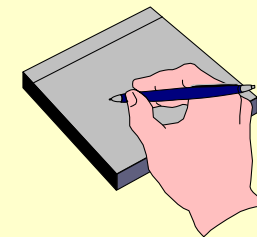
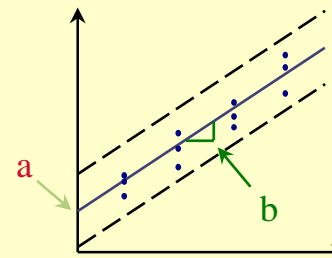
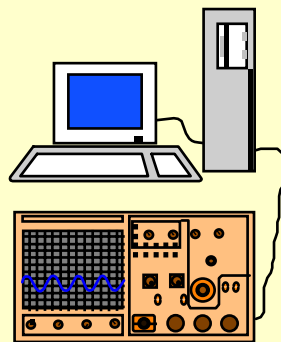


Validation concepts for pesticide residues in food of animal origin

EU AQC Guidelines vs CD 2002/657/EC



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Two sets of AQC

1. Quality Control Procedures for Pesticide Residues Analysis
(Document No. **SANCO/10232/2006**)
2. **Commission Decision 2002/657/EC**
Implements Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products

Fundamentals of CD 2002/657/EC (1)

- **Subject matter and scope (article 1)**
 - testing of official samples taken pursuant to article 15 (1) sentence 2 of DC 96/23/EC
 - This Decision shall not apply to substances for which more specific rules have been laid down in other Community legislation
- **Analytical methods (article 3)**
 - are documented in test instructions, preferably according to ISO 78-2
 - comply with part 2 of the Annex to this Decision
 - validated according to the procedures described in Part 3 of the Annex
 - comply with the relevant minimum required performance limits (MRPL)

Fundamentals of CD 2002/657/EC (3)

- **MRPL-values (article 4)**
 - establishing of **m**inimum **r**equired **p**erformance **l**imits (MRPL) of analytical methods to be used for substances for which no permitted limit has been established
- **Quality control (article 5)**
 - The Member States shall ensure the quality of the results of the analysis of samples taken pursuant to Directive 96/23/EC, in particular by monitoring tests and/or calibration results according to chapter 5.9 of ISO 17025 (1)

Fundamentals of CD 2002/657/EC (4)

- **Interpretation of results (article 6)**
 - The result of an analysis shall be considered non-compliant if the **decision limit (CC α)** of the confirmatory method for the analyte is exceeded
 - If a MRL has been established for a substance, the **decision limit** is the concentration **above** which it can be decided with a statistical certainty of $1 - \alpha$ that the permitted limit has been truly exceeded ($\alpha = 5 \%$)
 - If no permitted limit has been established for a substance, the **decision limit** is the lowest concentration level at which a method can discriminate with a statistical certainty of $1 - \alpha$ that the particular analyte is present. ($\alpha = 1 \%$)

Fundamentals of CD 2002/657/EC (5)

- **Detection capability ($CC\beta$ - 1.12)**
 - **Detection capability ($CC\beta$)** means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β
 - MRL-substances: the detection capability is the concentration at which the method is able to detect MRL-concentrations with a statistical certainty of $1 - \beta$ ($\beta = 5\%$)
 - Substances with no permitted limit: the detection capability is the lowest concentration at which a method is able to detect truly contaminated samples with a statistical certainty of $1 - \beta$ ($\beta = 5\%$)

Fundamentals of CD 2002/657/EC (6)

- **Alpha (α - 1.2)**
 - error means the probability that the tested sample is compliant, even though a non-compliant measurement has been obtained ("**false non-compliant decision**")
- **Beta (β - 1.4)**
 - error means the probability that the tested sample is truly non-compliant, even though a compliant measurement has been obtained ("**false compliant decision**")
- **Handling of samples (2.1.1)**
 - Samples shall be obtained, handled and processed in such a way that there is a maximum chance of detecting the substance
 - Sample handling procedures shall prevent the possibility of accidental contamination or loss of analytes

CD 2002/2002/657

Designed to distinguish between

- compliant and
- not compliant

samples

Fundamentals of CD 2002/657/EC (7)

- **Recovery** (2.1.2.1)
 - recovery shall be determined in each batch of samples, if a **fixed recovery correction factor** is used
 - If the recovery is within limits, the **fixed correction factor** may then be used
 - Otherwise the **recovery factor** obtained for that specific batch shall be used

