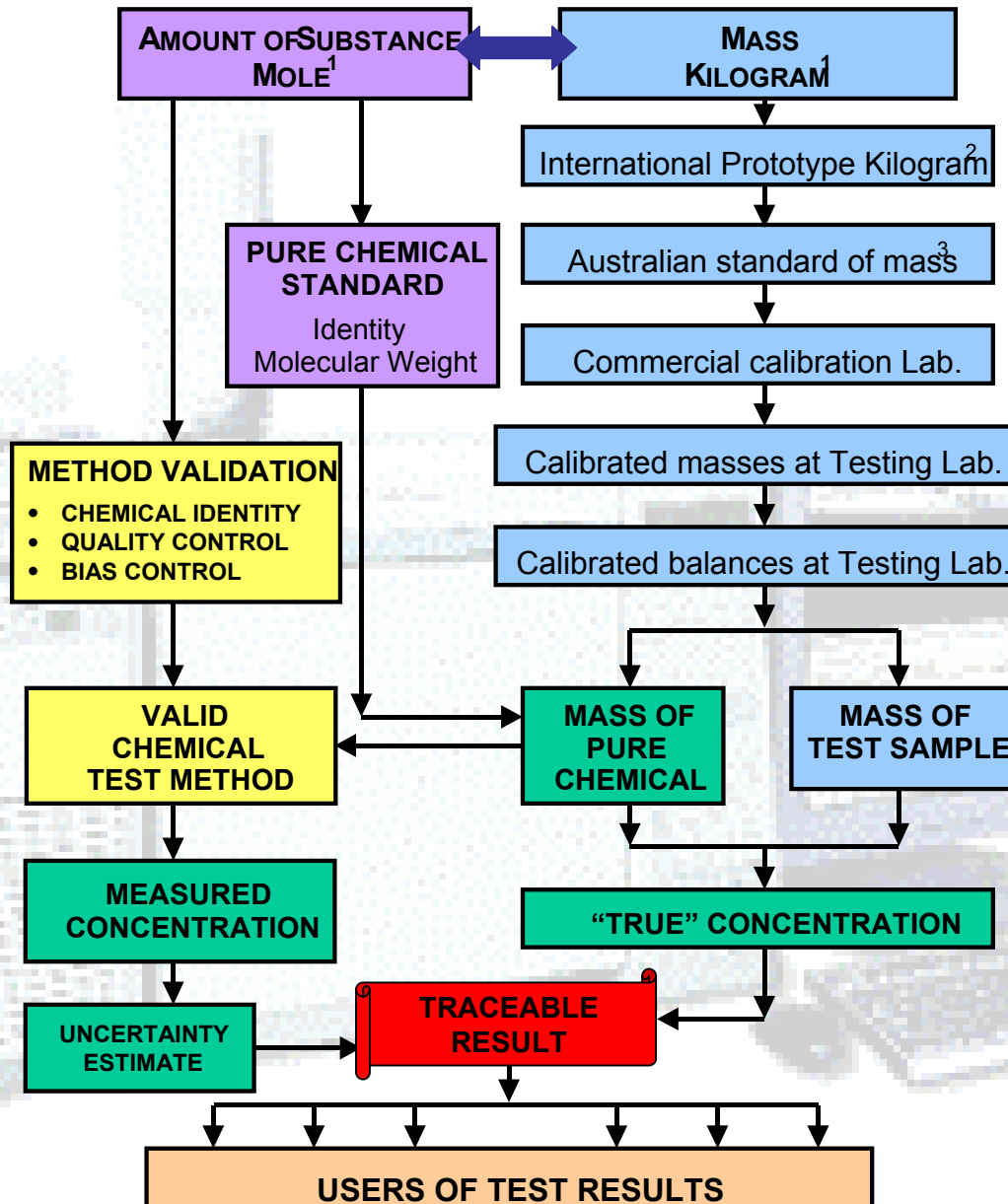
**‘test results must be traceable to stated references, usually national or international standards, through an unbroken chain of comparisons, all having stated uncertainties’ (Eurachem/CITAC 2003)**



# TRACEABILITY: Chemical Test Results

Chemical Measurements

Physical Measurements



# ESTIMATING MU



**Does it have to be a difficult exercise?**

# USEPA Method 555.2 ' HAA in Drinking Water'

## Method Summary

- 40 mL water sample adjusted to pH = 5
- extract with MTBE solvent (4.0 mL)
- esterify to form methyl esters
- add internal standard to 1.0 mL extract
- determine by GLC-ECD

**internal standard quantitation**

**matrix-matched calibration standards**

**surrogate standard to monitor method performance**

**Theoretical ‘Bottom Up’ approach recommended by the ISO GUM ‘bible’ on uncertainty:**

*‘Guide to Expression of Uncertainty of Measurement’, ISO (1993)*

**This may not be the best way to go about estimating the MU associated with results generated by a complex chemical test method.**

**Let’s look at how this approach may be used so that you may decide for yourself. (It’s a good idea to have some knowledge of estimating MU from first principles)**

# Theoretical 'Bottom Up' approach recommended by the ISO GUM 'bible' on uncertainty:

*'Guide to Expression of Uncertainty of Measurement', ISO (1993)*

$$\text{TCA}(\text{mg/L}) = \frac{A_{\text{TCA}} \times C_{\text{IS}}}{A_{\text{IS}} \times \text{RRF}}$$

$A_{\text{TCA}}$  = *area TCA peak*

$A_{\text{IS}}$  = *area internal standard peak*

$C_{\text{IS}}$  = *concentration internal standard*

$\text{RRF}$  = *average relative response factor*

$$\left(\frac{u_{TCA}}{TCA}\right)^2 = \left(\frac{u_{A_{TCA}}}{A_{TCA}}\right)^2 + \left(\frac{u_{C_{IS}}}{C_{IS}}\right)^2 + \left(\frac{u_{A_{IS}}}{A_{IS}}\right)^2 + \left(\frac{u_{RRF}}{RRF}\right)^2$$

$$u_{TCA} = TCA \sqrt{\left(\frac{u_{A_{TCA}}}{A_{TCA}}\right)^2 + \left(\frac{u_{C_{IS}}}{C_{IS}}\right)^2 + \left(\frac{u_{A_{IS}}}{A_{IS}}\right)^2 + \left(\frac{u_{RRF}}{RRF}\right)^2}$$

Each uncertainty ( $u$ ) must be quantified as a standard uncertainty (standard deviation, 's')

$u_{A_{TCA}}$  and  $u_{A_{IS}}$  may be estimated from  $s$  of replicate injections of the final sample extract

## Concentration of Internal Standard, $C_{IS}$

100 mg IS is weighed into a 100 mL std. flask.  
250  $\mu$ L of stock is then diluted to 10 mL in std. flask.  
Final concentration of working solution = 25  $\mu$ g/mL

$$C = \frac{W \times P}{V_F} \times \frac{V_s}{V_f}$$

$$u_c = C \sqrt{\left(\frac{u_W}{W}\right)^2 + \left(\frac{u_P}{P}\right)^2 + \left(\frac{u_{V_F}}{V_F}\right)^2 + \left(\frac{u_{V_S}}{V_S}\right)^2 + \left(\frac{u_{V_f}}{V_f}\right)^2}$$

## Weights

Balance calibration  $\pm 0.0004\text{g}$  (95% CI)

s from replicate weighings of 100mg weights =  $0.00004\text{g}$

## Internal Standard

Certified purity =  $99.9 \pm 0.1\%$

## Volumetric Glassware

Tolerance 100mL flask =  $0.1\text{mL}$  at  $20^\circ\text{C}$

s from 'fill and weigh' experiments =  $0.02\text{mL}$

Tolerance 10mL flask =  $0.02\text{mL}$ ;

s of volume delivery =  $0.003\text{mL}$

Tolerance 500  $\mu\text{L}$  syringe =  $2.5 \mu\text{L}$

s from replicate delivery of 250  $\mu\text{L}$  aliquots =  $2.1 \mu\text{L}$

Coefficient of volume expansion for MTBE =  $0.001$  per  $^\circ\text{C}$

Lab temperature controlled to  $20 \pm 2^\circ\text{C}$

To estimate  $u_w$  we need to consider;

