Use of the combined uncertainty of measurement result for testing compliance of food commodities with legal limits

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in cooperation with

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Objectives

- Interpret the maximum limits from the prospectives of producers and official control.
- Use of combined uncertainty for establishing action and decision limits by the producers and buyers, respectively.
- Illustrate the principles with practical examples.



Basic definitions – legal limits

- **Maximum level (ML)**

  For contaminants, naturally occurring toxicants and nutrients, the maximum concentration of a substance recommended by the Codex Alimentarius Commission to be legally permitted in a given commodity. It applies to the avearge concentration of the chemical in samples meeting the minimum mass and sample size requirements

- **Maximum residue limit (MRL, ML) for pesticide residues**

  The maximum concentration of a pesticide residue (expressed as milligrams per kilogram) to be legally permitted in or on food commodities and animal feed. MRLs for meat and poultry apply to a bulk sample derived from a single primary sample, whereas MRLs for plant products, eggs and dairy products apply to the average residue in a specified portion of the composite bulk sample derived from 1-10 primary samples.

Control of the commodities

There are two distinctly different situations which needs different sampling plans:

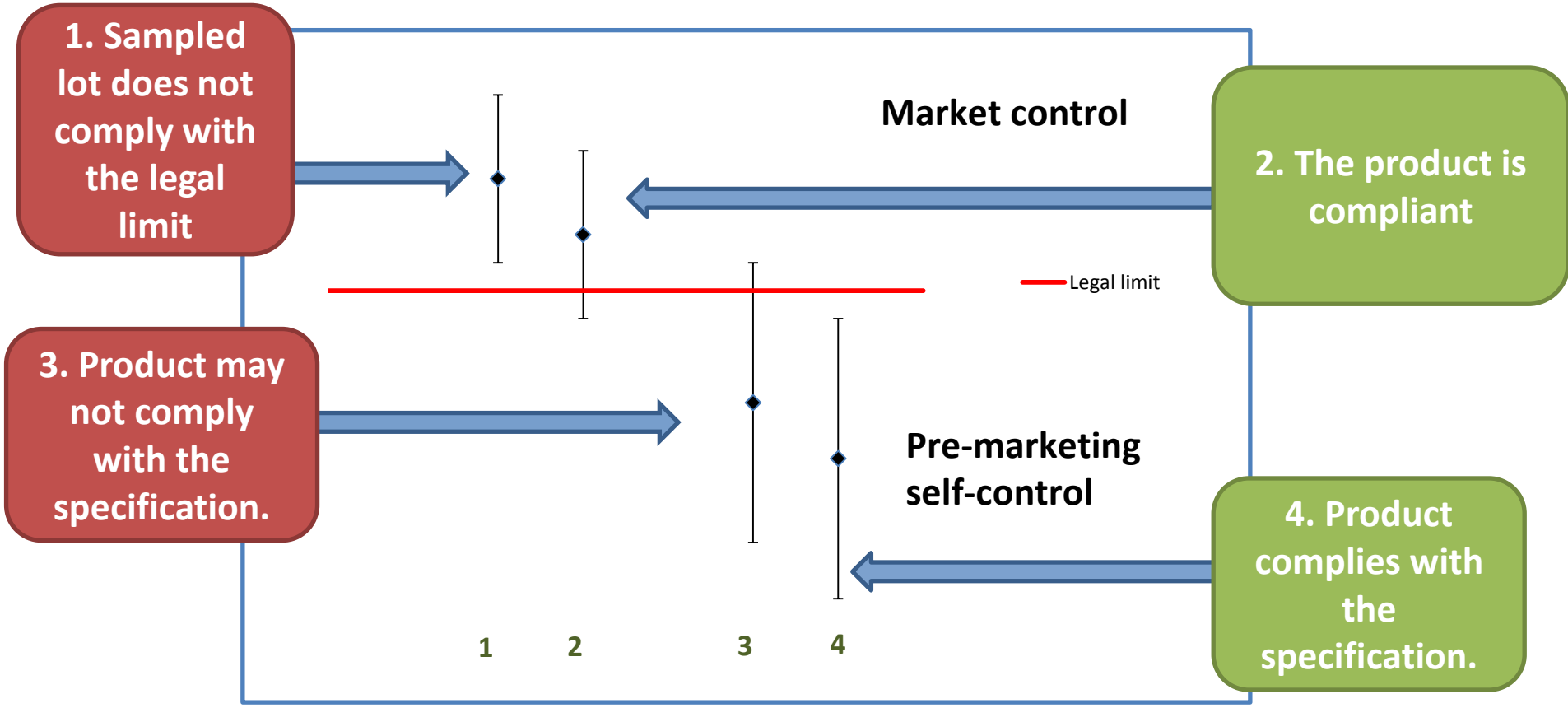
Premarketing self-control

- it has to be certified that at least a specified proportion of the product in terms of the minimum size and mass of bulk/laboratory sample complies with the legal limit