Fundamentals of CD 2002/657/EC (7)

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(unless the specific recovery factor of the analyte in the sample is to be applied in which case the standard addition procedure (see 3.5) or an internal standard shall be used for the quantitative determination of an analyte in a sample)
- **Consequence: all results must be corrected using the recovery rate!**

Fundamentals of CD 2002/657/EC (7)

Trueness of quantitative results (2.3.2.1)

Minimum trueness of quantitative methods

Mass fraction	Range
$\leq 1 \mu\text{g}/\text{kg}$	-50% to +20%
$> 1 \mu\text{g}/\text{kg}$ to $10 \mu\text{g}/\text{kg}$	-30% to +10%
$\geq 10 \mu\text{g}/\text{kg}$	-20% to +10%

With certified reference materials (CRM)

If no CRM is available: recovery of additions of known amounts of the analyte(s) to a blank matrix

Conventional validation

Validation approaches, e.g. according to

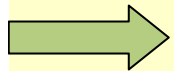
- AOAC
- Codex alimentarius
- ISO Standards
 - ISO Guide 25
 - ISO 5725-2
 - ISO 11843

are based on repeatability and reproducibility standard deviation.

they consider differences between identical samples only. All differences due to major changes (species, matrix) are considered as systematic effects.

Consequence:

validation data shall be available for each matrix and each species, separately!



Conventional validation procedures (1)

- **Recovery (3.1.2.1)**
 - Analyse 6 replicates of a certified reference material (CRM)
 - Select 18 aliquots of a blank material and fortify 6 aliquots at each
 - of 0,5 1.0 and 1.5 times the minimum required performance limit
 - of 0.5, 1 and 1.5 times the permitted limit
 - Calculate recovery and cv

- **Recovery (standard addition method)**
 - The complete procedure for determination of the recovery by mean of the standard addition method is described in 3.5

Conventional validation procedures (2)

- **Repeatability (3.1.2.2)**
 - Repeat the procedure for the recovery on at least two other occasions
 - Calculate the overall mean concentrations and CVs for the fortified samples

- **Within-laboratory reproducibility (3.1.2.3)**
 - Select 18 aliquots of a blank material and fortify 6 aliquots at each
 - of 0.5, 1.0 and 1.5 times the minimum required performance limit
 - of 0.5, 1 and 1.5 times the permitted limit
 - Repeat the procedure for the recovery on at least two other occasions (with different operators, equipment, samples...)
 - Calculate the mean concentration, standard deviation and the coefficient of variation (%) of the fortified samples

Conventional validation procedures (3)

- **Reproducibility (3.1.2.4)**
 - participate in collaborative studies according to ISO 5725-2
- **Decision Limit (CC_{α}) (3.1.2.5) – MRL components**
 - By the calibration curve procedure according to ISO 11843
 - blank material shall be used, which is fortified around the permitted limit in equidistant steps. Analyse the samples.
 - Plot the signal against the added concentration.
 - The corresponding concentration at the permitted limit plus 1.64 times the standard deviation of the within-laboratory reproducibility equals the decision limit ($\alpha = 5\%$)
 - *Analyse at least 20 blank materials per matrix fortified with the analyte(s) at the permitted limit. The concentration at the permitted limit plus 1.64 times the corresponding standard deviation equal the decision limit ($\alpha = 5\%$)*

Conventional validation procedures (4)

- **Detection capability $CC\beta$ (3.1.2.6) – MRL–Stoffe**
 - By the calibration curve procedure according to ISO 11843
 - blank material shall be used, which is fortified around the permitted limit in equidistant steps. Analyse the samples.
 - Plot the signal against the added concentration.
 - The corresponding concentration at the decision limit plus 1.64 times the standard deviation of the within-laboratory reproducibility equals the detection capability ($\beta = 5\%$)
 - Analyse at least 20 blank materials per matrix fortified with the analyte(s) at the decision limit. The concentration at the decision limit plus 1.64 times the corresponding standard deviation equal the detection capability ($\beta = 5\%$)

Conventional validation procedures (5)

- **Ruggedness (major changes) (3.1.2.7)**
 - The analytical method should be tested under different experimental conditions
 - The changes introduced should be major (e.g. different species, different matrices or different sampling conditions)
 - The importance of these changes can be evaluated, for instance, using the Youden approach
 - Each performance characteristic should be determined for all major changes that have been shown to have a significant effect on the performance of the assay