- Balance calibration
- Weighing precision

**Calibration**  $\pm 0.0004\text{g}$  (95% CI)

$$s = \frac{0.0004}{2} = 0.0002\text{g}$$

**Precision**  $s = 0.00004\text{g}$

**Combining calibration & Precision**

$$u_w = \sqrt{(0.0002)^2 + (0.00004)^2}$$

$$u_w = 0.000204\text{ g}$$

To estimate the uncertainty in the volume of the 100 mL volumetric flask,  $u_{VF}$  we need to consider;

- Tolerance of the flask,
- Precision of filling to the mark, and
- The effects of temp (the expansion coefficient of MTBE)

Tolerance (20°C) =  $\pm 0.1$  mL       $s = \frac{0.1}{\sqrt{3}} = 0.058$  mL

Precision       $s = 0.02$  mL

Coefficient of volume expansion for MTBE = 0.001 per °C

temp =  $\pm 2$ °C,       $s = \frac{2}{\sqrt{3}} = 1.15$

Uncertainty in volume due to temp =  $100 \times 0.001 \times 1.15 = 0.115$  mL

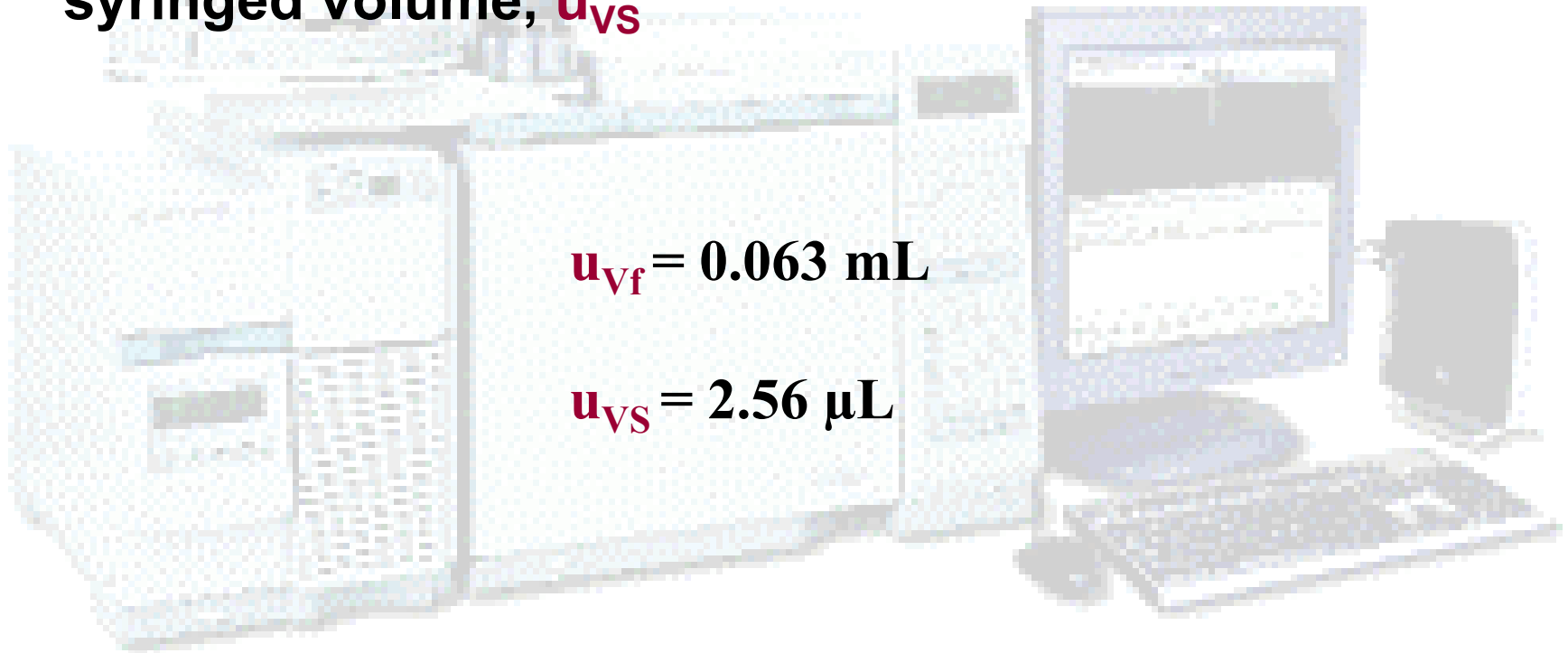
$$u_{VF} = \sqrt{(0.058)^2 + (0.02)^2 + (0.115)^2}$$

$$u_{VF} = 0.13 \text{ mL}$$

Similar consideration of the manufacturer's tolerance, precision of volume delivery and temperature (coefficient of expansion) provides an estimate of the uncertainty in the volume of the 10mL standard flask,  $u_{vf}$  and the uncertainty in the 250  $\mu\text{L}$  syringed volume,  $u_{vs}$

$$u_{vf} = 0.063 \text{ mL}$$

$$u_{vs} = 2.56 \mu\text{L}$$



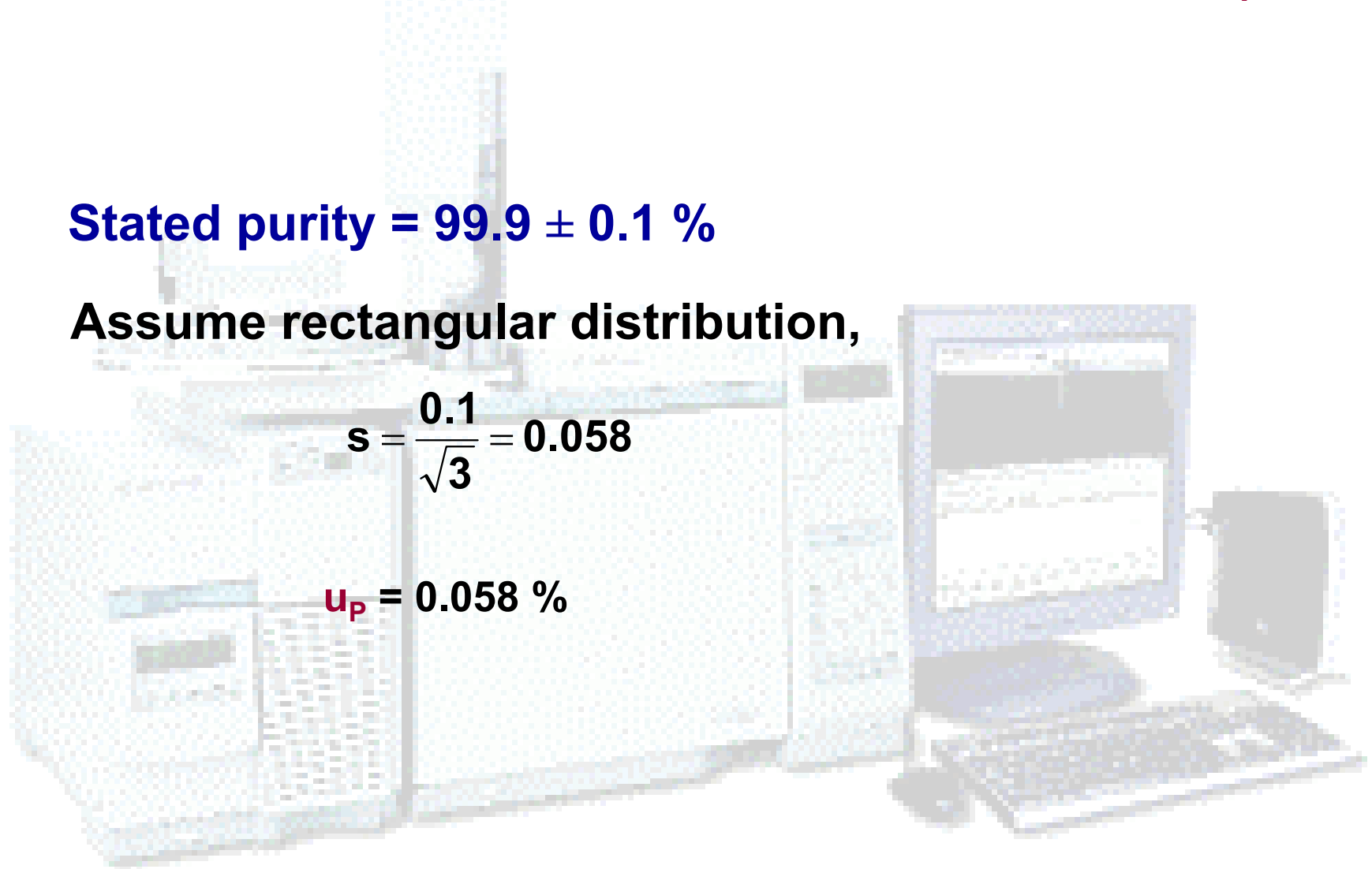
# Uncertainty in the purity of Internal Standard, $u_p$

