IMPORTANCE OF SAMPLING STEP TO INTERPRET ANALYTICAL RESULTS IN FOOD SAFETY

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Outline

- Introduction
- Sampling and Sampling Plans
- ISO 17025 and Sampling
- Combined Measurement Uncertainty
- Sampling Uncertainty
- Compliance Assesment/Interpretation of Results
- Conclusion

1.Introduction

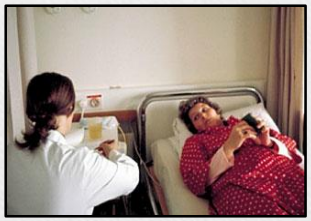
➢ Definition of Food Safety

✓"Food shall be deemed to be unsafe if it is considered to be:

a) injurious to health;

(b) unfit for human consumptin *(EC Regulation 178/2002)*"

➢Unsafe food ontaining harmful bacteria, viruses, parasites or chemical substances is linked to the deaths of an estimated 2 million people annually including many children *(www.who.int)*



➢10 food safety hazards *in 2016 RASFF Report*

✓ *pathogenic microorganisms, allergens, foregin bodies, heavy metals, mycotoxins, pesticide residues, food additives and flovoring, biocontaminants, chemical migration from food contact materials(https://ec.europa.eu/food);*

Food Processing

Legal Limits

Compalince Assesment: *Laboratory Analysis*

Foods in national and international trade

Reliable and comparable analytical results

Primary Agricultural and Animal Production

Validation /verification studies and QA/QC

Measurement uncertainty

Common Steps in Analysis for Food Safety

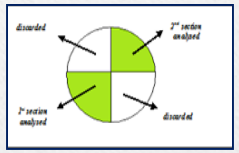
External operations

Laboratory operations

Sampling

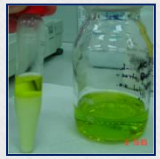
Sample preparation

Sample size reduction

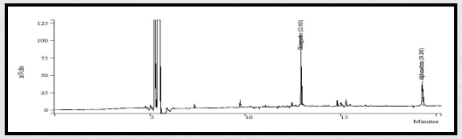


Sample processing

Exctraction and clean-up



Determination



Reporting

1.5±0.5 mg/kg

2.Sampling and Sampling Procedure

- **Sample** is defined as one or more units selected from a population of units, or a portion of material selected from a larger quantity of material
- Analytical measurements for food safety analysis are primarily performed in small amount of sample representing the whole material
- Therefore representative sample reflecting the properties of the parent population should be taken to get reliable results for the end-user
- An essential starting point should be selecting an appropriate sampling procedure, which is a procedure used to draw and constitute a sample

(Thompson, 1998, IUPAC, 1990; Codex, 1999; EC, 2002Lyken et al., 1957; Thompson and Ramsey, 1995; Nocerino, et al., 2005).

- An international recognized method should be selected

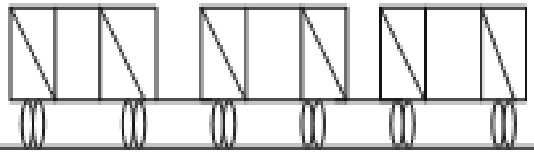
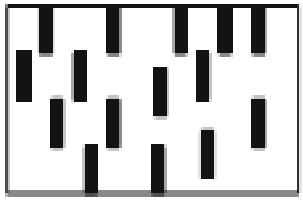
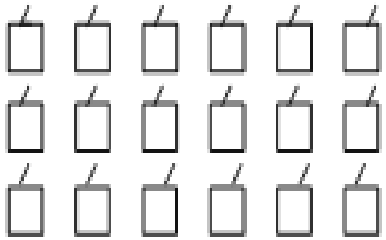
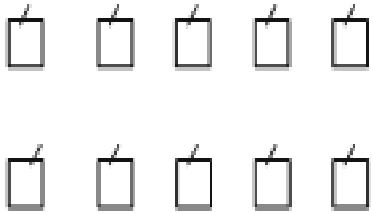
  - ✓For the control of levels of mycotoxins: Commission Regulation (EC) 401/2006
  - ✓For the control of levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs: Commission Regulation (EC) No 333/2007
  - ✓For the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs: Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014
  - ✓For the control of levels of nitrates: Commission Regulation (EC) 1882/2006
  - ✓General Guidelines on Sampling (CAC/GL 50- 2004under revision
  - ✓Commission Directive 2002/63/EC of 11 July 2002 Sampling for the official control of pesticide residues in and on products of plant and animal

➢ISO has released sampling standards both for specific products and general application;

- ✓ ISO 18593:2004 Microbiology of food and animal feeding stuffs -- Horizontal methods for sampling techniques from surfaces using contact plates and swabs
- ✓ ISO 23611-1 &2 :2006 Soil quality -- Sampling of soil invertebrates
- ✓ISO 18400-102:2017 Soil quality -- Sampling -- Part 102: Selection and application of sampling techniques
- ✓ISO 5667-1-24 6:2014 Water quality – Sampling
- ✓ ISO 28590:2017 Sampling procedures for inspection by attributes -- Introduction to the ISO 2859 series of standards for sampling for inspection by attributes
- ✓ ISO 28591:2017 Sequential sampling plans for inspection by attributes
- ✓ISO 28592:2017Double sampling plans by attributes with minimal sample sizes, indexed by producer's risk quality (PRQ) and consumer's risk quality (CRQ)
- ✓ISO 28593:2017 Acceptance sampling procedures by attributes -- Accept-zero sampling system based on credit principle for controlling outgoing quality
- ✓……..