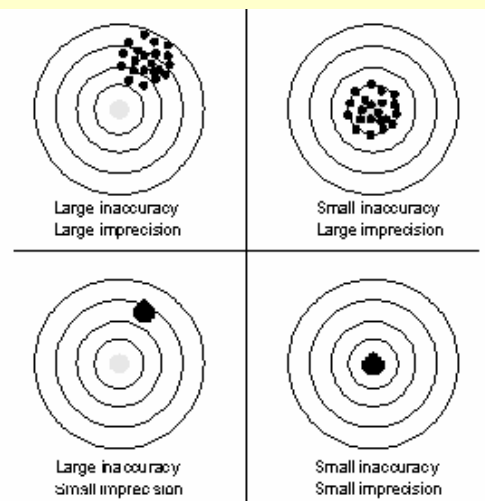
Error probabilities depend on the calculation of accuracy and precision

Underlying model:

Measurement value
= true value
+ systematic error (Bias)
+ random error

⇒ Accuracy
⇒ Precision

Trueness =
Accuracy + Precision



Typical factors in residue analyses

- Species (e.g. cattle, pig, turkey, salmon)
- Compartment (e.g. plasma, muscle, shrimps, liver, eggs, milk, honey)
- Staff
- Condition of sample (fresh - not fresh)
- Homogenisation (lyophilised yes/no)
- Storage conditions (duration of storage, temperatures, frozen....)
- Storage of extract before measurement
- Type and condition of instruments
- Time between experiments
- Condition of columns
- ...

The idea: systematic and simultaneous assessment of noise factors

In order to assess the impact of the noise factors to the precision of test results, a large number of measurements is required if the samples are selected randomly.



More cost-effective: systematic assessment of error (see EURACHEM Guide)

Conventional versus alternative approach

Conventional:

Random selection of samples

⇒ random variation of noise factors

⇒ Precision = random variation of measurement result

⇒ Many samples required

Alternative:

Selection of samples by factorial design

⇒ systematic variation of noise factors

⇒ Precision = factorial effects + remaining random variation

⇒ Reduced number of samples

Orthogonal designs

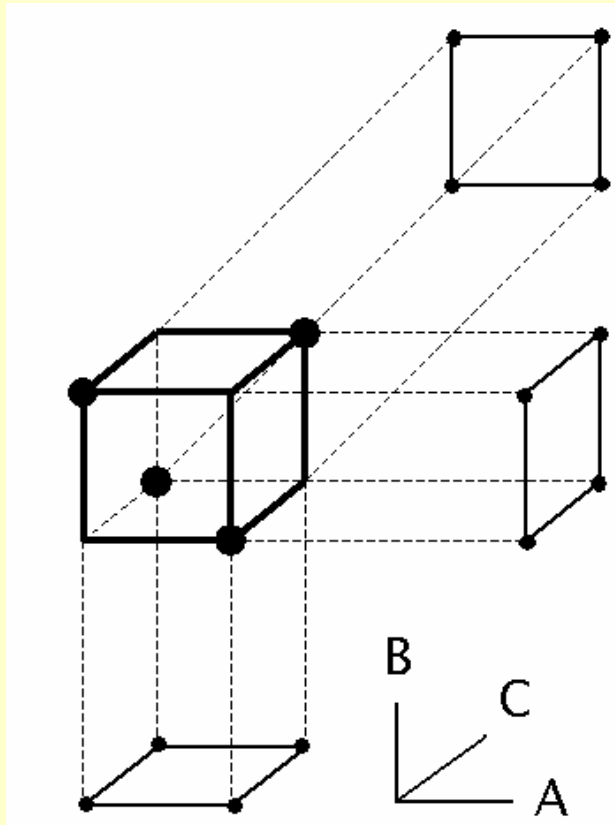
Simultaneous variation of several factors reduces experimental effort considerably.

But: with 7 factors each with 2 factor levels there are $2^7=128$ different factor settings – far to much

Therefore a special selection of settings is required, according to the principle of orthogonality.