**Stated purity =  $99.9 \pm 0.1$  %**

**Assume rectangular distribution,**

$$s = \frac{0.1}{\sqrt{3}} = 0.058$$

$$u_p = 0.058 \%$$



**The estimated standard uncertainty in Concentration of the internal standard solution is therefore,**

$$u_c = 25 \sqrt{\left(\frac{0.058}{99.9}\right)^2 + \left(\frac{0.0002}{0.100}\right)^2 + \left(\frac{2.56}{250}\right)^2 + \left(\frac{0.13}{100}\right)^2 + \left(\frac{0.063}{10}\right)^2}$$

$$= 0.31 \mu\text{g/L}$$

$$\left(\frac{u_{TCA}}{TCA}\right)^2 = \left(\frac{u_{A_{TCA}}}{A_{TCA}}\right)^2 + \left(\frac{u_{C_{IS}}}{C_{IS}}\right)^2 + \left(\frac{u_{A_{IS}}}{A_{IS}}\right)^2 + \left(\frac{u_{RRF}}{RRF}\right)^2$$

$$u_{TCA} = TCA \sqrt{\left(\frac{u_{A_{TCA}}}{A_{TCA}}\right)^2 + \left(\frac{u_{C_{IS}}}{C_{IS}}\right)^2 + \left(\frac{u_{A_{IS}}}{A_{IS}}\right)^2 + \left(\frac{u_{RRF}}{RRF}\right)^2}$$

✓                      ✓                      ✓

Each uncertainty (u) must be quantified as a standard uncertainty (standard deviation, 's')

$u_{A_{TCA}}$  and  $u_{A_{IS}}$  may be estimated from s of replicate injections of the final sample extract

**RRF = average relative response factor derived from 5 points on the calibration curve**

$$= \frac{1}{5} (\text{RRF}_1 + \text{RRF}_2 + \text{RRF}_3 + \text{RRF}_4 + \text{RRF}_5)$$

$$u_{\text{RRF}} = \sqrt{\left(u_{\text{RRF}_1}\right)^2 + \left(u_{\text{RRF}_2}\right)^2 + \left(u_{\text{RRF}_3}\right)^2 + \left(u_{\text{RRF}_4}\right)^2 + \left(u_{\text{RRF}_5}\right)^2}$$

**But, at each point on the calibration curve,**

$$\text{RRF}_n = \frac{A_{\text{TCA}} \times C_{\text{IS}}}{A_{\text{IS}} \times C_{\text{TCA}}}$$



**This approach can be tedious; let's call it quits at this stage and consider other approaches. Before we do, it is worth noting;**

- how we would report the estimated MU, and**
- the thoughts of an internationally respected expert on laboratory quality assurance.**



# Reporting MU

We first need to calculate the expanded Uncertainty  
....to give a stated level of confidence

$$U = k u_{TCA}$$

$$U = 2 u_{TCA} \text{ (95\% Confidence Interval)}$$

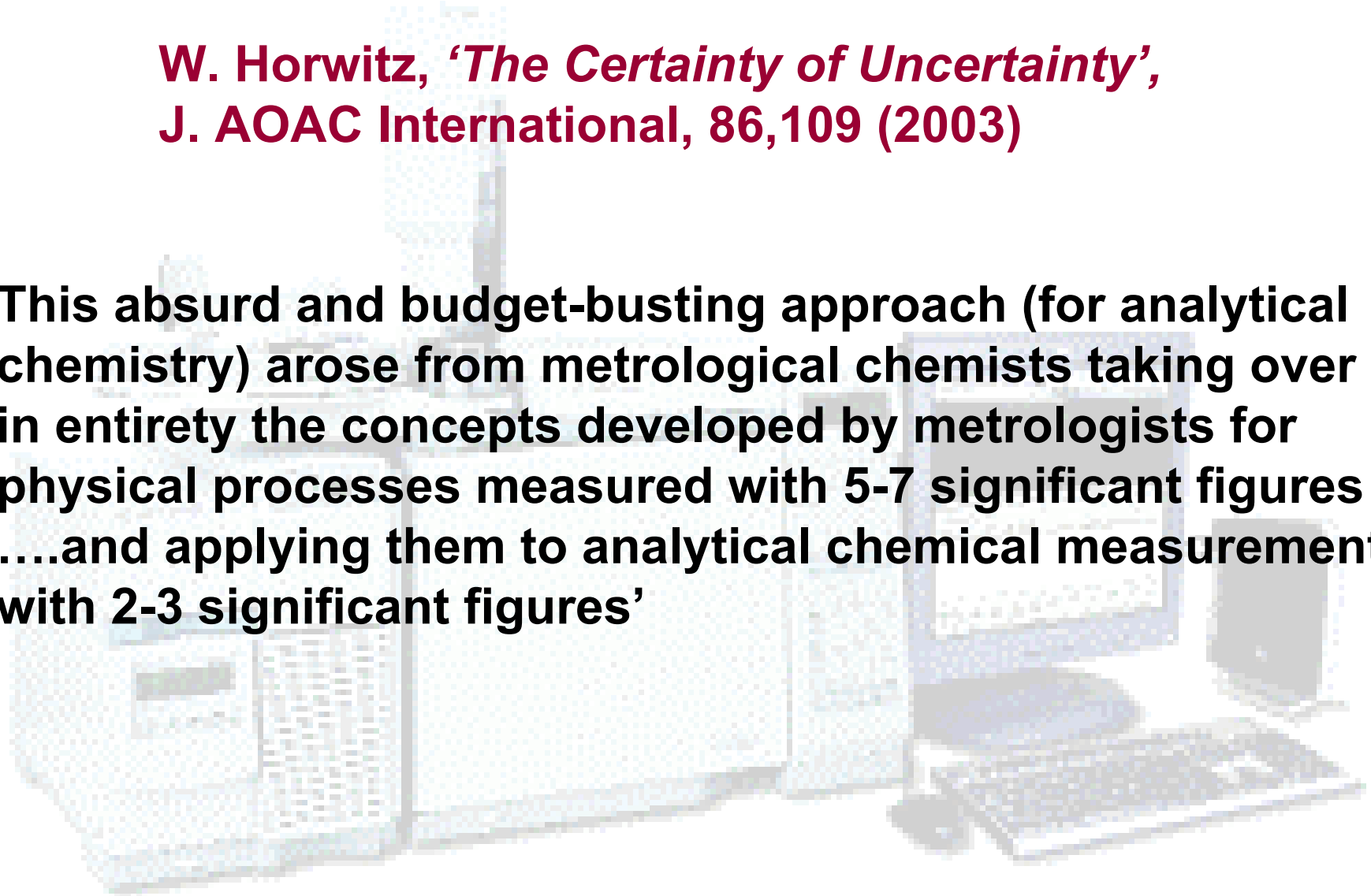
For example,

$$TCA = 50 \pm 20 \mu\text{g/L}$$

applying a coverage factor of 2 to give approximately 95% confidence.

