

# IPPC Discharges Monitoring Workshop

## Analytical Procedures and Method Validation

Peter Webster

Regional Chemist (EPA Cork)



# Contents ...

- Overview of data quality
- Selection of suitable Analytical Methods
  - Choosing your method
  - Use of test kits for regulatory reporting
  - Fitness for purpose
- Method Validation
  - Determining Performance Characteristics and suitability
  - Demystifying detection / reporting limits
  - Determining Measurement Uncertainty

# Overview of EPA monitoring

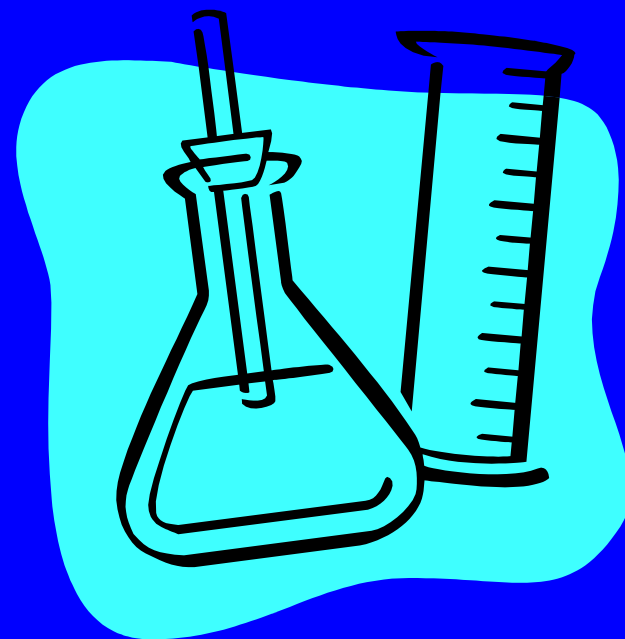
- EPA monitor over 800 IPPC facilities and approx 150 Waste Management facilities annually
- Urban Wastewater Treatment plants legislation introduced in 2009 will add a further 520 WWTPs by 2012
- All of these facilities have a requirement for ‘Self-Monitoring’
- Quality of regulatory data varies significantly between facilities hence the need for this workshop

# Typical problems observed

- No documented operating procedures for test methods
- Limited training on test methods given to WWTP staff
- In-house 'Variations' from standard/ reference methods
- Performance characteristics of method not determined
- Lack of adequate / suitable Quality Control
- No calibration checks or maintenance of equipment
- Method sensitivity not adequate to determine compliance
- Delays in submission of samples to contract labs
- No checks on competency of external contractors

# Choosing your test method

- Suitable for matrix (effluent)
- Appropriate measurement range
- Free from likely interferences
- Ease of application
- Does it need 'specialist' equipment



## Choosing your test method contd.

- Is an approved / regulatory method based on CEN / ISO / AWWA APHA 'Standard Methods' available for use?
- What will the results be used for?
  - e.g. Regulatory Compliance or Process Control
- Is the Limit of Quantitation achievable at least 1/10<sup>th</sup> of the regulatory ELV
- Use a 'standard' method / test kit / or develop in-house?

# Choosing your test method contd.

- **BS ISO 17381:2003** - “Water Quality – Selection and Application of ready-to-use test kit methods for water analysis”
  - Useful guide to when test kits may be suitable for use
- **BS 1427:2009** – Guide to on-site test methods for the analysis of waters
  - Provides practical guidance on the choice and limitations of test kits available for many routine water and IPPC / wastewater analysis such as COD / BOD / Nutrients etc.







# A (cautionary) word on use of Test Kits

- Wide range of proprietary Test kits available
- Convenient for use by non-scientific staff
- No reagent preparation necessary
- Can be cost-effective for in-situ or even lab analyses
- **BUT**
- Interferences may not always be well documented
- Quality of instructions varies significantly
- May suffer from interferences due to colour / turbidity
- Limitations need to be appreciated and understood

## Fit for purpose?

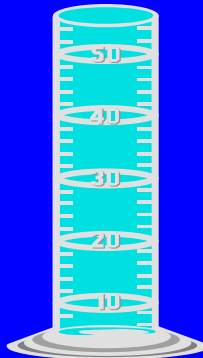
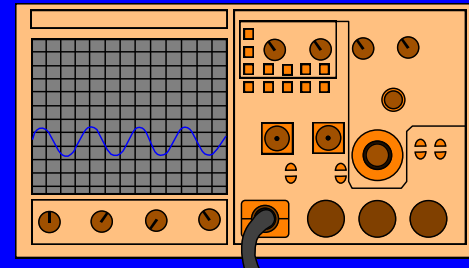
“The degree to which data produced by a measurement process enables the user to make technically and administratively correct decisions for a stated purpose”