Sampling Procedure

A- For pesticide residue analysis too comply with MRL (CAC 1999; EC 2002)

1. Sampling Consignment		**A lot ;** delivered at one time; having the same origin, variety, type of packing, packer, consignor or markings.
Lot		1, 3, 5, or 10 primary samples taken from an equal number of randomly chosen positions
Bulk Sample		Unit(s) compromising the bulk sample
Laboratory Sample	 	Minimum size of laboratory sample *large sized fresh products*; 5 units *small sized fresh products*; 10 units

Minimum number of primary samples (CAC 33, 1999; EC 2002/63)

	Minimum number of primary samples to be taken from the lot
a) Meat and poultry	
a non-suspect lot	1
a suspect lot	determined according to Table 2 given in the guideline (CAC, 1999)
b) Other products	
i) Products, packaged or in bulk, which can be assumed to be well mixed or homogenous	1
ii) Products, packaged or in bulk, which may not be well mixed or homogenous	
either:	
Weight of lot, kg	
<50	3
50-500	5
>500	10
or	
Number of cans, cartons or other containers in the lot	
1-25	1
26-100	5
>100	10

Description of primary samples and minimum size of laboratory samples for fresh food commodities (CAC, 1999).

	Examples	Nature of primary samples to be taken	Minimum size of each laboratory sample
Class A, primary food commodities of plant origin			
1. All fresh fruits and vegetables			
Small sized fresh products units generally >25 g	berries	Whole units, or packages, or units taken with a sampling device	1 kg
	peas		
	olives		
Medium sized fresh products units generally 25-500 g	apples	Whole units	1 kg (at least 10 units)
	oranges		
Large sized fresh products units generally>250 g	cabbages	Whole units	2 kg (at least 5 units
	cucumbers		
	grapes (bunches)		

[1] details for other commodities can be found in Table 4 of the Codex guideline (CAC, 1999).

B- For mycotoxins (EC 401/2006; EC Guidance Documentt for aflatoxin sampling 2010)

General survey of the method of sampling for dried figs, groundnuts and nuts

Table 1

Subdivision of lots into sublots depending on product and lot weight

Commodity	Lot weight (tonnes)	Weight or number of sublots	Number of incremental samples	Aggregate sample weight (kg)
Dried figs	≥ 15	15-30 tonnes	100	30
	< 15	—	10-100 (*)	≤ 30
Groundnuts, pistachios, brazil nuts and other nuts	≥ 500	100 tonnes	100	30
	> 125 and < 500	5 sublots	100	30
	≥ 15 and ≤ 125	25 tonnes	100	30
	< 15	—	10-100 (*)	≤ 30

(*) Depending on the lot weight — see Table 2 of this part of this Annex.

3.ISO 17025:2017 and Sampling

3. Terms and Definition

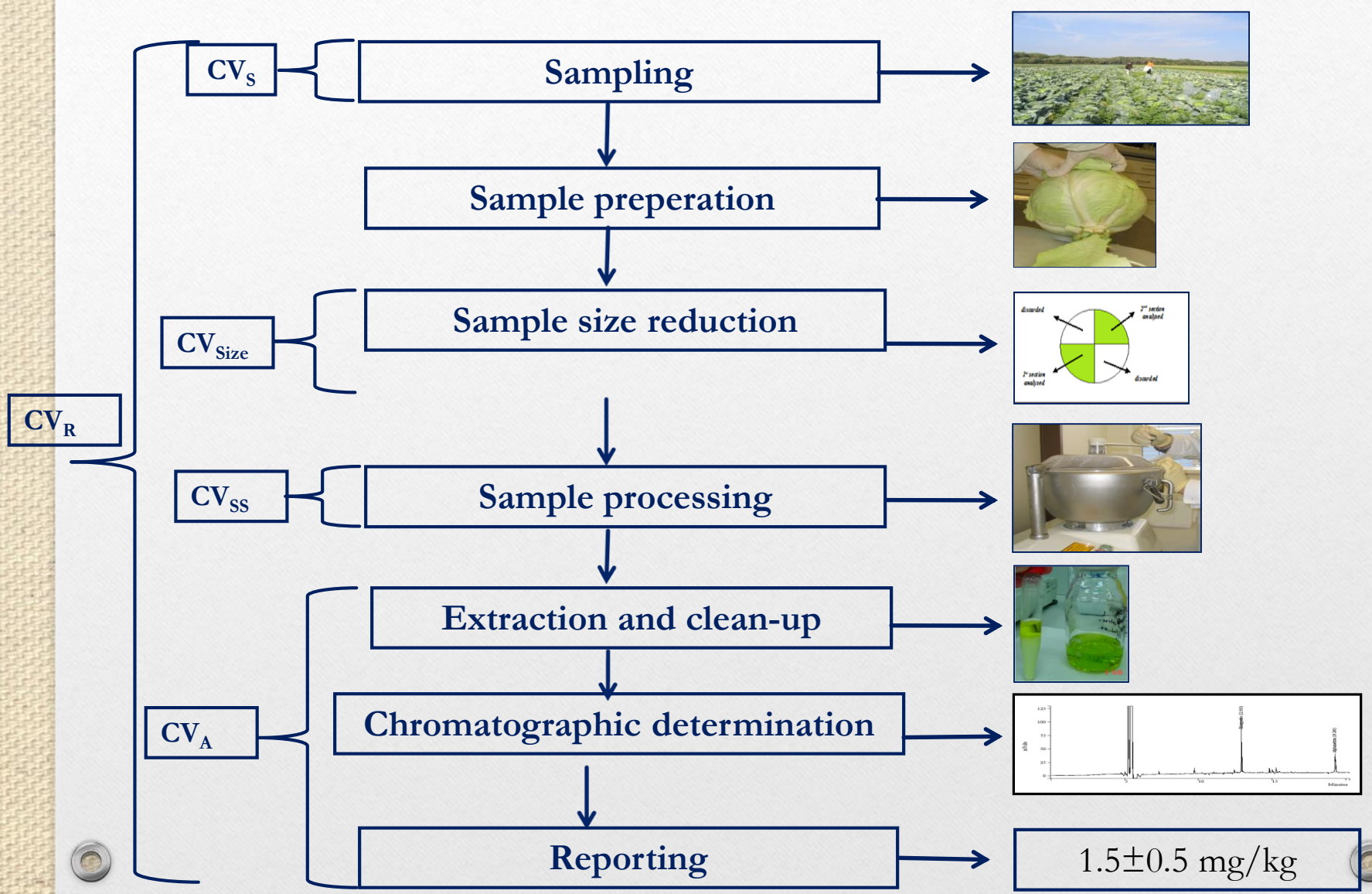
- Laboratory; Body that performs one or more of the following activies
  - ✓ test; calibration; and sampling, associated with subsequent testing or calibration