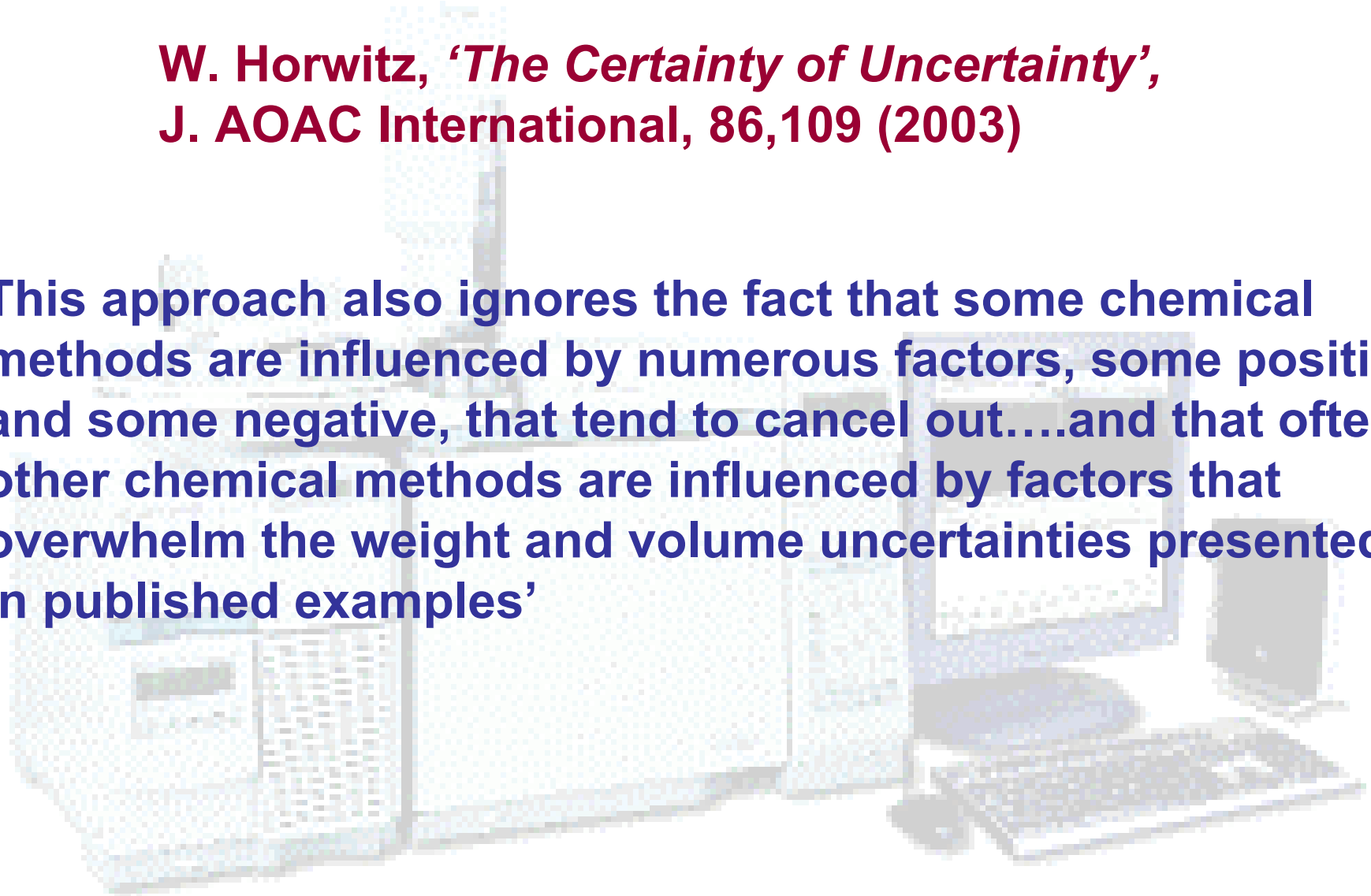
**W. Horwitz, *'The Certainty of Uncertainty'*,  
J. AOAC International, 86,109 (2003)**

**'This absurd and budget-busting approach (for analytical chemistry) arose from metrological chemists taking over in entirety the concepts developed by metrologists for physical processes measured with 5-7 significant figures ....and applying them to analytical chemical measurements with 2-3 significant figures'**



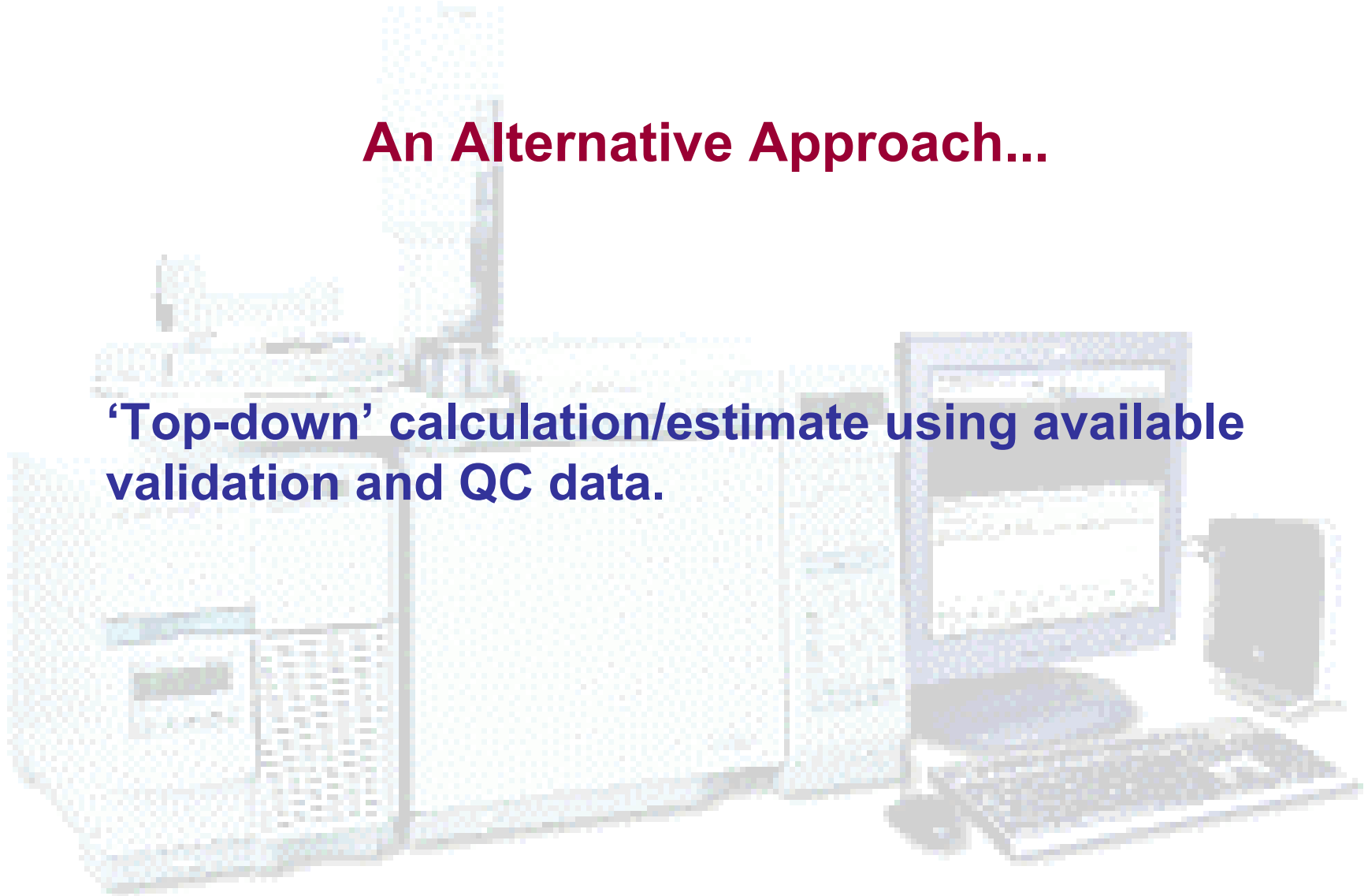
**W. Horwitz, *'The Certainty of Uncertainty'*,  
J. AOAC International, 86,109 (2003)**