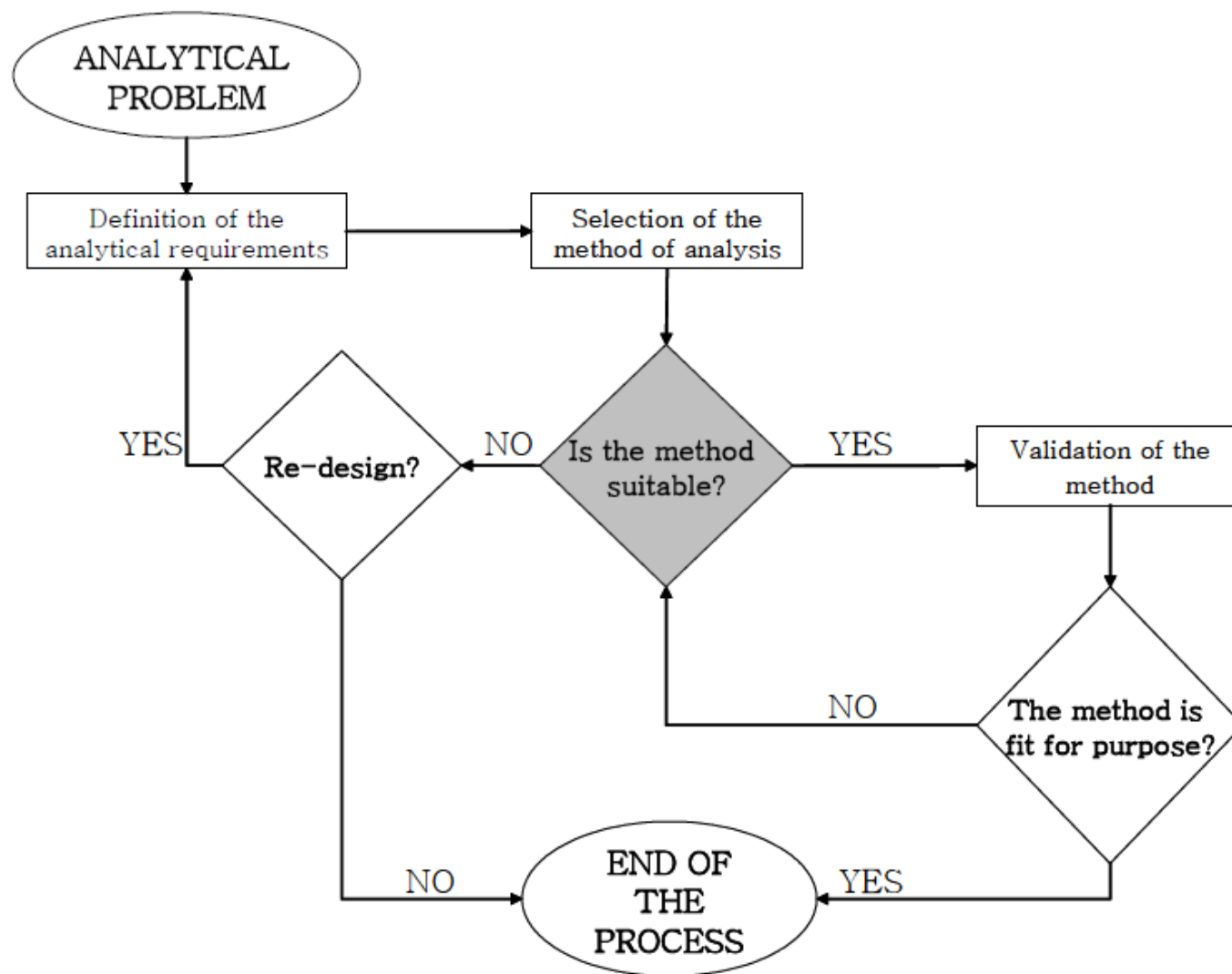
IUPAC Compendium of Analytical Nomenclature ...  
the “Orange book” ISBN 0-632-05127-2

[http://old.iupac.org/publications/analytical\\_compendium/](http://old.iupac.org/publications/analytical_compendium/)

# Fit for purpose?

- What do require the method to be capable of?
- Performance characteristics should be adequate to meet the needs of the user / Regulator as appropriate
- Should be specific to the analyte of interest (interferences known)
- Determined by evaluating **actual** performance and not just that stated by the supplier / manufacturer
- Ref: **“The Fitness for Purpose of Analytical Methods”**  
**EURACHEM**





**Figure 1.** Fitness for purpose concept. Adapted from the EURACHEM *The Fitness for Purpose of Analytical Methods* [4].

# Main causes of 'unfit for purpose' results

- Operator competence (what training provided?)
- Matrix interferences with your particular sample type
- Inappropriate test kit employed ... Needs to meet your client needs
- Contamination (of sample and /or equipment)
- Inappropriate sampling protocol / sample pre-treatment (needs to be agreed with your client)
- Wrong range of measurement ... aim for your 'critical limit' to be 60 – 80 % of test kit range

# Definition of 'Validation'

- Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled (*ISO/IEC 17025:2005 cl. 5.4.5.1*)
- A process of evaluating method performance and demonstrating that it meets a particular requirement
- In essence, it is knowing what your method is capable of delivering, particularly at low concentrations

# Method Validation .. When ?

- When should a method be validated to verify its performance parameters are adequate for use for a particular analytical problem?
  - New method ... no previous performance history
  - Revised or established method adapted to a new problem
  - When a review of AQC indicates an established method is changing with time
  - When an established method is used in a different laboratory, with different analysts or with different equipment
  - To demonstration of the equivalence between two methods, e.g. a new method vs. a standard method.

# Principal Components

- Selectivity and Specificity
  - Accuracy
  - Precision (Within batch and Between batches)
  - Range and Linearity
  - Detection Limit
  - Limit of Quantitation
  - Robustness & System suitability
  - Uncertainty of Measurement
- 
- **INAB Guide to Method Validation for Quantitative Analysis in Chemical Testing Laboratories**





# Selectivity and Specificity

- Selectivity

- This is the ability to discriminate between the analyte or species to be determined and other materials in the test sample

- Specificity

- The ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample such as degradation products
- Need to be aware of potential interferences ...especially for in- house developed methods. These are usually well documented for ‘standard’ methods

# Selectivity and Specificity

- **Selectivity and Specificity** can be a real problem with gas / liquid chromatographic methods
  - May be necessary to confirmation on more than one column or by use of different eluents
- Ion Chromatographic analysis for anions / cations offers good separation but there is often interference in early eluting peaks from organic compounds
  - e.g. Acetate / Formate elute just ahead of Fluoride on carbonate eluent IC columns

# Accuracy

- **Accuracy = Trueness**

**Accuracy is the closeness of agreement between a test result and the accepted reference or true value of the property being measured**

- Can be evaluated as '**Bias**' by
  - use of certified reference materials as controls
  - the use of a traceable reference material or material prepared 'in-house'
  - the use of a standard / reference method with little or no systematic error
  - the use of the method when participating in a proficiency testing scheme
  - the use of spiked samples, based on blank or positive samples
  - from assessment of routine AQC Charts

# Precision

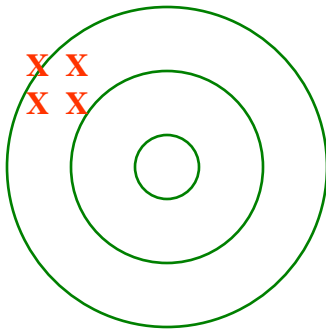
- Precision = Repeatability
- Combination of Within batch (Internal) and Between batches variability
  - Influenced by changes in analyst, instrument conditions, reagents etc.
  - Can be assessed (in simplest way) by analysis at least 10 times of known material singly or in replicate. The SD of single measurements gives an estimate of  $S_b$  whereas SD of replicate measurements gives an indication of  $S_w$
- Long term assessment can be derived from AQC charts
- **ISO 5725-2** “Accuracy (trueness and precision) of measurement methods and results