- **7.3 Sampling, 7.3.1**
  - ✓ The laboratory shall have a sampling plan and method when it carries out sampling of substances, materials or products for subsequent testing or calibration
  - ✓Sampling plans shall, whenever reasonable, be based on appropriate statistical methods.

- **7.2.2.1** Validation can include sampling step as well

- **7.6.1** Laboratories shall identify the contributions to measurement uncertainty. When evaluating measurement uncertainty, all contributions that are of significance, including those arising from sampling, shall be taken into account using appropriate methods of analysis.

4.Combined Measurement Uncertainty



$$S_R = \sqrt{(S_S)^2 + ((S_{Sizereduction})^2 + (S_{Sp})^2 + (S_A))^2}$$

$$CV_R = \sqrt{(CV_S)^2 + (CV_L)^2}$$

External steps

Analytical Steps in laboratory (S_L)

$$S_R = \sqrt{(S_S)^2 + (S_L)^2}$$

$$CV_L = \sqrt{(CV_{Sizereduction})^2 + (CV_{Sp})^2 + (CV_{Ex})^2 + (CV_{Clean-up})^2 + (CV_{ch})^2}$$

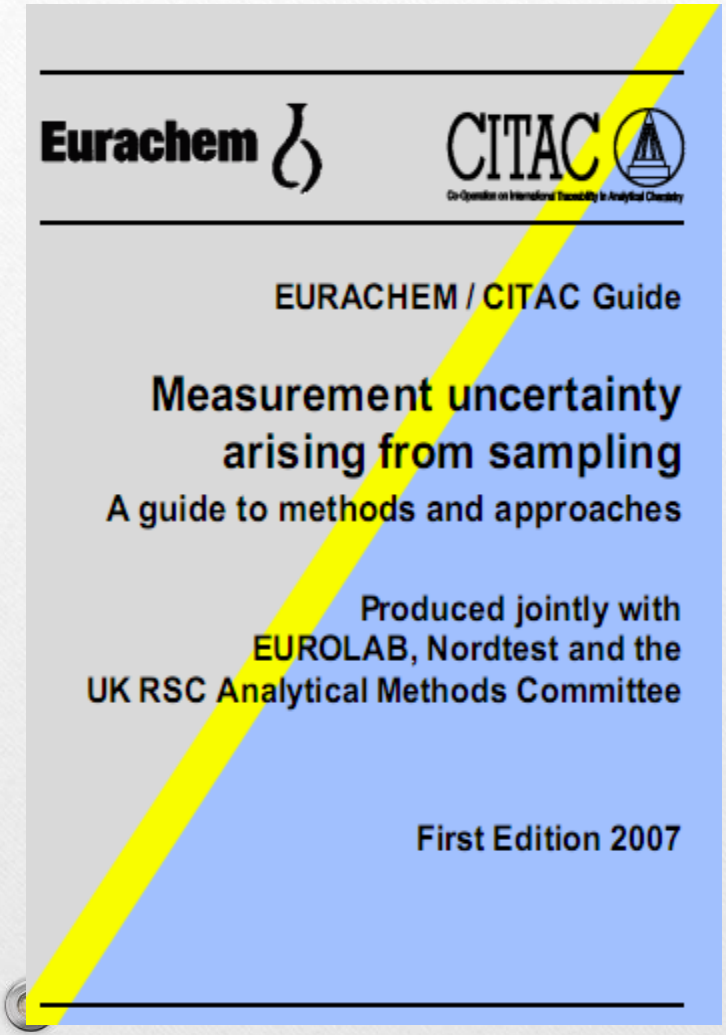
$$CV_{Lc} = \sqrt{CV_{SS}^2 + CV_{Sp}^2 + CV_A^2}$$