**'This approach also ignores the fact that some chemical methods are influenced by numerous factors, some positive and some negative, that tend to cancel out....and that often other chemical methods are influenced by factors that overwhelm the weight and volume uncertainties presented in published examples'**



## **An Alternative Approach...**

**'Top-down' calculation/estimate using available validation and QC data.**



# **ACCURACY: Trueness (bias) and Precision**

a 'reasonable' estimate of MU may be obtained by considering the uncertainties associated with imprecision and bias

## **Validation of USEPA Method 552.2 (Table 4)**

Data from 7 replicate analyses of water spiked with TCA at 2.0  $\mu\text{g/L}$ , conducted during method validation:

Mean result = 1.74  $\mu\text{g/L}$  (87% recovery)

Standard deviation,  $s = 0.144 \mu\text{g/L}$  (8.3% RSD)

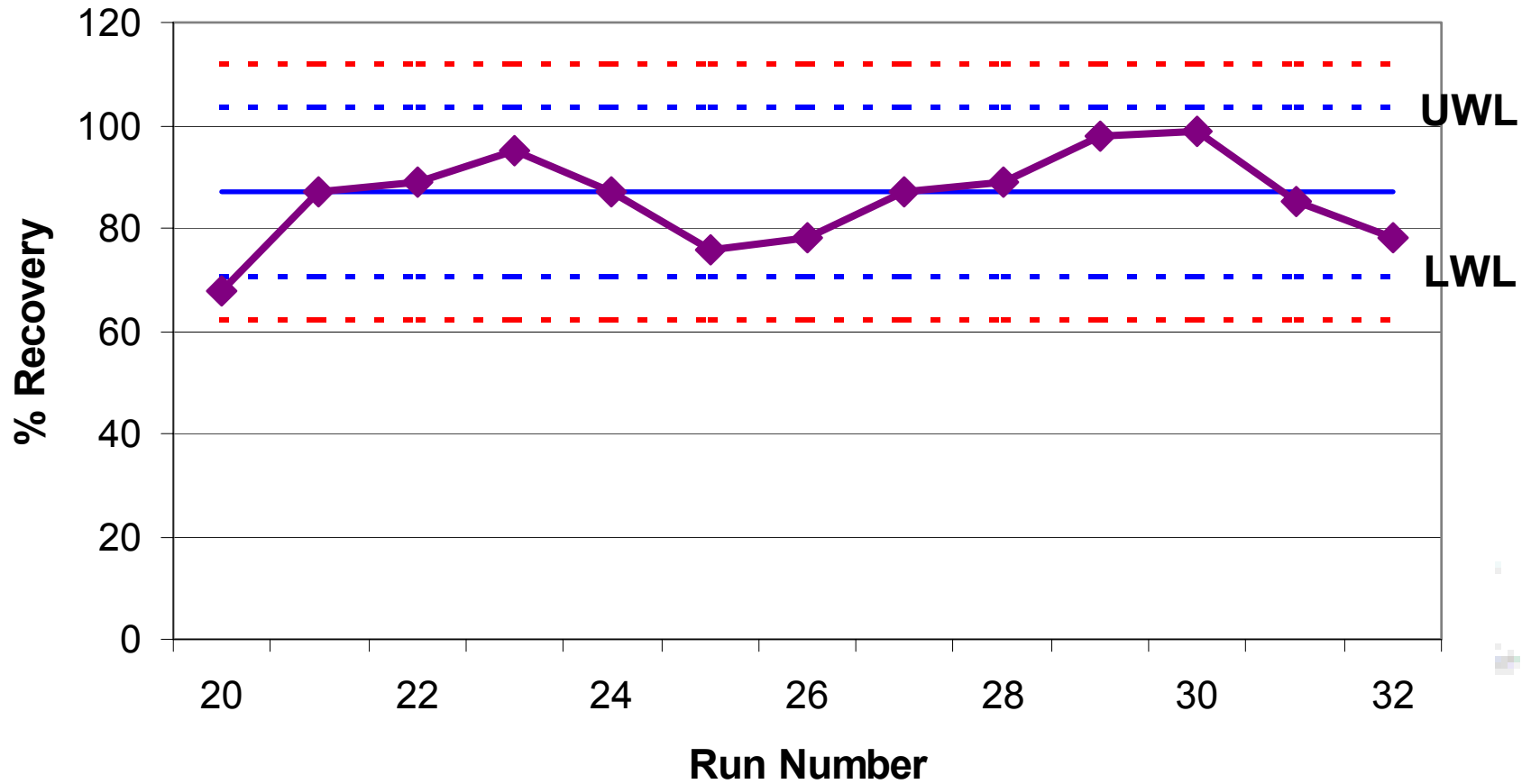
$$\mathbf{u}_{\text{TCA}} = \sqrt{\left(\mathbf{u}_{\text{Precision}}\right)^2 + \left(\mathbf{u}_{\text{bias}}\right)^2}$$

**Ideally we should consider the precision and bias of each analytical run, but this is not practical or cost-effective**

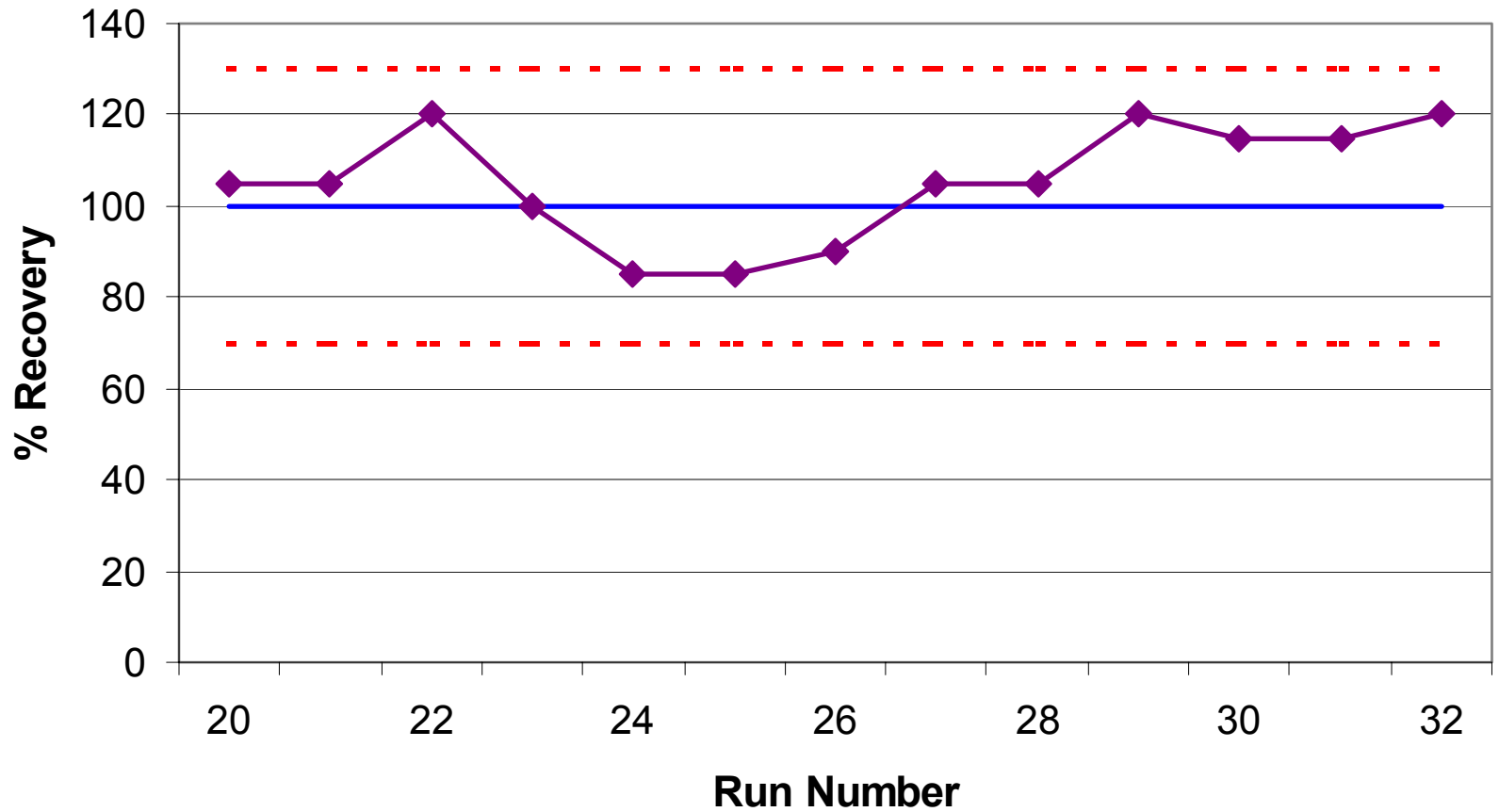
**A reasonable estimate of precision is the standard deviation of replicate tests over the short-medium term**