# Accuracy vs. Precision

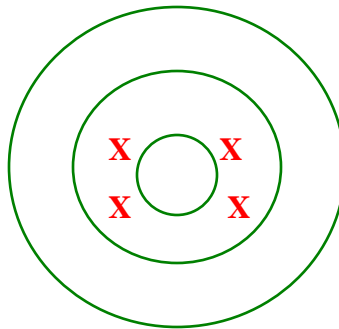
**Accuracy = Closeness to true value (error = Bias)**

**Precision = Measure of ...**

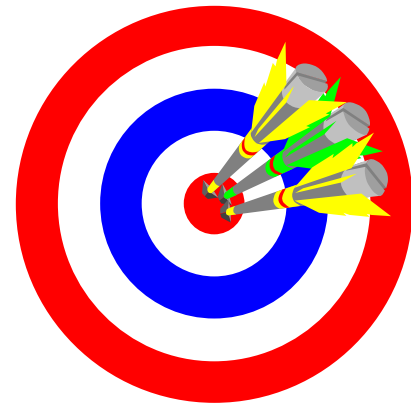
- **Repeatability (Within Batch)**
- **Reproducibility (Between batch)**



**Precise but not  
Accurate**



**Accurate but not  
Precise**



**Perfection !!**  
Only rarely achieved.

# Range and Linearity

- **All methods have an upper and lower boundary of applicability and may not be linear over all concentrations**
  - E.g. ISE electrodes exhibit non-linearity at ca  $10^{-5}$  Molar
- **Choose method whose range brackets those found in your samples (or dilute samples to fit range)**
  - E.g. COD vials come in a series of ranges. If your effluent is typically 50 there is a greater error (typically ca. 5%) when using a 10 -1500 mg/l range tube than when using a 10 -150 mg/l range tube. Also check the spectrometer preset calibration at regular intervals using known standards
- **Check using known standards at appropriate intervals**

# Detection Limits

- The most controversial part of any Method Validation
- Can be generally derived in three main ways
  - **Use of a multiple of Blank SD within a single batch**
    - Simple, Quick but dependant on one analyst set up of instrument
    - May not be reproducible between analysts or over time
  - **From slope of calibration (assumes linear / quadratic fit)**
  - **Calculation based on multiple batches in replicate**
    - Based on multiple batches in replicate
    - Robust and generally gives more reliable estimate than above
    - **EPA recommended approach**

# Detection Limit ... Definitions

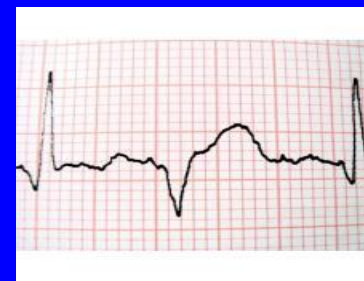
- The lowest concentration that can be measured with reasonable statistical certainty (AOAC)
- The lowest concentration of analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test (NATA Tech, Note #3)
- The smallest concentration that can be determined statistically different from a blank at a specified level of confidence (typically 95%). This corresponds to the critical level. (Currie, 1988 Am. Chem. Soc)
- The true net concentration or amount of the analyte in the material to be analysed which will lead with probability  $(1-\beta)$ , to the conclusion that the concentration of the analyte in the analysed material is larger than that of the blank matrix (ISO DIS 11843-1)
- **The output signal or value above which it can be affirmed with a stated level of confidence, for example 95 %, that a sample is different from a blank sample containing no determinand of interest. (ISO 13530 :2009)**



# Detection Limit ... which one?

- **Instrumental detection limit**

Equates to the smallest signal above background noise that can be detected reliably. Typically 3x Signal/ Noise ratio but this is of little practical value in measurement terms



- **Method Detection Limit (MDL)**

Min. concentration reportable to 99% CL that analyte > zero. Determined from analysis of a low sample concentration in given matrix. SD x student's t value or **3.14 for 7 measurements**. (40 CFR Part 136, Appendix. B)

MDL = “Criterion of Detection” (ASTM)

# Detection Limits and LoQ

- **(Statistical) Limit of Detection (LoD)**

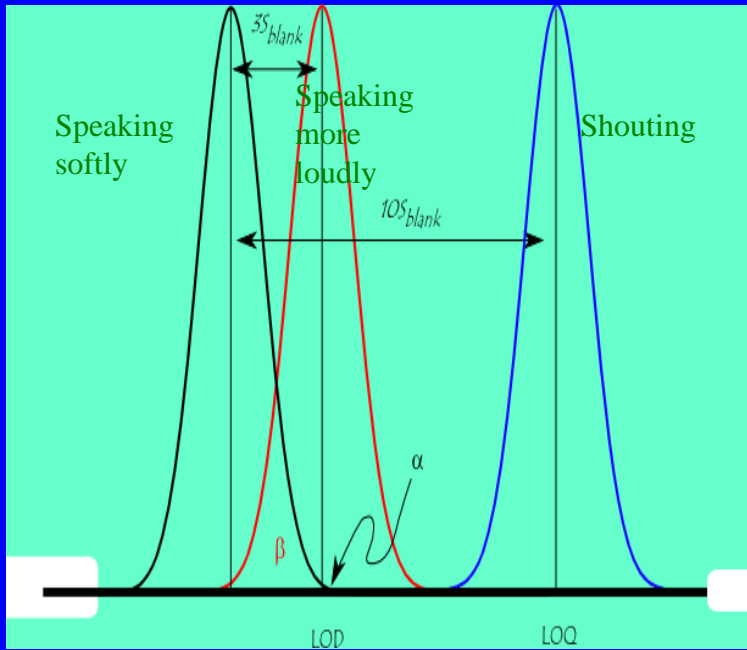
*The output signal or value above which it can be affirmed with a stated level of confidence, for example 95 %, that a sample is different from a blank sample containing no determinand of interest* **ISO 13530 :2009**

- **$LOD = 2\sqrt{2} \cdot t(df, \alpha = 0.05) \cdot s_w$**

- *For a one-sided test with 11 degrees of freedom  $t = 1.796$  and this transposes as  **$LOD = 5.08s_w$***

- **Ref: ISO 13530 :2009 “Water Quality – Guide to analytical quality control for water analysis”**

- *However Directive 2009/90/EC on WFD criteria cites LoD as only  **$3s_w$***



It is often difficult to understand the concept of detection limit. The following example may help to clarify some of the concepts defined previously.