Orthogonal designs

3 Factors A, B and C, each with 2 levels + und -



A	B	C
+	+	+
+	+	-
+	-	+
+	-	-
-	+	+
-	+	-
-	-	+
-	-	-

Orthogonal designs

7 Factors A, B, C, E, F, G each with 2 levels + and -

A	+	+	+	+	-	-	-	-
B	+	+	-	-	+	+	-	-
C	+	-	+	-	+	-	+	-
D	+	+	-	-	-	-	+	+
E	+	-	+	-	-	+	-	+
F	+	-	-	+	+	-	-	+
G	+	-	-	+	-	+	+	-

Each combination ++, +-, -+ and – of each factor pair appears twice.
This design is performed with 4 concentration levels,
i.e.: **32 measurements in total** (plus blanks)

Principles of in-house validation experiments

- Factor levels shall be determined so that the full bandwidth of realistic conditions is covered, e.g.
 - operator: experienced / unexperienced
 - storage of extracts: 0d / 1d
 - fat content: low / high
 - etc.
- Apart from one factor with up to 4 levels only 2 levels per factor shall be used (proper definition of factors required)
- Randomisation of order of experiments to avoid effects of temporal trends.
- Not all experiments within one week

Model components

true concentration value

+

systematic deviations

method, matrix, lab, run

+

random deviations

=

measurement value

classic <-> inhouse

Overview	classical approach	inhouse
No. Samples	about 100	32
Matrices <i>Liver / kidney</i>	1	2
Species <i>Cattle / swine</i>	1	2
effective samples	about 100	8

High number of analyses for classical validation for CD 2002/657/EC can be reduced by inhouse-validation concept giving nearly same results

(8 beef livers + 8 pork livers + 8 beef kidneys + 8 pork kidneys=

In case of MRL analytes additional experiments are necessary for the calculations of LOD/LOQ

Applicability

1. Pesticide Guidelines - SANCO/10232/2006:
Pesticides in all food products

2. Commission Decision 2002/657/EC:
Organic residues and contaminants:

veterinary drugs, OCs, OPs, carbamates and pyrethroids, some
contaminants and dyes

in meat, fish, eggs, milk and honey
for samples taken pursuant Directive 96/23/EC

Lex specialis

- Commission Regulation **1883/2006 (for dioxins)** constitute “more specific rules laid down in other Community legislation” in sense of Art. 1 of 2002/657CD. As a consequence, the **analytical methods covered by these Regulations are not subject to 2002/657/CD.**
- For **pesticides**, the legal service of the Commission considers that the current **guidelines** under 395/2005 **cannot** be considered for the moment **more specific legislation** and therefore the **validation** of methods for analysis of pesticides in food of animal origin in the framework of Council Directive 96/23 will still be covered **by 2002/657/CD**
- One aim of the CRL-AO: “lex specialis” for pesticides

Main differences 2002/657/EC <-> AQC Guidelines

- CD 2002/657/EC: comprehensive validation of methods for identifying non compliant samples before use required; few requirements within the CD for continuous quality control after introduction at use in routine
- Pesticide AQC Guidelines: few requirements for validation before use; comprehensive requirements of continuous quality control during use

Main differences 2002/657/EC <-> AQC Guidelines

	Pesticide AQC Guidelines	Commission Decision 2002/657EC
Matrices to be validated	key matrices	all matrices
Recovery correction	no (required 70 % <-> 110 %)	Yes
Number of mass ID-points	3 2 if m/z >200	3
Estimation of uncertainty	inter-laboratory reproducibility	Within-laboratory reproducibility
Standard uncertainty	25 %, two sided	Individual, single sided
Reference point for addition / subtraction of uncertainty	measured value	MRL

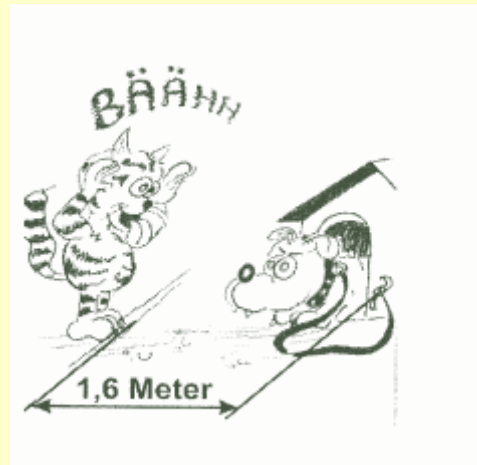
Main differences 2002/657/EC <-> AQC Guidelines

Different interpretation of measurement uncertainty

	Pesticide AQC Guidelines	Commission Decision 2002/657EC
MRL	100 µg/kg	100 µg/kg
Standard uncertainty (U)	25 %	25 %
Extended uncertainty (2 U)	50 %	50 %
Non compliant for measured values	> 200 µg/kg	> 150 µg/kg

Need for harmonization

It is important to know the measurement uncertainty



Thank you for your attention