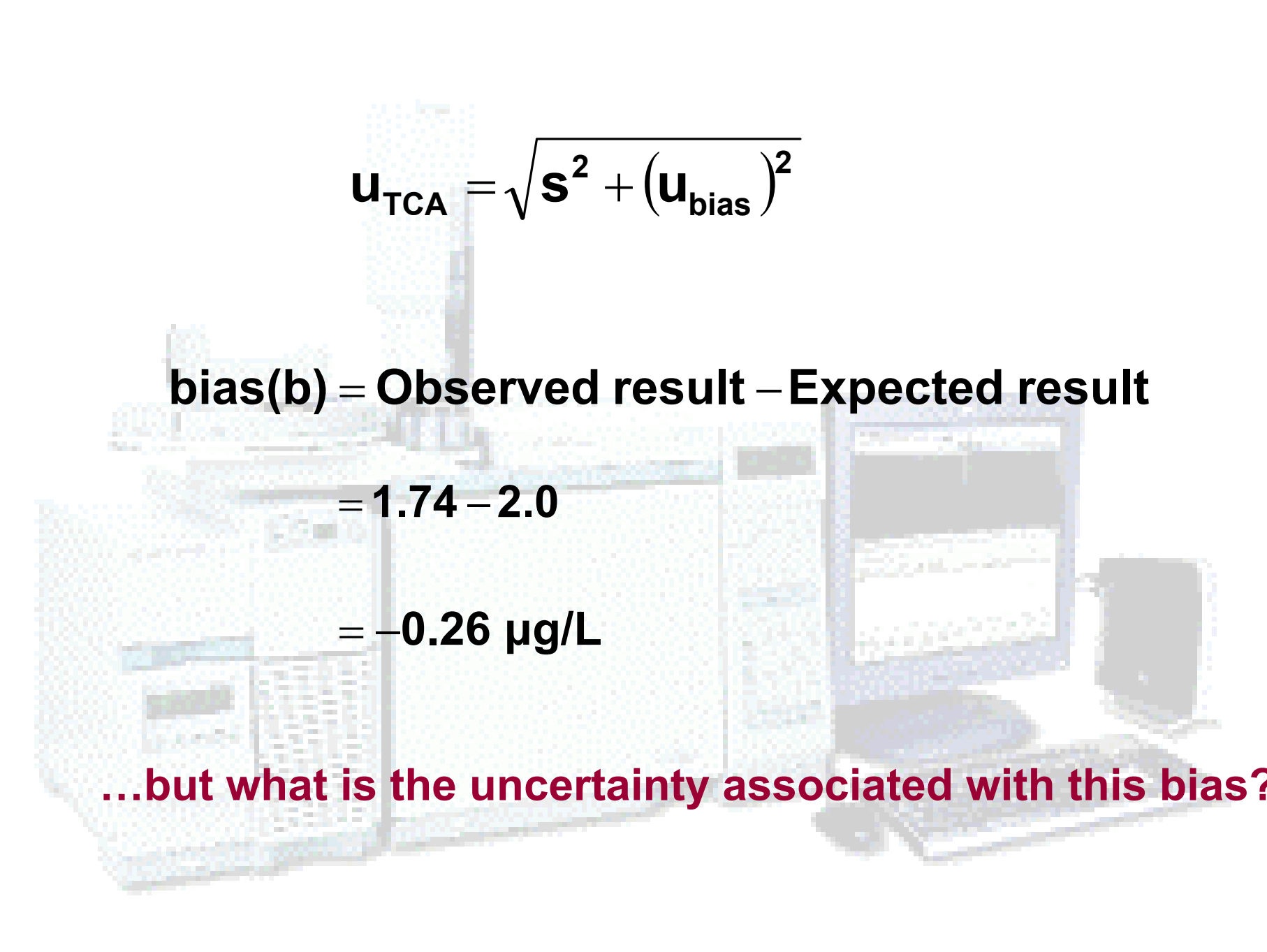
**Providing we can be confident the method is under control, a reasonable estimate of bias is the average bias recorded over the short-medium term**

# Control Chart: Internal QC



# Surrogate Recovery: Run to Run




$$u_{\text{TCA}} = \sqrt{s^2 + (u_{\text{bias}})^2}$$

**bias(b) = Observed result – Expected result**

$$= 1.74 - 2.0$$

$$= -0.26 \mu\text{g/L}$$

**...but what is the uncertainty associated with this bias?**

**bias(b) = Observed result – Expected result**

$$\mathbf{u_b} = \sqrt{(\mathbf{u_o})^2 + (\mathbf{u_E})^2}$$

$$\approx \sqrt{(\mathbf{u_o})^2}$$

$$\mathbf{u_o} = \mathbf{sdm} = \frac{\mathbf{0.144}}{\sqrt{\mathbf{7}}} = \mathbf{0.0544 \mu g/L}$$

$$\mathbf{u_b} = \mathbf{0.0544 \mu g/L}$$

$$b = -0.26 \mu\text{g/L}$$

$$u_b = 0.0544 \mu\text{g/L}$$

*'Is this Bias, taking into account the uncertainty associated with it, significant?'*