(Source : Wikipedia)

Suppose you are at an airport with lots of noise from jets taking off. If the person next to you speaks softly, you will probably not hear them. Their voice is less than the LOD. If they speak a bit louder, you may hear them but it is not possible to be certain of what they are saying and there is still a good chance you may not hear them. Their voice is  $>LOD$  but  $<LOQ$ . If they shout then you can understand them and take action on what they are saying and there is little chance you will not hear them. Their voice is then  $>LOD$  and  $>LOQ$ . Likewise, their voice may stay at the same loudness, but if the noise from jets is reduced allowing their voice to become  $>LOD$  you will hear them.

**Detection limits are thus dependent on both their voice (signal intensity ) and background noise (jet noise)**

# Limit of Quantitation / Practical Reporting Limit

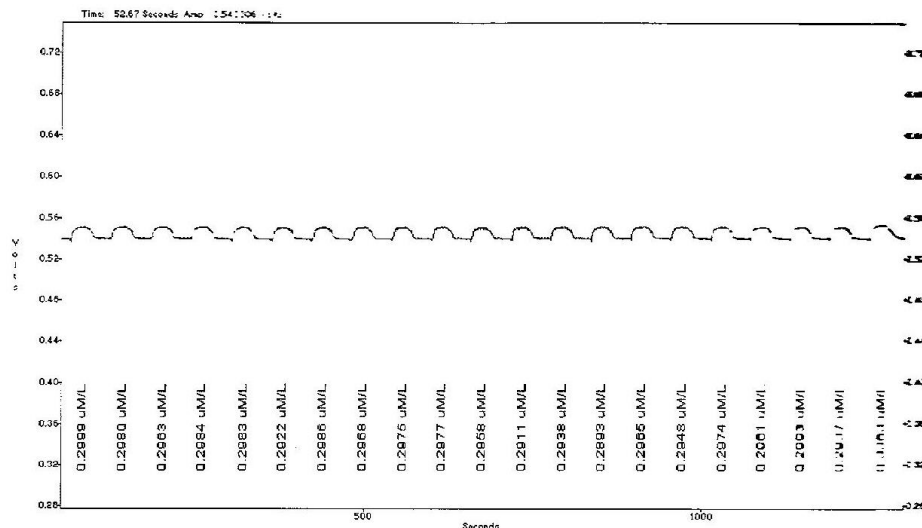
- **Practical Reporting Limit / Limit of Quantitation (PRL or LoQ)**

Stated multiple of the Limit of Detection, for example, 2 - 3 x LoD, at a concentration of the determinand that can reasonably be determined with an acceptable level of accuracy and precision.

The **Limit of Quantification** can be calculated using an appropriate standard or sample, and may be obtained from the lowest calibration point on the calibration curve (excluding the blank).

*ISO 6107-2:2006 – Water Quality – Vocabulary Part 2*

- Referred to (but not specifically defined) in ISO 13530 as  **$10s_w$**
- **LoQ / PRL is often assigned as lowest calibration standard in accredited laboratories as this can be shown to have a clearly defined, rather than theoretical, response.**

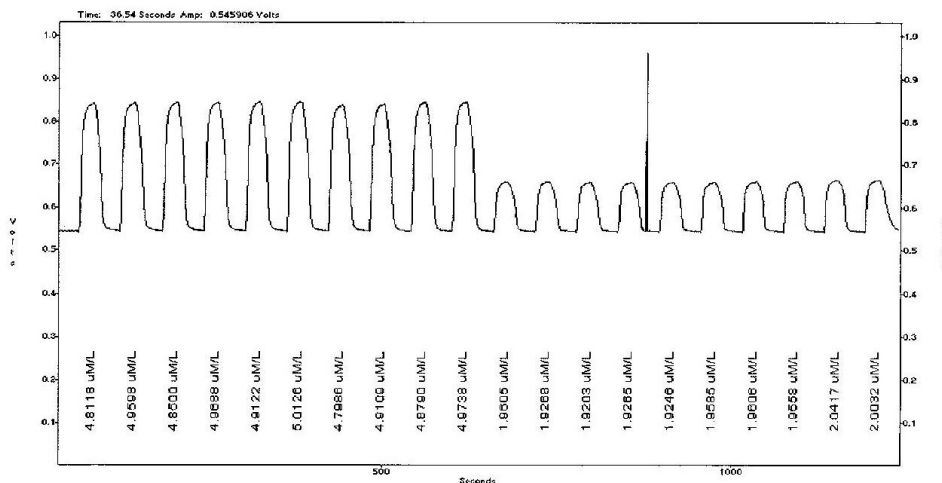


Method Detection Limit for phosphorus using 0.25  $\mu\text{M}$  P as  $\text{PO}_4$  standard

**MDL = 0.025**

Standard Deviation (s) = 0.009, Mean (x) = 0.29  $\mu\text{M}$ , Known value = 0.25  $\mu\text{M}$

Acq. Date: 14 May 1998



Precision data for phosphorus using 5.0  $\mu\text{M}$  P as  $\text{PO}_4$  and 2.0  $\mu\text{M}$  P as  $\text{PO}_4$  standard

**% RSD = 1.47**

Standard Deviation (s) = 0.072, Mean (x) = 4.91, Known value = 5.0  $\mu\text{M}$

**% RSD = 1.96**

Standard Deviation (s) = 0.038, Mean (x) = 1.96, Known value = 2.0  $\mu\text{M}$

Acq. Date: 14 May 1998

MDL extracted from Lachat test method for ortho-P in seawater

Standard used 0.25  $\mu\text{M}$  (7.8  $\mu\text{g/l}$  P)

Mean 0.29  $\mu\text{M}$ , Sw = 0.009  $\mu\text{M}$

MDL applied = 0.025  $\mu\text{M}$  (0.8  $\mu\text{g/l}$  P)