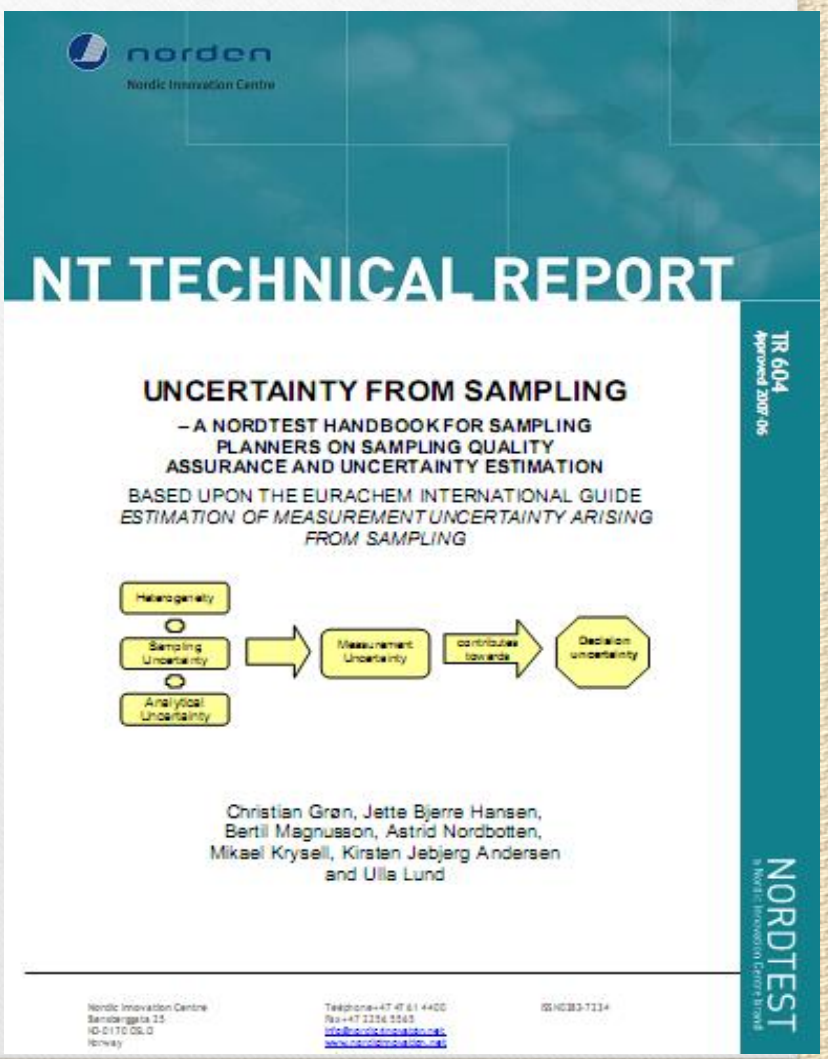
Uncertainty of laboratory phase

$$U = 2 \times S_R$$

$$\text{Result} = x \pm U \text{ (mg/kg)}$$

5. Sampling Uncertainty





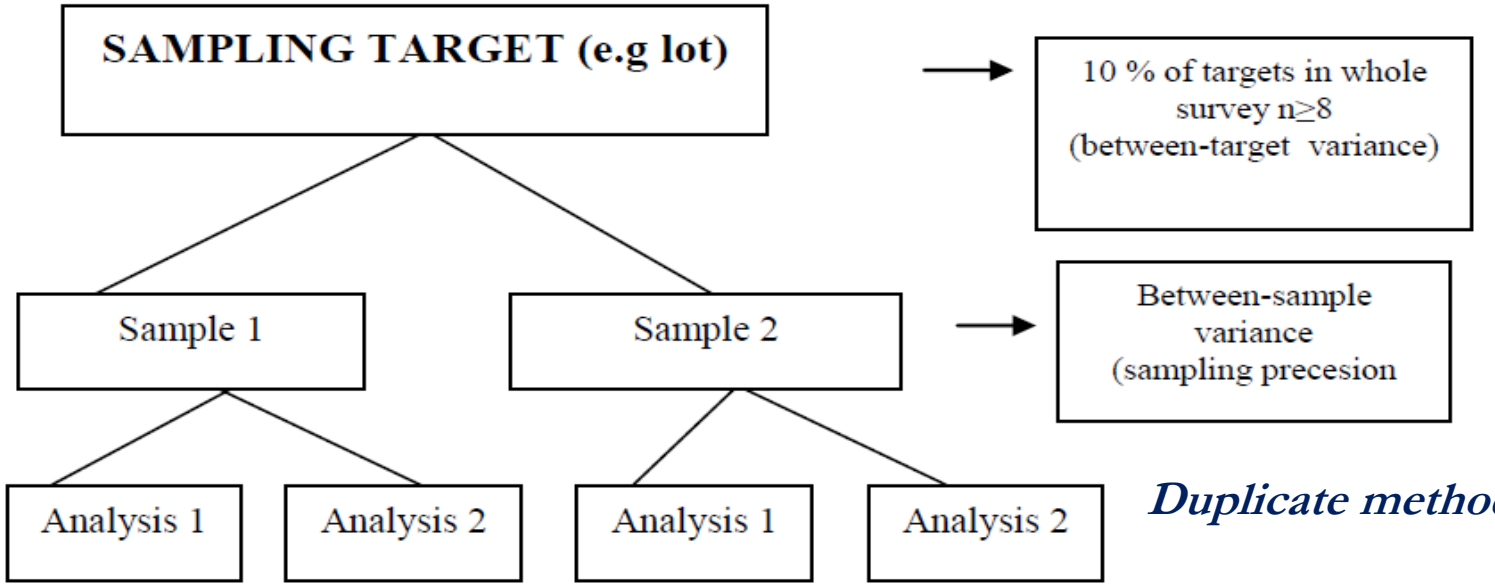
Approaches to estimate sampling uncertainty

(EUROCHEM CITAC Guide 2007)