$$|b| > 2u_b \quad \text{YES}$$

**So we need to:**

(i) Correct for bias as well as consider  $u_b$  in our estimate of  $\mu$ , or

**PREFERRED OPTION**

(ii) Expand our estimate of uncertainty to account for uncorrected bias

**G.E.O'Donnell & D.B.Hibbert**  
***Analyst*, 130, 721-729 (2005)**

$$U_{TCA} (95\%CI) = 2u_{TCA} + |b|$$

$$U_{TCA} = 2 \sqrt{s^2 + (u_b)^2} + |b|$$

$$= 2 \sqrt{(0.144)^2 + (0.0544)^2} + 0.26$$

$$= 0.57 \mu\text{g/L}$$

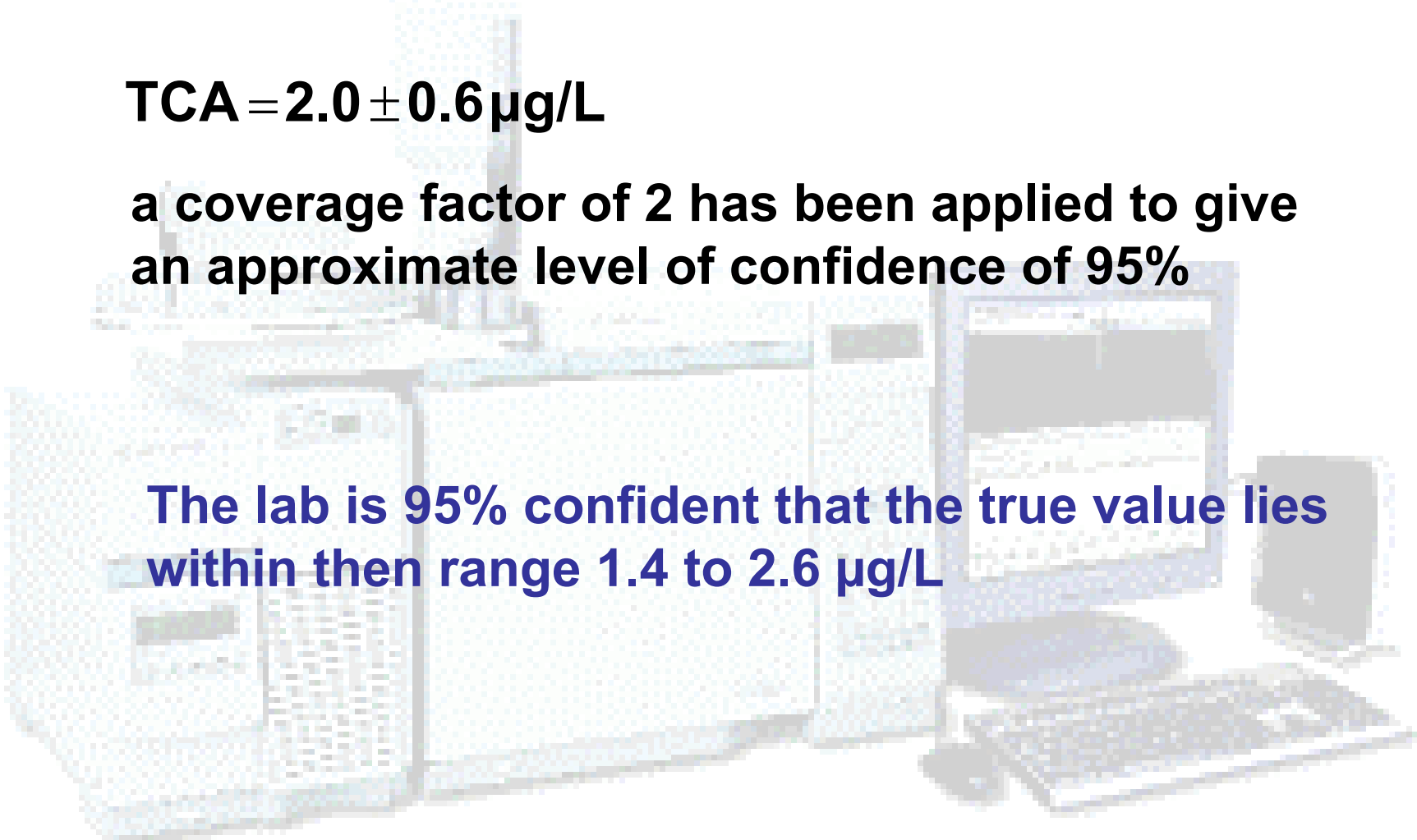


# Reporting the Result with its estimated MU

$$\text{TCA} = 2.0 \pm 0.6 \mu\text{g/L}$$

a coverage factor of 2 has been applied to give an approximate level of confidence of 95%

The lab is 95% confident that the true value lies within then range 1.4 to 2.6  $\mu\text{g/L}$



# Other Approaches to Estimating MU

Twice Reproducibility from inter-lab studies

Horwitz function

**Useful References:**

**Eurachem/CITAC Guide (2000)**

**NORDTEST Report TR537 (2003)**

**[www.measurementuncertainty.org](http://www.measurementuncertainty.org)**

# USEPA Method 552.2

**Specified QA/QC Requirements:**  
**Initial Demonstration of Capability (section 9.3.2)**  
**Run QC (sections 9.7 & 9.8)**

**Mean Recovery 80-120%,  $s < 20\%$**

**Individual Run Recovery 70-130%**

**Surrogate Recovery 70-130%**

**What is the likely MU associated with results produced by a method operating within these specifications?**

Assuming a lab achieved a mean recovery = 85% with  $s = 15\%$  for the determination of TCA in water, (well within the acceptable limits imposed by the method):

**If results** are not corrected for bias:

at 50  $\mu\text{g/L}$ ,  $42 \pm 21 \mu\text{g/L}$  (95% CI)

at 100  $\mu\text{g/L}$ ,  $85 \pm 42 \mu\text{g/L}$  (95% CI)

**Regulatory Limit**

**If results** are corrected for bias:

at 50  $\mu\text{g/L}$ ,  $50 \pm 14 \mu\text{g/L}$  (95% CI)

at 100  $\mu\text{g/L}$ ,  $100 \pm 27 \mu\text{g/L}$  (95% CI)

**Regulatory Limit**

**If results** are corrected for bias & tested in duplicate:

at 50  $\mu\text{g/L}$ ,  $50 \pm 10 \mu\text{g/L}$  (95% CI)

at 100  $\mu\text{g/L}$ ,  $100 \pm 20 \mu\text{g/L}$  (95% CI)

**Regulatory Limit**

From this hypothetical (but realistic) example, if single test results are not corrected for bias,

results approximating 50 µg/L will be associated with an uncertainty of about ± 25 µg/L in order to provide 95% confidence of encompassing the true result.

Lab A	38 µg/L	(38 ± 19 µg/L)
Lab B	63 µg/L	(63 ± 32 µg/L)

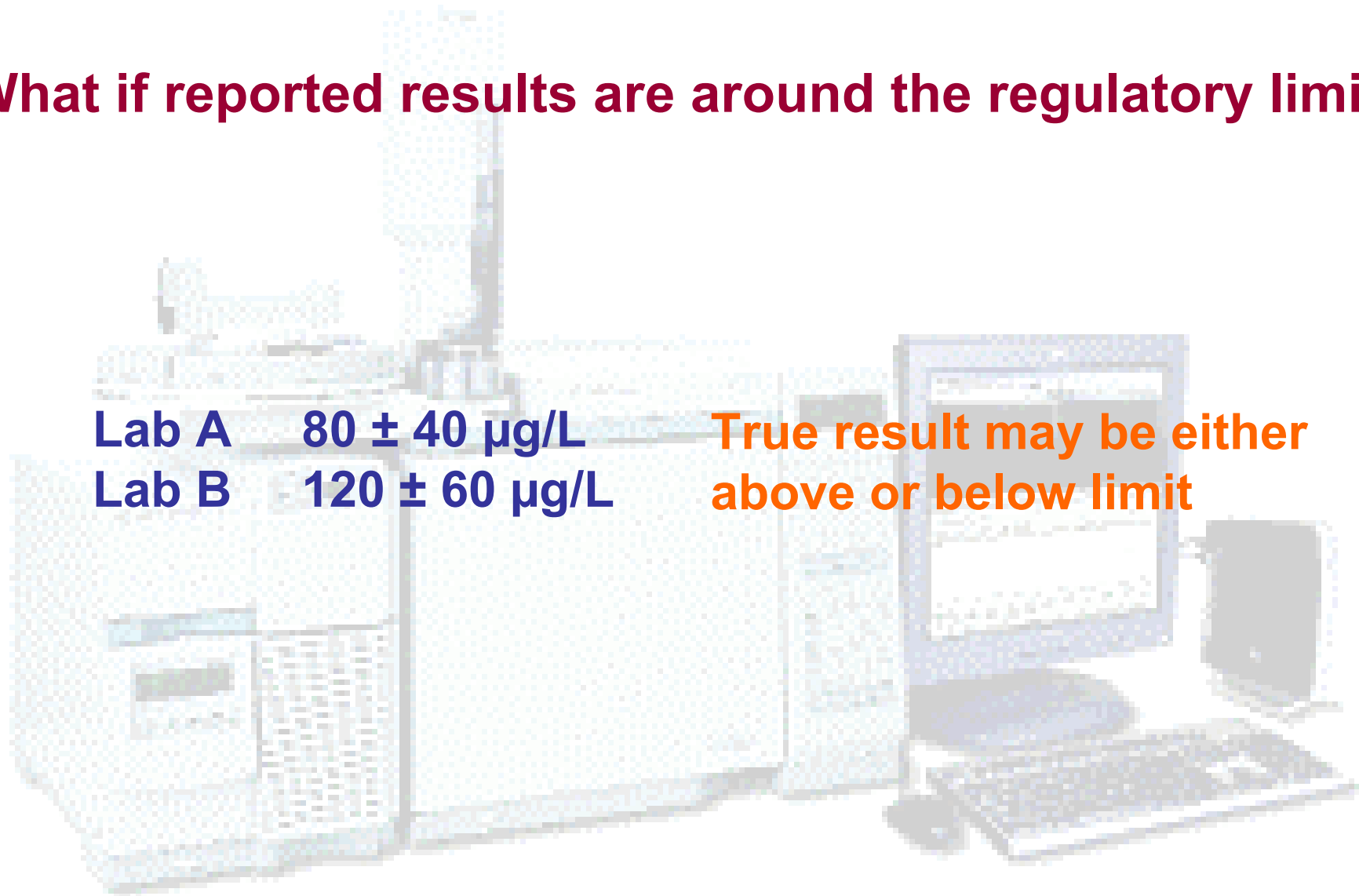
95% confidence ranges  
'overlap'; both labs' results  
may encompass true result