LoD 5.08sw = 1.5  $\mu\text{g/l}$  P

**PRL = probably 5  $\mu\text{g/l}$  P (low std)**

Precision extracted from Lachat test method for ortho-P in seawater

5  $\mu\text{M}$  (150  $\mu\text{g/l}$  P), Mean 4.91

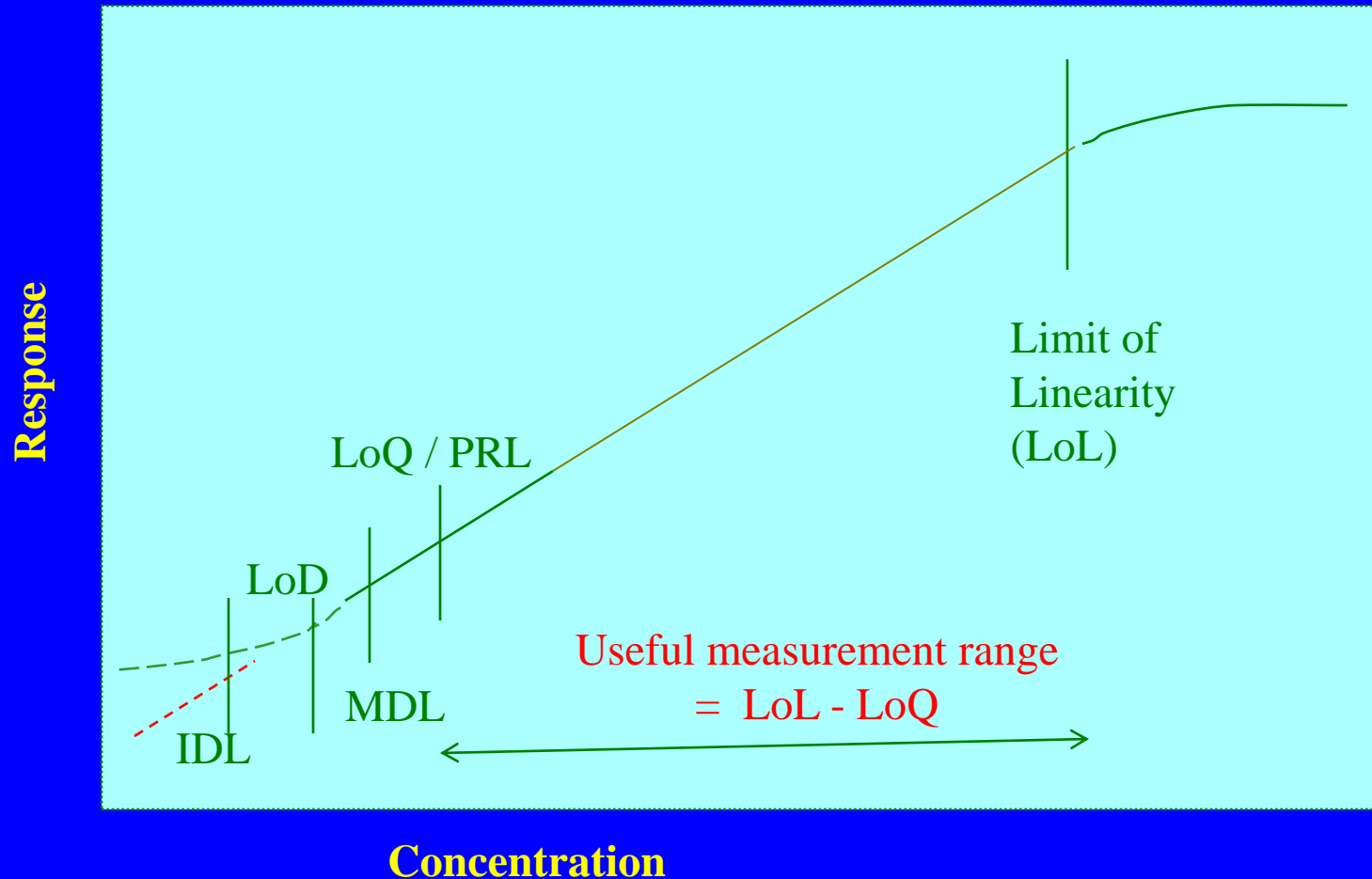
Sw = 0.072, RSD = 1.47%

2  $\mu\text{M}$  (60  $\mu\text{g/l}$  P) Mean 1.96

Sw = 0.038, RSD = 1.96%

**These look good but are they repeatable!**

# What these 'Limits' mean in practice



# Robustness / System suitability

- The analytical method should not be sensitive to small changes in procedures e.g. flow rates / reagents etc.
  - Identify any variables which may have an effect of data and set up experiments using known materials to determine effects
  - Set performance criteria for methods to achieve based on e.g. Minimum slope / Lowest standard response / Sensitivity etc.
- Robust methods will generally produce similar results for the same sample when used in independent laboratories.
- ‘Standard’ methods will generally fall into this category hence their recommended use
- Ref: **“The Fitness for Purpose of Analytical Methods “**  
**EURACHEM**

# Uncertainty of Measurement

- **No analytical measurement is an absolute value ... They all have an uncertainty which is influenced by environmental / physical factors!**
- Uncertainty can be calculated from a metrology perspective taking account of all contributory factors but this is long winded and not readily applicable to chemical analysis
- It is much simpler to use the information you will (hopefully) already have to determine UM in the event you require it.



# Uncertainty of measurement references

- The GUM ‘Bible’ ... An extensive reference but fairly unreadable even if you have a degree in statistics!
  - **EURACHEM / CITAC Guide** “*Quantifying Uncertainty in Analytical Measurement*” [www.measurementuncertainty.org](http://www.measurementuncertainty.org)
- Better
  - **Nordtest Report TRV 537** “*Handbook for calculation of Measurement Uncertainty in Environmental Laboratories Ed.2*” [www.nordtest.org](http://www.nordtest.org)
  - **Nordic Committee for Food Analysis (NMKL)** “*Estimation and Expression of Measurement Uncertainty in Chemical analysis*” [www.nmkl.org](http://www.nmkl.org)
  - **A Beginners Guide to Uncertainty of Measurement (NPL)** [www.npl.co.uk](http://www.npl.co.uk)

# But best (in my opinion) is the new Draft Std. ISO 11352

Draft for Public Comment		Form 38 Version 10.1
		DPC: 10/30174938 DC
BSI Group headquarters 389 Chiswick High Road London W4 4AL Tel: +44 (0)20 8996 9000 Fax: +44 (0)20 8996 7400 www.bsigroup.com		Date: 29 March 2010 Origin: International
Latest date for receipt of comments: 30 June 2010		Project no.: 2007/03288
Responsible committee: EH/3/2 Physical chemical and biochemical methods		
Interested committees: EH/3		
Title: Draft BS ISO 11352 Water quality - Determination of measurement uncertainty based on validation data		
Supersession information: If this document is published as a standard, the UK implementation of it will supersede NONE and partially supersede. NONE If you are aware of a current national standard which may be affected, please notify the secretary (contact details below).		
<b>WARNING: THIS IS A DRAFT AND MUST NOT BE REGARDED OR USED AS A BRITISH STANDARD. THIS DRAFT IS NOT CURRENT BEYOND 30 June 2010.</b>		