➢**The modeling approach** (theoretical, predictive, bottom up) uses a predefined model that identifies each of the component parts of the uncertainty, quantifies the contribution from each source and than combines all of the contributions to give an estimate of the combined standard uncertainty

➢**The empirical approach** (experimental, top-down) uses repeated sampling and analysis (duplicate analysis), under various conditions, to quantify the effects caused by the factors such as the heterogeneity of the analyte in the sampling target and variations in the application of one or more sampling protocols

✓***Duplicate method:*** single sampler, should take duplicate samples from at least 8 sampling target (lot) which is a portion of material at a particular time that the sample is intended to represent (i.e. 10% of the total number of sampling target, but no less than eight targets)



- *Calculations:* ANOVA (Analysis of Variance) and Range

- Lyn *et al.* (2007b) observed that increasing the number of duplicate samples from 8 to 16 caused a slight decrease in the confidence interval of sampling uncertainty values. are generally applicable.

- Farkas et al. (2014) indicated that taking a minimum of 6 replicate samples from at least 8–12 lots is recommended to obtain a relative 95% range of sampling uncertainty within 50%.

The Sources that contributes sampling uncertainty

- Distribution of the analayte in the lot
- Heterogeneity
- Size of the primary sample/incremental sample
- Type of the commodotiy
- Effects of specific sampling strategy (e.g, random, strafied random, proportional etc)
- Effects of movement of bulk medium (Particularly density or size selection)
- Physical state of bulk (solid, liquid, gas)
- Temperature and pressure effects
- Effect of sampling process on composition (e.g differential adsorption in sampling system)
- Contamination
- Transportaion and preservation of sample *(Ramsey and Ellison, 2007)*

Sampling uncertainty for contaminant and residue

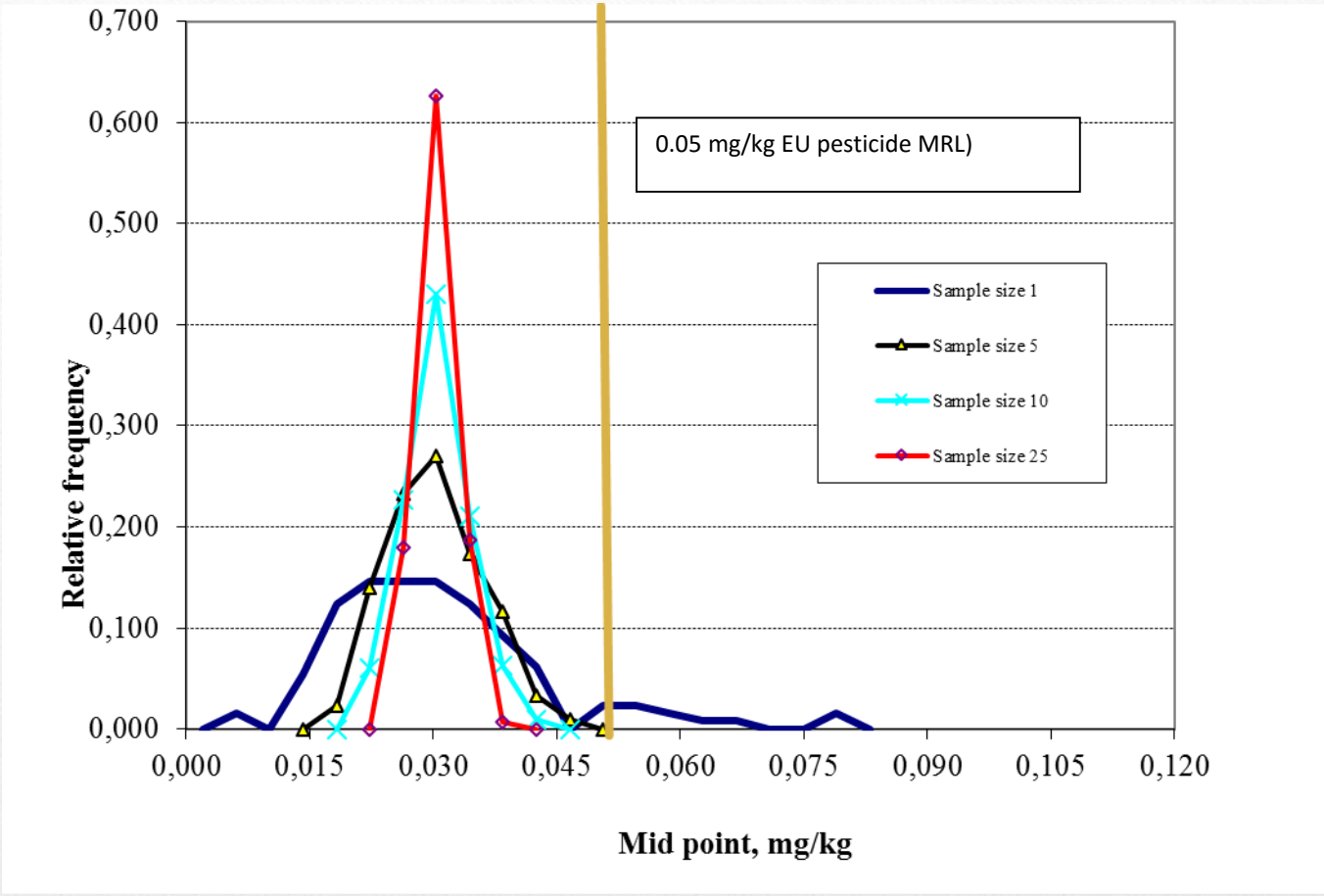
➢Squire *et al.* (2000) concluded that sampling uncertainty with one sampler was 60.08% in the contaminated soil