**What if reported results are around the regulatory limit?**

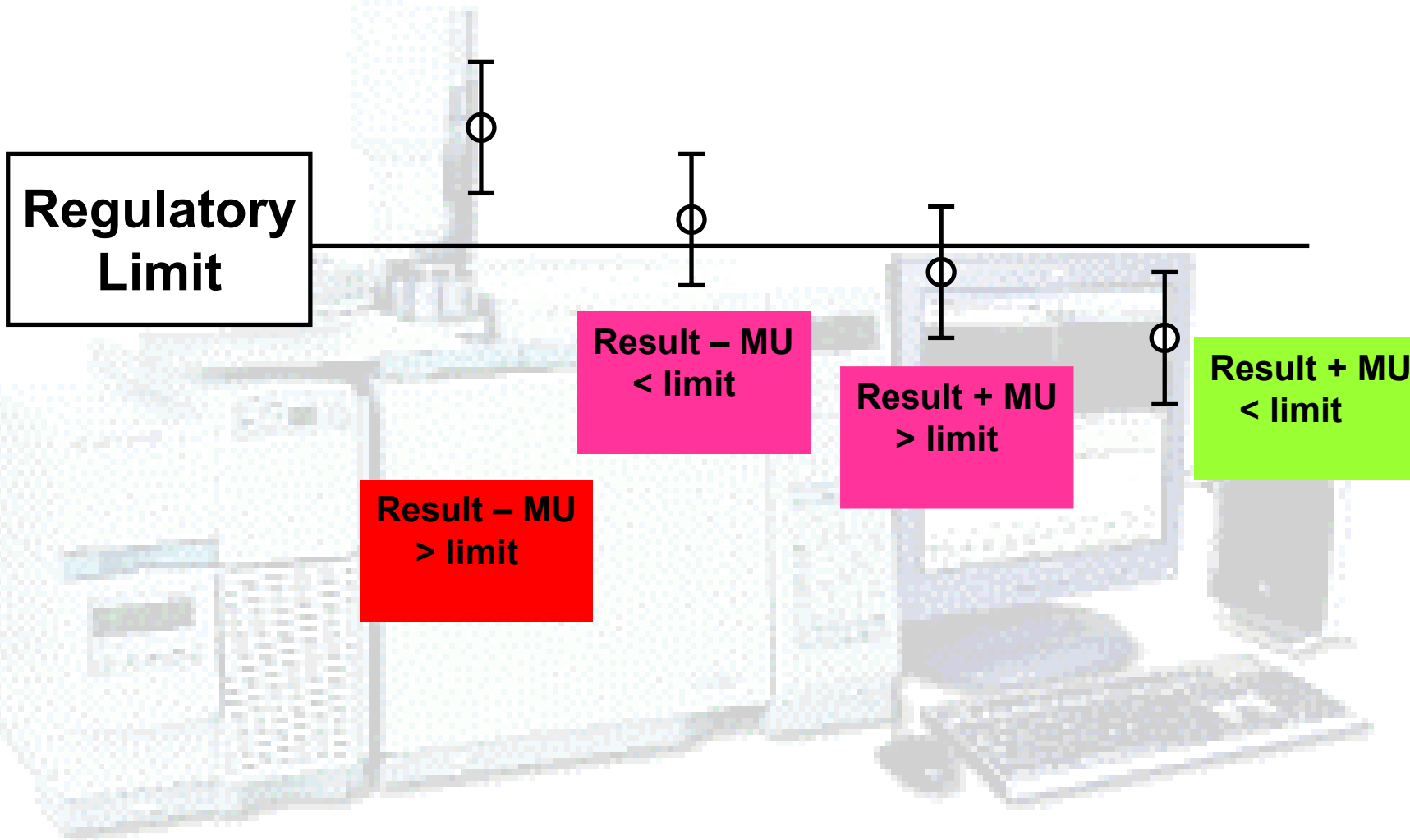
**Lab A**      **$80 \pm 40 \mu\text{g/L}$**

**Lab B**      **$120 \pm 60 \mu\text{g/L}$**

**True result may be either  
above or below limit**



# The Regulator's Dilemma



# The regulator's dilemma: Response to equivocal results

## For consideration:

risks associated with exceeding limit

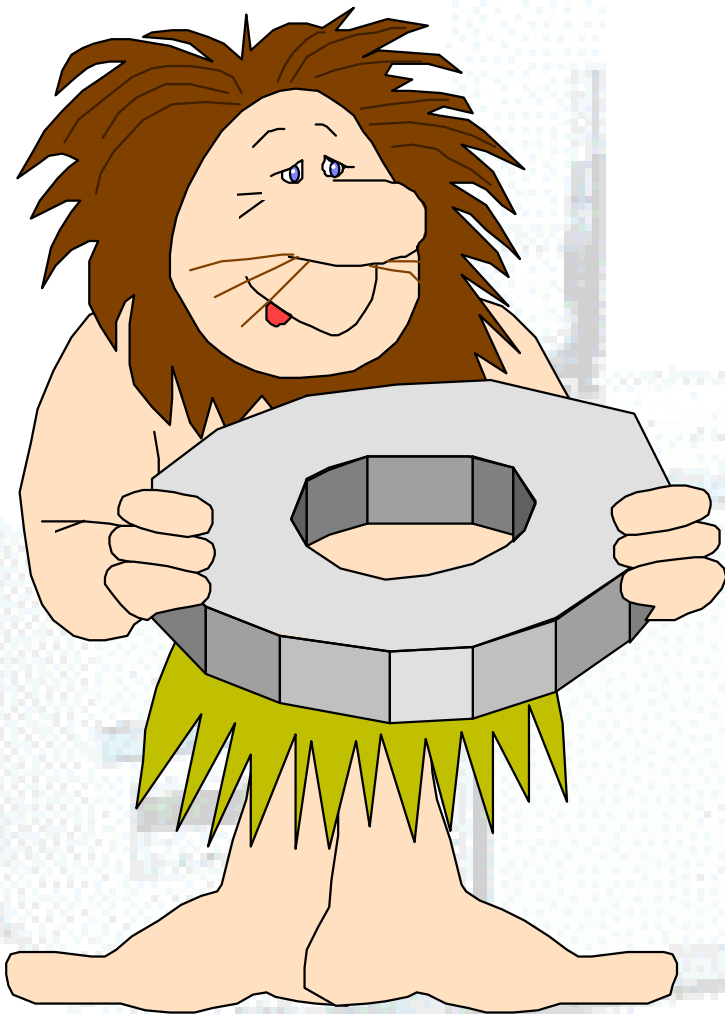
whether event is transient or lasting

is 'flexible' or 'conservative' approach warranted

## Responses could include:

disregard MU





**Original Specification  
'before MU'**

**hole size  $10.0 \pm 0.2$  mm**

**Measurement Uncertainty**

**U (95% CI) =  $\pm 0.2$  mm**

**Revised Specification  
'if MU taken into account'**

**hole size  $10.0 \pm 0.4$  mm**

# The regulator's dilemma: Response to equivocal results

## For consideration:

risks associated with exceeding limit

whether event is transient or lasting

is 'flexible' or 'conservative' approach warranted

## Responses could include:

disregard MU

no action unless  $(\text{result} - \text{MU}) > \text{limit}$

action if  $(\text{result} + \text{MU}) > \text{limit}$

re-test or more regular testing

specifications for acceptable MU at regulatory limit



**Thanks for  
your  
attention**