This is a very readable document which gives clear examples of UM as it relates to laboratory data

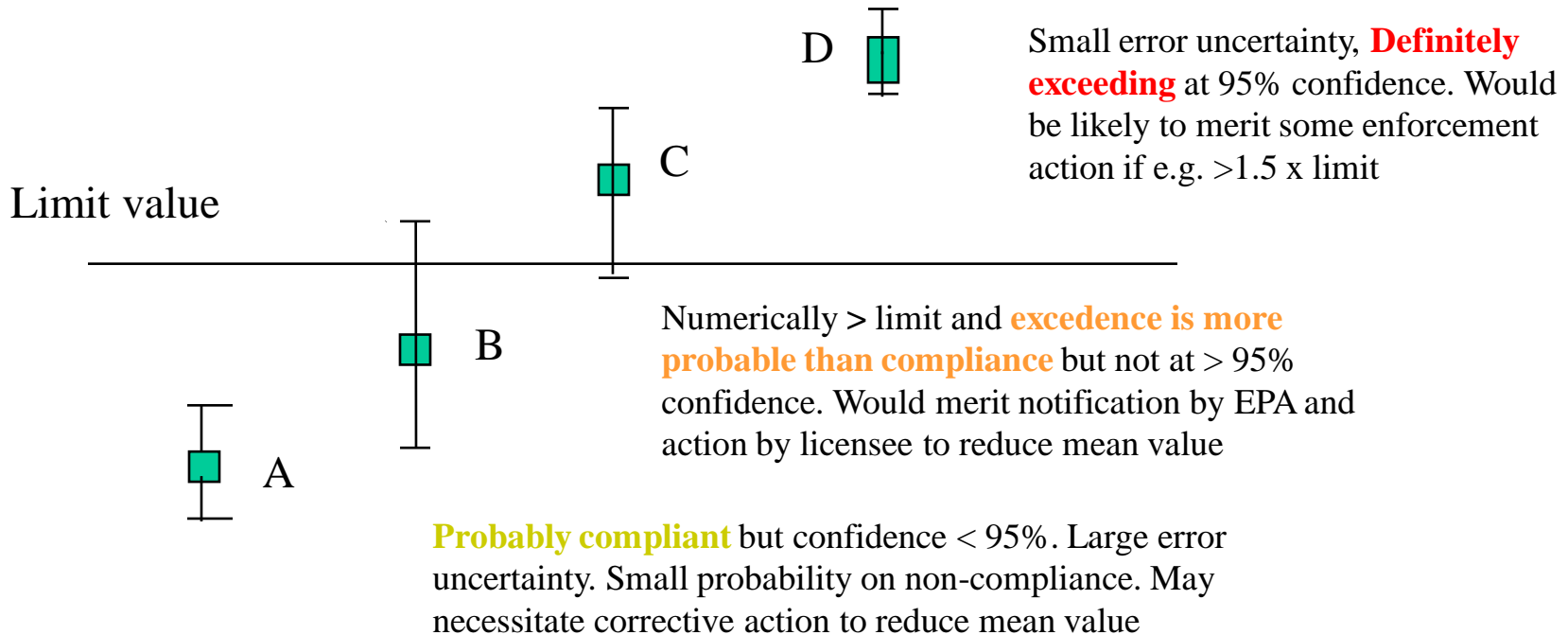
# Uncertainty of Measurement - demystified

- Assess using routine control chart precision and bias data
- Use information from reference materials, inter-lab performance tests, % recovery data
- Each input is additive
- Several good worked examples in ISO 11352
- UoM (MU) is important from a regulatory perspective because ....

# Interpreting exceedences

Assessing whether a value exceeds a regulatory limit requires consideration of the **Uncertainty of Measurement** associated with the result(s). Assessment based on single measurements (most of our data) is difficult since there is no information on the range of repeatability of the actual measurement.

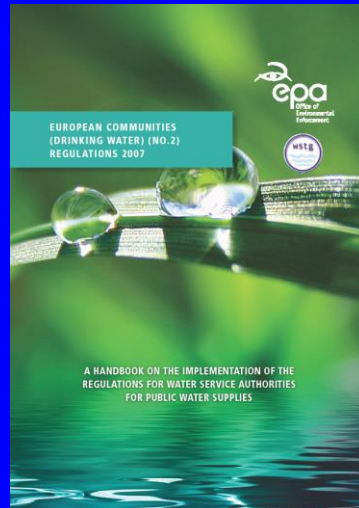
Variation has often to be inferred from historic data from replicate analysis / QC data ..  
Replicate measurements are better in “difficult” situations.



# Determining Performance Characteristics

- The EPAs preferred approach to Method Validation , particularly for anyone involved in regulatory Drinking Waters analysis, is outlined in the EPA “Handbook on the Implementation of the Regulations (...the Drinking Water Regs. 2007) for Water Services Authorities and Private Water Supplies ... Section 5”