➢It has been shown that sampling uncertainty is the biggest contributor to the total variance in aflatoxin analysis due to the large variability among the contaminated units (Whitaker *et al.*, 2007a; Whitaker *et al.*, 2007b)

➢It has been revealed that sampling contributed about 99% of the total variance when a 10 kg sample was taken from a hazelnut lot with an aflatoxin concentration of 10 ng/g total aflatoxin; 0.42% for sample preperation and 0.14% for analysis

➢The uncertainty of sampling in pesticide residue analysis may contribute to the 80–90% of the combined uncertainty of the results (*Ambrus and Lantos 2002; Ambrus 2011;Farkas vd 2014; Ambrus 2009)*

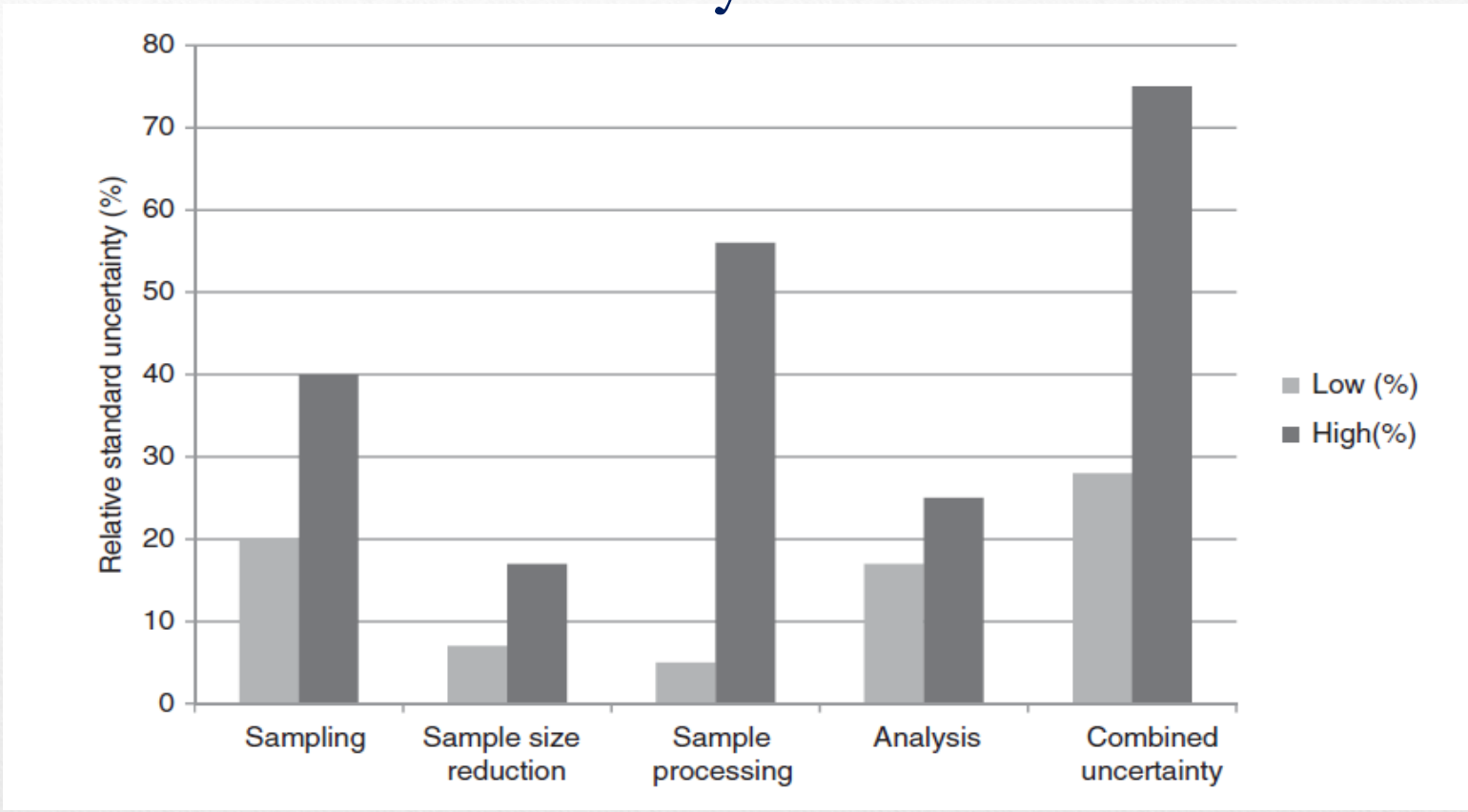
- ✓Middle and small size crops (n≤250 g, apple): CV_s= %25 (n=10)
- ✓Large size crops (n>250 g, cabbage): CV_s= %33 (n=5)
- ✓ Root vegetable crops (carrot): CV_s=%19 (n=10)
- ✓Green leafy vegetables (n>250 g, lettuce): CV_s=%20 (n=5)



- *Sampling from the same cabbage field for analysis of chlrorphyriphos-methyl residue*

$$S_n = \frac{S_i}{\sqrt{n}}$$

The contribution of individual steps to combined measurement unceraitny in pesticide residue analysis



(Yolci Omeroglu 2013c; Ambrus vd. 2011;Farkas vd 2014; Ambrus 2009)

6. Compliance Assesment

- Article 14(6) of Regulation (EC) 178/2002 provides that "where any food which is
  - ✓unsafe is part of a batch, lot or consignment of food of the same class or description,, it shall be presumed that all the food in that batch, lot or consignment is also unsafe, unless following a detailed assessment there is no evidence that the rest of the batch, lot or consignment is unsafe".
- Therefore each lot should be sampled seperately
- Between fields, variation of residues in composite samples is usually two to three times larger than the variation within field due to the differences in mean values of residue
- In a mixed consignment, a lot containing residues/contaminant above the legal limit can easily remain unobserved.
- Consequently, sampling of mixed lots should be avoided as far as practically possible *(Hill, 2000; Ambrus, 2004)*.