- the combined uncertainty including sampling uncertainty (**CV_R**) shall be taken into account

Control of commodities on the market

- a lot is considered non-compliant if the measured analyte concentration corrected for recovery, where specified, minus the expanded uncertainty of the results are above the legal limit.
- the combined uncertainty of the measured concentration (**CV_L**) shall only be taken into account (excluding the sampling uncertainty)

Illustration of the consideration of combined uncertainty of the measurement result



Distributions of contaminants in food

If the measured value is compared to the legal limit the chance of wrongly declaring a lot to be compliant depends on the distribution of the measurand in the tested food.

- if the tested commodity is homogenous in term of the contaminant (aflatoxin M1 in milk), then the uncertainty of the analytical measurement (e.g. 15% for ELISA-based detection of aflatoxin M1) need only to be considered ;
- the pesticide residues in fruits and vegetables, and ochratoxin in pistachio are distributed approximately following lognormal distribution; in case of pesticide residues the CV_R of 35-45% shall be taken into account.
- due to the very patchy distribution of aflatoxins in nuts, cereals, etc. their distribution can be best described with negative binominal function; the CV_R around 60-70% can be expected.







OBJECTIVE: Combined uncertainty of results **(S_{Res})** should be as low as practically posible

$$S_{Res} = \sqrt{S_S^2 + S_L^2}$$

$$CV_{Res} = \sqrt{CV_S^2 + CV_L^2}$$

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

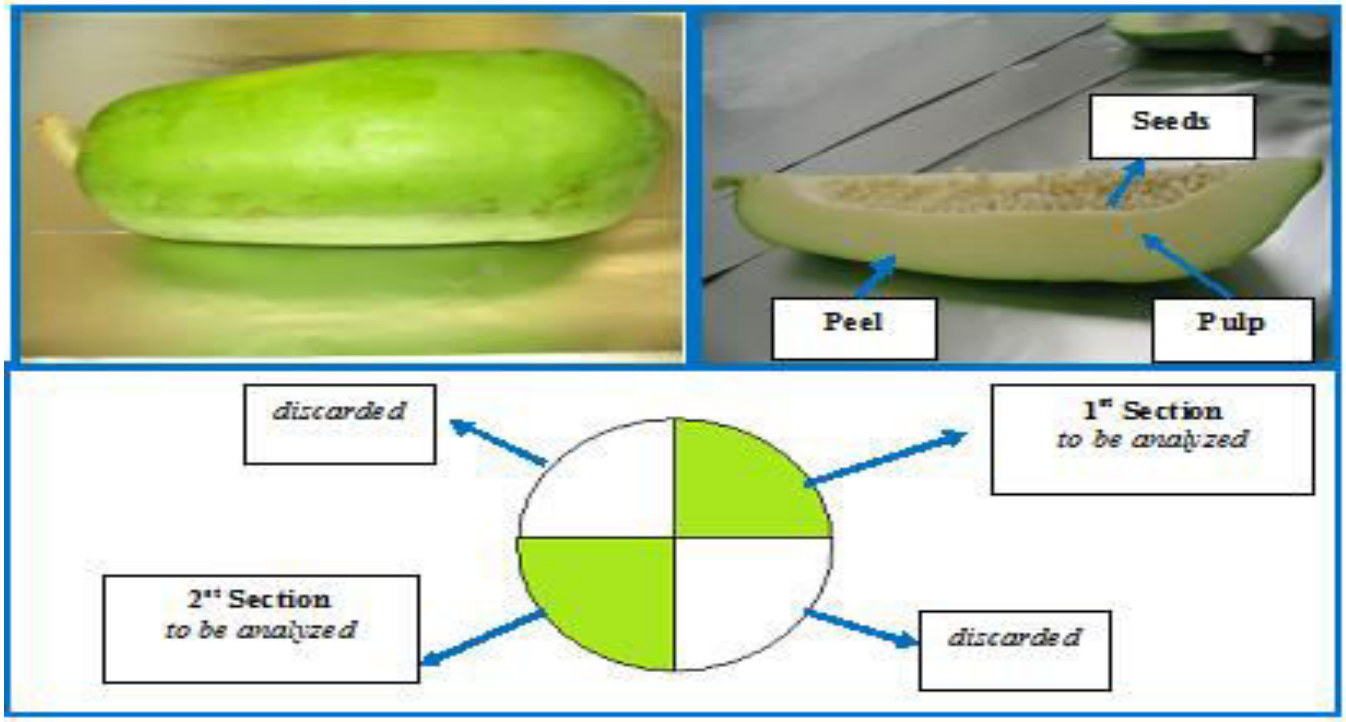
Ring tests, proficiency tests and internal quality control provide information only for CV_A