<http://www.epa.ie/downloads/pubs/water/drinking/publicwatersupplieshandbook/>

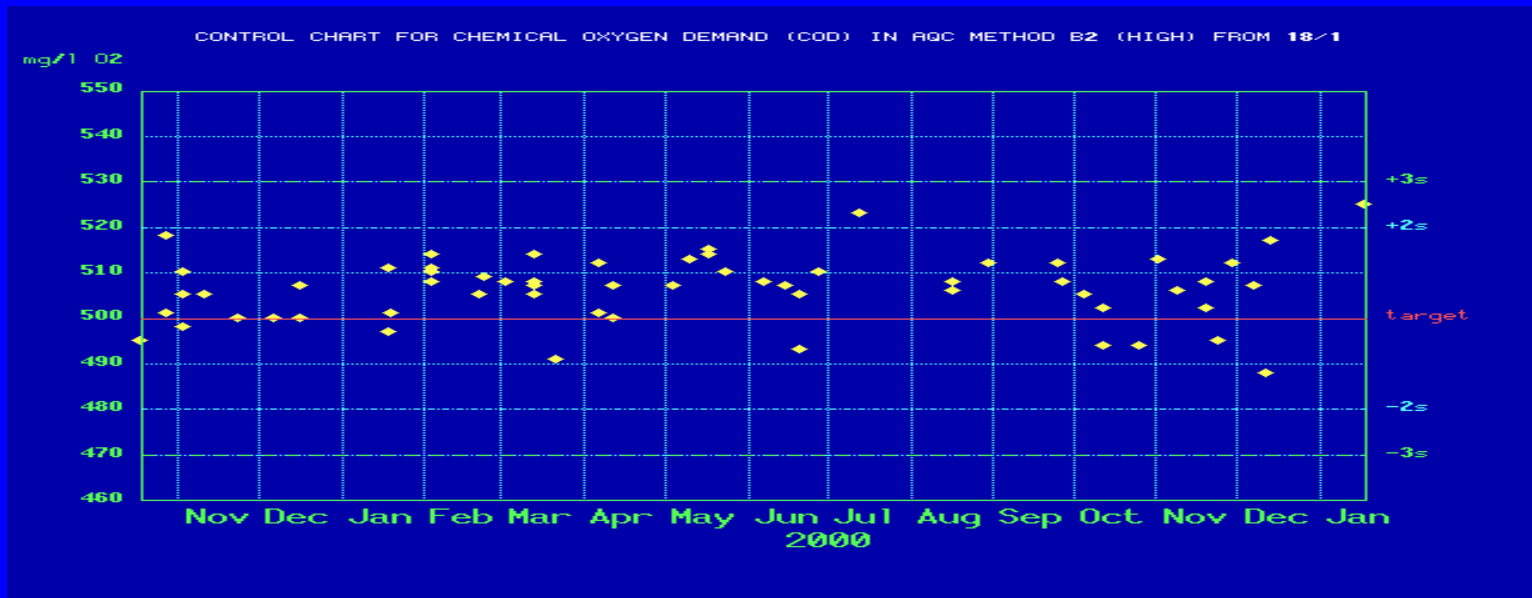


## Section 5 explained

- **Based on WRc publication NS30 (M. Gardner / A. Wilson )**
- Blank, Low Standard (20%), High Standard (80%), Low sample, Spiked sample (+ ca. 50%)
- Analyse in Duplicate over 11 batches over several days with as many analysts as possible to reflect lab practices
- Calculate the Standard deviations and Recovery from each relevant matrix e.g. Waters, Sewage, Trade wastes etc.
- EPA defines LoD as
  - **3x  $S_w$  of low sample or ...**
  - **5 x  $S_w$  of the blank solution (not calibration blank)**
- Recommends the use of Shewart Control Charts and rules

# Control Charts

- In-house Standards / Certified Reference materials
- Use of Mean and/or Mean / Range charts
- Log Difference charts for microbiology (especially if using Lenticules)



## What performance is acceptable?

- How long is a piece of string? ... It really depends on the use to which the data will be put.
- General 'Rule of Thumb' is aim for a Maximum Error Threshold of 20% comprising Bias (10%) and Std. Dev. (5%)
- Most analytical methods will meet the Bias comfortably but some will may have difficulty with the Std. Dev.
- Threshold is typically 30% for trace organics (10% SD)

Parameter	Method	% Uncert	Parameter	Method	% Uncert	Parameter	Method	% Uncert
COD	B1	5.1	TOC	B17	7.0	Silicate (300)	B44	18.5
	B2	3.2	Ammonia	B19	6.8	Silicate (3000)		13.3
PH	B3	0.08	Nitrite	B29	6.7	Salinity	B47	1.6
Conductivity	B4	1.8	Phosphate		9.8	Ammonia	B48	4.8
BOD	B5	13.2	Ammonia		10.3	Nitrite	B48	6.8
Alkalinity	B6	1.5	Total Solids	B30	3.9	Phosphorus	B48	4.7
Suspended. Solids	B7	7.2	TDS		3.7	Chloride	B48	3.3



# Useful References

- **NS30** *A Manual of Analytical Quality Control for the Water Industry, WRc*
- **ISO/TR 17944:2004** – *Water Quality – Criteria for establishing the equivalence between microbiological methods.*
- **UK Drinking Water Inspectorate** *Guidance Note on Interpretation of Aspects of AQC*

<http://www.dwi.gov.uk/stakeholders/guidance-and-codes-of-practice/aqc-ar1.pdf>

- **ISO 13530** *Water quality — Guidance on analytical quality control for chemical and physicochemical water analysis*
- **Environment Agency (UK)** *Performance Standard for Organisations Undertaking Sampling and Chemical Testing of Water (v1.1 May 2009)*
- **Environment Agency (UK)** *Monitoring of Discharges to Water and Sewer (TGN M18)*

<http://www.environment-agency.gov.uk/business/regulation/89197.aspx>

## Extract from Appendix B of EA's Performance Standard document

	Test sample	Sewage effluent sample	spiked sewage sample	Recovery	Trade effluent sample	Spiked trade effluent sample	Recovery
Batch	Replicate						
1	1	0.327	5.073	4.746	9.133	22.899	13.766
1	2	0.450	5.311	4.861	9.550	22.330	12.780
	Batch Mean	0.3885	5.1920	4.80350	9.3415	22.6145	13.273
	Batch S.Dev.	0.08697	0.16829	0.08132	0.29486	0.40234	0.69721
2	1	0.614	5.431	4.817	9.688	24.227	14.539
2	2	0.519	5.138	4.619	9.376	23.380	14.004
	Batch Mean	0.5665	5.2845	4.7180	9.5320	23.8035	14.2715
	Batch S.Dev.	0.06718	0.20718	0.14001	0.22062	0.59892	0.37830
3	1	0.281	5.427	5.146	9.560	23.637	14.077
3	2	0.412	5.394	4.982	9.417	24.336	14.919
	Batch Mean	0.3465	5.4105	5.0640	9.4885	23.9865	14.498
	Batch S.Dev.	0.09263	0.02333	0.11597	0.10112	0.49427	0.59538
4	1	0.430	5.870	5.440	9.770	21.871	12.101
4	2	0.557	6.086	5.529	9.564	21.039	11.475
	Batch Mean	0.4935	5.9780	5.48450	9.6670	21.4550	11.788
	Batch S.Dev.	0.08980	0.15274	0.06293	0.14566	0.58831	0.44265
5	1	0.600	5.000	4.501	10.100	22.114	12.026
Overall mean		0.53391	5.410		9.874	23.080	
Overall mean recovery				4.876			13.206

## Conclusion of above data table

Precision test (From ANOVA)	Sewage effluent	Spiked Sewage effluent	Trade effluent	Spiked trade effluent
Mean	0.53391	5.410	9.874	23.080
Within-Batch sd	0.104619	0.249369	0.293543	0.594442
Between-Batch sd	0.121437	0.186605	0.365231	0.534918
Total sd	0.160288	0.311459	0.468574	0.799687
Relative sd %	30.02%	5.76%	4.75%	3.46%
Target sd:	0.125	0.2705	0.4937	1.154
Tabulated F 0.05 value	1.67	1.60	1.69	1.64
Calculated F-Value	1.64	1.33	0.90	0.48
Estimate degrees freedom	15.14	18.02	14.68	16.86
Assessment	PASS	PASS	PASS	PASS

This is obtained from statistical tables for the estimated degrees of freedom at the 5% probability level ( $p=0.05$ )

This value is calculated as  $(\text{total sd} / \text{target sd})^2$ .