MRL and ML values

- The Maximum Residue Level (MRL) and Maximum Level (MLs) are defined as the maximum legally permissible average concentration of chemical contaminants in a composite sample with specified minimum mass and size in terms of the number of primary samples (single-sample increments)
- The sample sizes defined in the related regulation

(Farkas et al., 2015;EC 1881/2006, 396/2005, etc.)

Use of Combined Uncertainty of Pesticide Residue Results for Testing Compliance with Maximum Residue Limits (MRLs)

(Z.Farkas, A.Slate, T.B. Whitaker, G. Suszter, Á. Ambrus, J. Agric. Food Chem. 63:4418-4428)

- Diffirent sampling plans are required for testing compliance with MRLs of pesticide residues in commodities before and after marketing

- **Pre-market control:** The combined uncertainty (CV_R)of residue data including the contribution of sampling (CVs) is used for calculation of an action limit, which should not be exceeded when compliance with maximum residue limits is certified as part of premarketing self-control programs.

- **Market control:** for testing compliance of marketed commodities the residues measured in composite samples should be greater than or equal to the decision limit calculated only from the combined uncertainty of the laboratory phase (CV_L) of the residue determination.

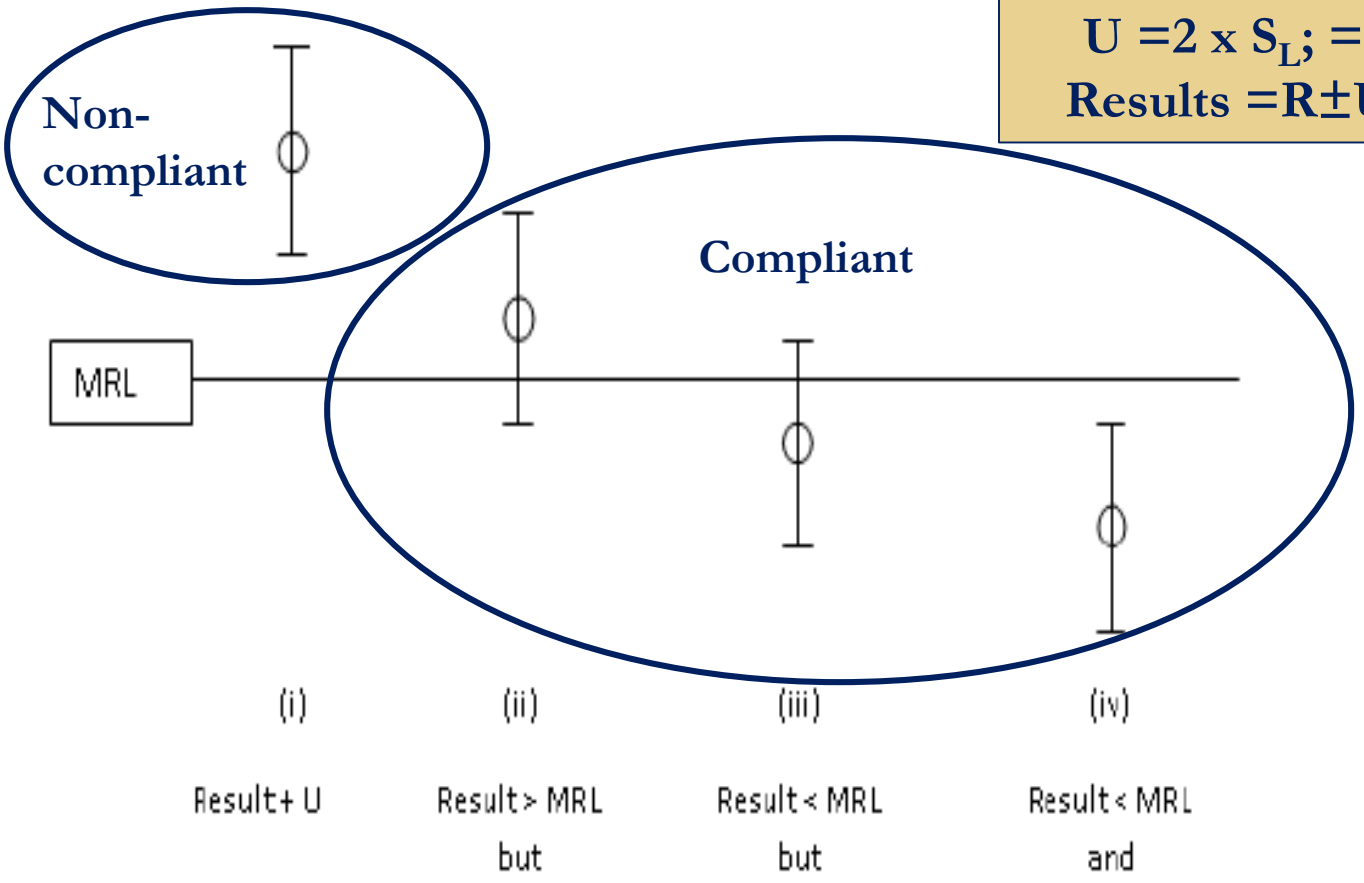
  - ✓CV_L is given as maximum 25% value for pesticide residue analysis in SANTE (2017) Guideline; Maximum standard uncertainty for myxcotoxin analysis is given as a function of LOD and concentration in EU 406/2006