What do we kow about the contribution of CV_S, CV_{SS}, CV_{Sp} ??

Sample size reduction CV_{SS}



Sample size reduction



Courtesy of Perihan Yolci

Internal quality control

Regularly re-analyse replicate test portions at different time intervals.

Select replicate results which are within the 95% critical range.

2 replicates:

$$C_{\max} - C_{\min} = CD = 2.8\, CV_{Ltyp}\, \overline{R}$$

3 replicates

$$C_{\max} - C_{\min} = CD = 3.3\, CV_{Ltyp}\, \overline{R}$$

Determination of CV_L

- Calculate their relative standard deviations from the results of replicate test portions :

$$R_{\Delta i} = 2(R_{i1} - R_{i2})/(R_{i1}+R_{i2})$$

$$CV_{Lab} = \sqrt{\frac{\sum_{i=1}^{n} R_{\Delta i}^2}{2n}} \qquad CV_L = \left(\frac{\sum R_{\Delta i}}{n}\right)/1.128$$

For 2 replicates

ν = n
(# : number of replicate test portions)

Effect of particle size

Gy's sampling RSD=CV_{Sp}

$$CV_{Sp} = Cd^3F = Cd^3\left(\frac{1}{M_{TP}} - \frac{1}{M_{As}}\right)$$

C: shape factor,

d: upper 95% of particle size,

M_{Tp} : extracted test portion,

M_{As} : mass of homogenised portion of sample

M_{AS}=1000 g M_{Tp}: 25g (**F=0.039**); 10g (**F=0.099**); 5g (**F=0.199**); 2 g (**F=0.499**)