Precision for sewage analysis is considerably poorer than the 5% target so an F-test is performed using a 'critical' concentration ... In this case 5 mg/l. An absolute target of 0.125 mg/l was applied rather than an RSD of 5% (equivalent to an SD of only 0.027 mg/l)

## Summary of performance

Recovery	Sewage effluent	Trade effluent
Expected recovery concentration	4.9995	14.9704
Mean measured recovery	4.8763	13.2057
Overall mean recovery	97.5%	88.2%
sd of mean recovery	5.5192	5.11
Standard error of mean recovery	1.664	1.5402
90 % Confidence interval of recovery	3.015	2.7909
Recovery range	94.52% - 100.55%	85.42% - 91.0%
Assessment	PASS	PASS

This value is the average of the mean recovery for each batch

This value is the relative sd of overall mean recovery divided by the square root of the number of batches

This value is the standard error of mean recovery multiplied by the Student's t value ( $p=0.05$  single sided) for degrees of freedom equal to number of batches - 1 ( $t=1.812$  for 11 batches)

- Performance characteristics for the Trade effluent sample are more variable and recovery is only just acceptable at 85%. It may be necessary to undertake some further checks on method interferences to see whether this can be improved.

# Performance Assessment Example: Environment Agency

Batch	Replicate	Sewage Efflt	Spiked Sewage	Recovery	Sewage Recovery %	Trade Efflt	Spiked T.Efflt	Recovery	Trade Efflt Recovery %
			(+ 5 mg/l)				(+15mg/l)		
1	1	0.327	5.073	4.746	94.9265	9.133	22.899	13.766	91.9560
1	2	0.45	5.311	4.861	97.2290	9.55	22.33	12.78	85.3910
	Batch Mean	0.3885	5.192	4.8035	96.07777	9.3415	22.6145	13.273	88.67349667
	Batch SD	0.08697	0.16829	0.08132		0.29486	0.40234	0.69721	
	Variance	0.00756	0.02832	0.00661		0.08694	0.16188	0.48610	
2	1	0.614	5.431	4.817	96.3523	9.688	24.227	14.539	97.1204
2	2	0.519	5.138	4.619	92.3904	9.376	23.38	14.004	93.5475
	Batch Mean	0.5665	5.2845	4.718	94.37133	9.532	23.8035	14.2715	95.33397333
	Batch SD	0.06718	0.20718	0.14001		0.22062	0.59892	0.37830	
	Variance	0.00451	0.04292	0.01960		0.04867	0.35870	0.14311	
3	1	0.281	5.427	5.146	102.9256	9.56	23.637	14.077	94.0379
3	2	0.412	5.394	4.982	99.6482	9.417	24.336	14.919	99.6483
	Batch Mean	0.3465	5.4105	5.064	101.28693	9.4885	23.9865	14.498	96.84310333
	Batch SD	0.09263	0.02333	0.11597		0.10112	0.49427	0.59538	
	Variance	0.00858	0.00054	0.01345		0.01022	0.24430	0.35448	
4	1	0.43	5.87	5.44	108.8086	9.77	21.871	12.101	80.8687
4	2	0.557	6.086	5.529	110.5911	9.564	21.039	11.475	76.6913
	Batch Mean	0.4935	5.978	5.4845	109.69987	9.667	21.455	11.788	78.78000667
	Batch SD	0.08980	0.15274	0.06293		0.14566	0.58831	0.44265	
	Variance	0.00806	0.02333	0.00396		0.02122	0.34611	0.19594	
5	1	0.698	5.289	4.591	91.8340	10.189	23.114	12.925	86.3704
5	2	0.744	5.899	5.155	103.1149	10.882	23.565	12.683	84.7710
	Batch Mean	0.721	5.594	4.873	97.47442	10.5355	23.3395	12.804	85.57071
	Batch SD	0.03253	0.43134	0.39881		0.49002	0.31891	0.17112	
	Variance	0.00106	0.18605	0.15905		0.24012	0.10170	0.02928	
6	1	0.495	5.395	4.9	98.0099	10.055	23.389	13.334	89.0944
6	2	0.415	5.845	5.43	108.6083	10.72	22.773	12.053	80.5677
	Batch Mean	0.455	5.62	5.165	103.3091	10.3875	23.081	12.6935	84.83108333
	Batch SD	0.05657	0.31820	0.37477		0.47023	0.43558	0.90580	
	Variance	0.00320	0.10125	0.14045		0.22111	0.18973	0.82048	
7	1	0.787	5.414	4.627	92.5557	9.239	22.304	13.065	87.2848
7	2	0.57	5.735	5.165	103.3114	9.678	23.836	14.158	94.5802
	Batch Mean	0.6785	5.5745	4.896	97.93357	9.4585	23.07	13.6115	90.93250333
	Batch SD	0.15344	0.22698	0.38042		0.31042	1.08329	0.77287	
	Variance	0.02354	0.05152	0.14472		0.09636	1.17351	0.59732	
8	1	0.94	5.391	4.451	89.0388	10.271	23.437	13.166	87.9788
8	2	0.647	5.201	4.554	91.0929	10.31	23.736	13.426	89.7129
	Batch Mean	0.7935	5.296	4.5025	90.06587	10.2905	23.5865	13.296	88.84581
	Batch SD	0.20718	0.13435	0.07283		0.02758	0.21142	0.18385	

# Too complicated?

- Not really ... It's worth the effort but very time consuming if doing it even using on Excel
- Simpler to purchase software to do the job e.g. mVal (from NMS)
- [www.vam.org.uk](http://www.vam.org.uk)
- <http://www.nmschembio.org.uk/PublicationArticle.aspx?m=113&amid=358> (NMS publications page)



Thank you

p.webster@epa.ie

021 486 0802