Commodotiy on the Market

$$CV_{Lc} = \sqrt{CV_{SS}^2 + CV_{Sp}^2 + CV_A^2}$$

$U = 2 \times S_L$; $= 2 \times CV_L \times R$

Results $= R \pm U$ (mg/kg)



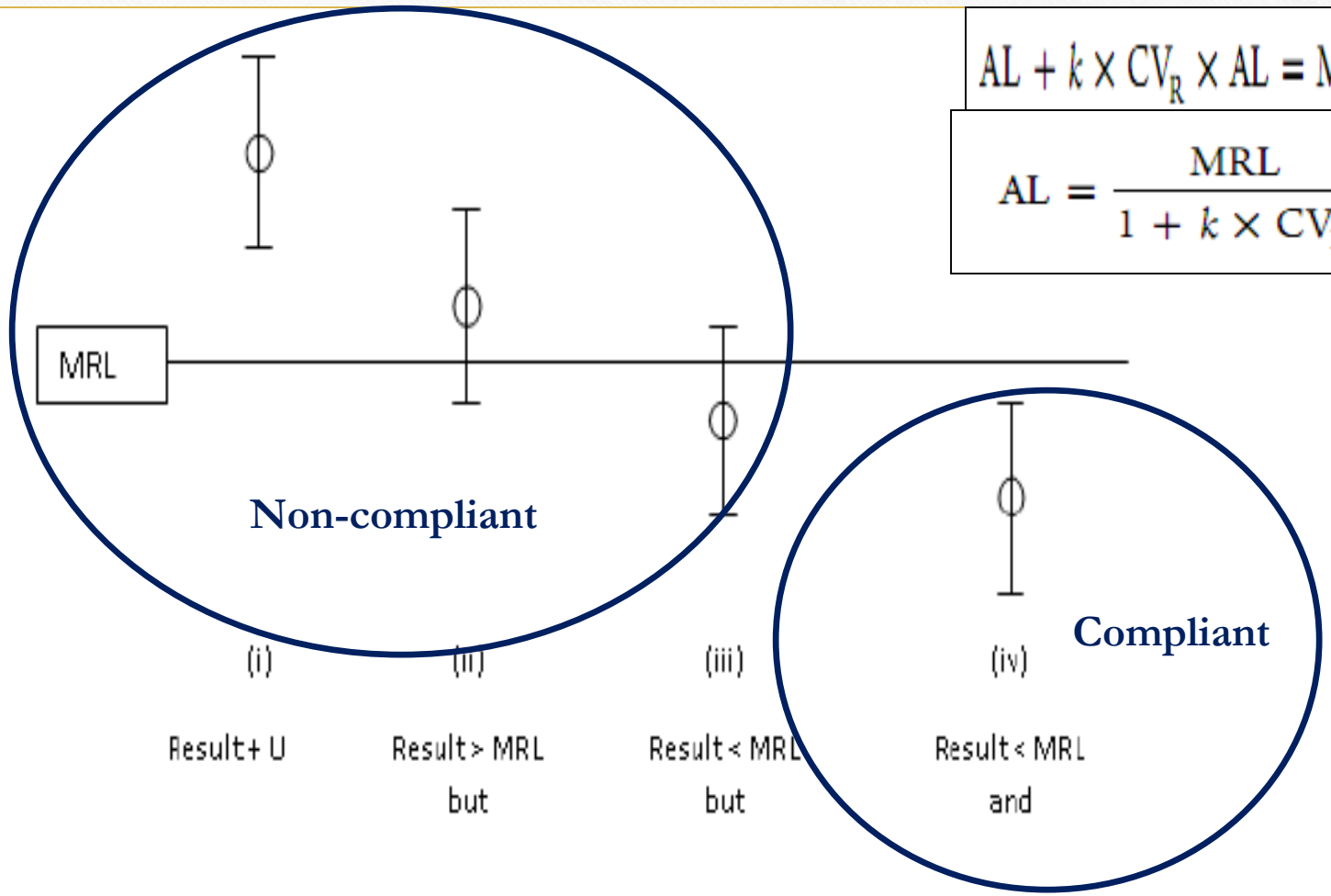
Desicion Limit=DL= 2MRL (for pesticide residue)

Pre-marketed control

$$CV_R = \sqrt{(CV_S)^2 + (CV_L)^2}$$

$$AL + k \times CV_R \times AL = MRL$$

$$AL = \frac{MRL}{1 + k \times CV_R}$$



Whitker model for AL for mycotoxin:http://www.fstools.org/mycotoxins/.

Conclusion

- The maximum allowable limits for residue and contaminants in food would at low levels (µg/kg or µg/l)
- Therefore, an ultimate attention should be given to select representative sample from a lot which has weight more than 1 tonnes
- Otherwise, all efforts and costly work spent for analytical steps may be wasted
- Sampling is very important step when controlling a commodity of a lot with legal limits
- Uncertainty associated with sampling are higher compared to uncertainties of sample preparation and analysis
- Uncertainty of sampling should be taken into account when controlling pre-maket commodity, and AL can be used based on the commodotiy, sample size and the allowable violation rate

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THANKS FOR YOUR ATTENTION !!!