Ingamells' sampling constant: $K_s = M_{Tp} CV_{Sp}^{\ 2}$

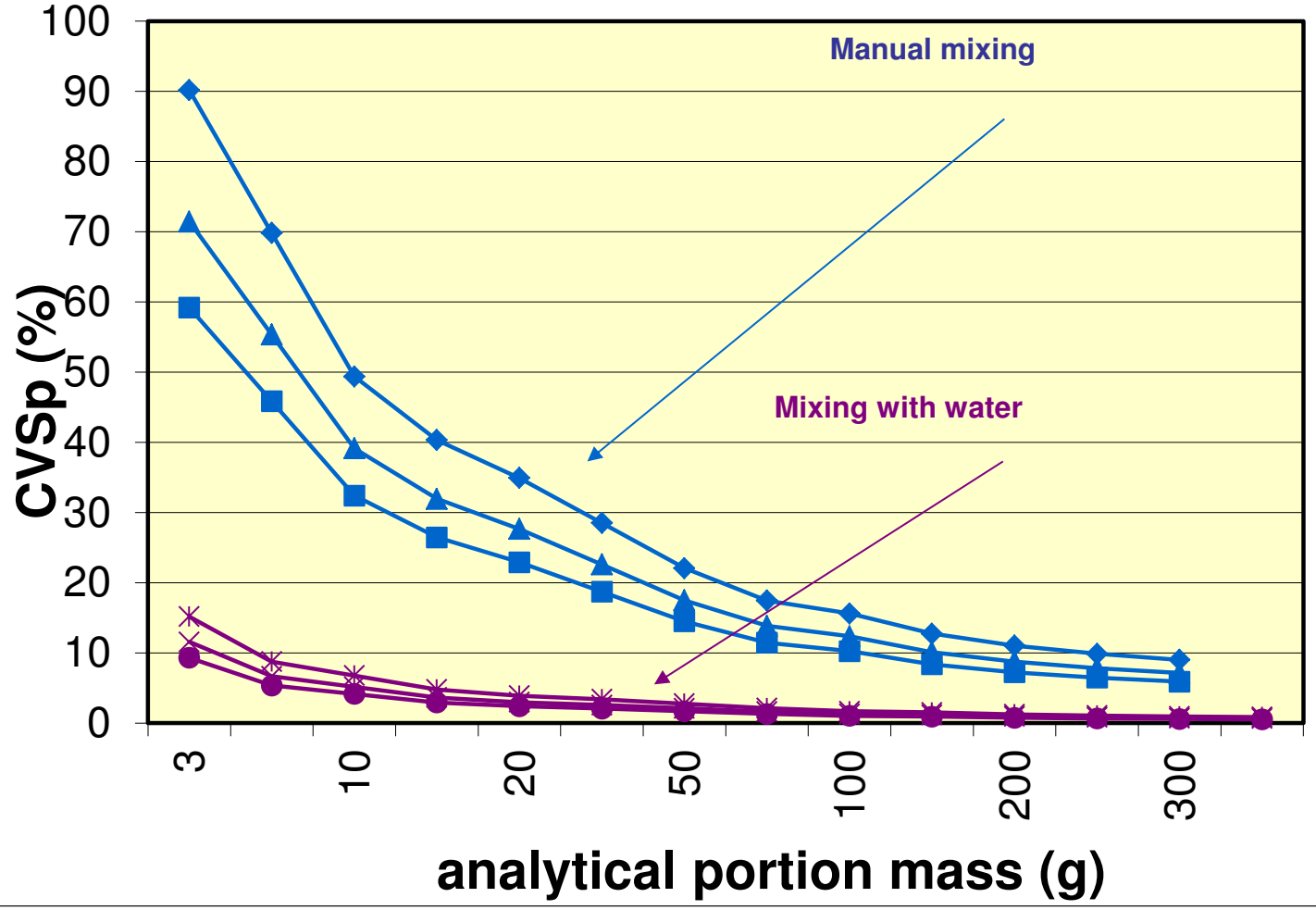
The effect of test portion size reduction

$$CV_L = \sqrt{CV_{ss}^2 + CV_{sp}^2 + CV_A^2} \qquad CV_L = \left(\frac{\sum \Delta}{n}\right) / 1.128$$

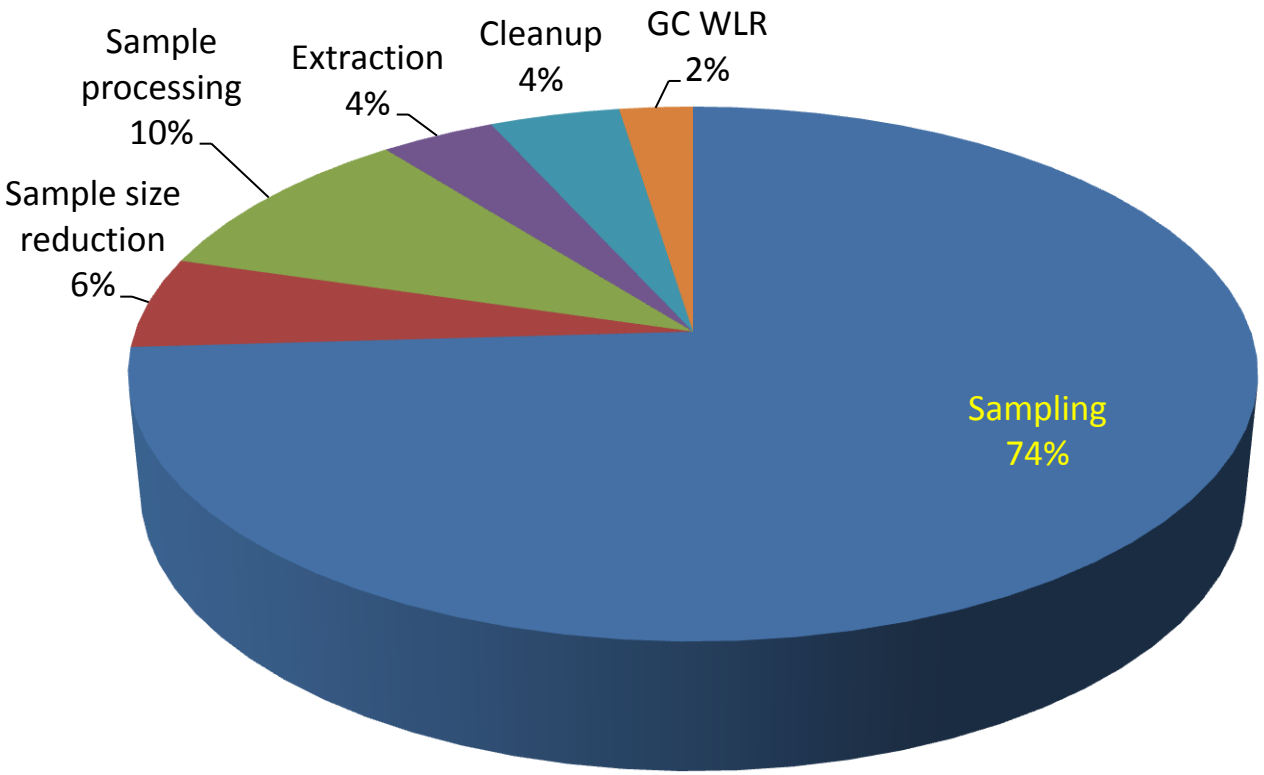
Gy's equation $$CV_S = Cd^3\left(\frac{1}{M_{Tp}} - \frac{1}{M_{Ls}}\right)$$

Test portion M_{Tp} [g]	Mass of laboratory sample (M_{Ls})		
	1 kg	2 kg	5kg
	Multiplying factor		
1	**15.2**	**15.1**	**15.0**
2	7.6	7.5	7.5
5	3.0	3.0	3.0
10	1.5	1.5	1.5
15	**1.0**	**1.0**	**1.0**
25	0.6	0.6	0.6
50	0.3	0.3	0.3

Comparison of sample processing error with water mixing and dry grinding & mixing



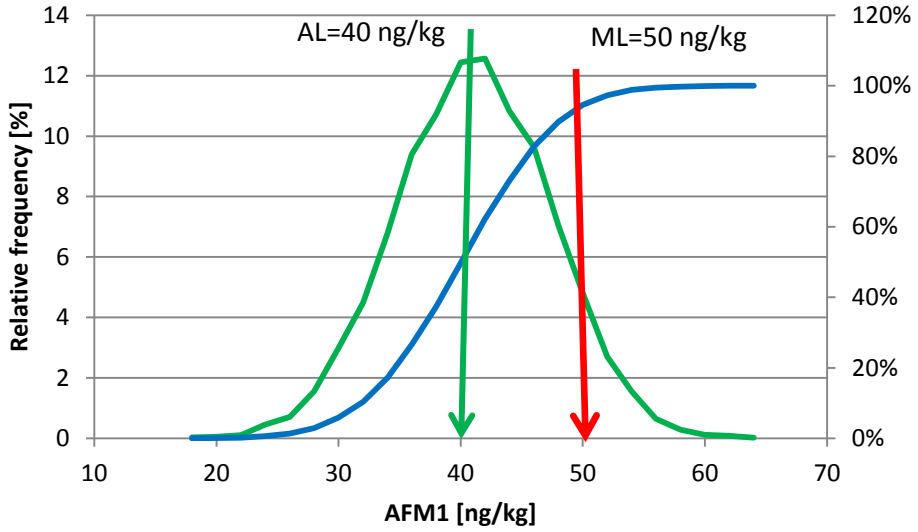
Typical contribution of the steps of pesticide residues determination (CV_R=0.38) to the combined uncertainty



The CV_A is only 11%

Interpretation of measure values

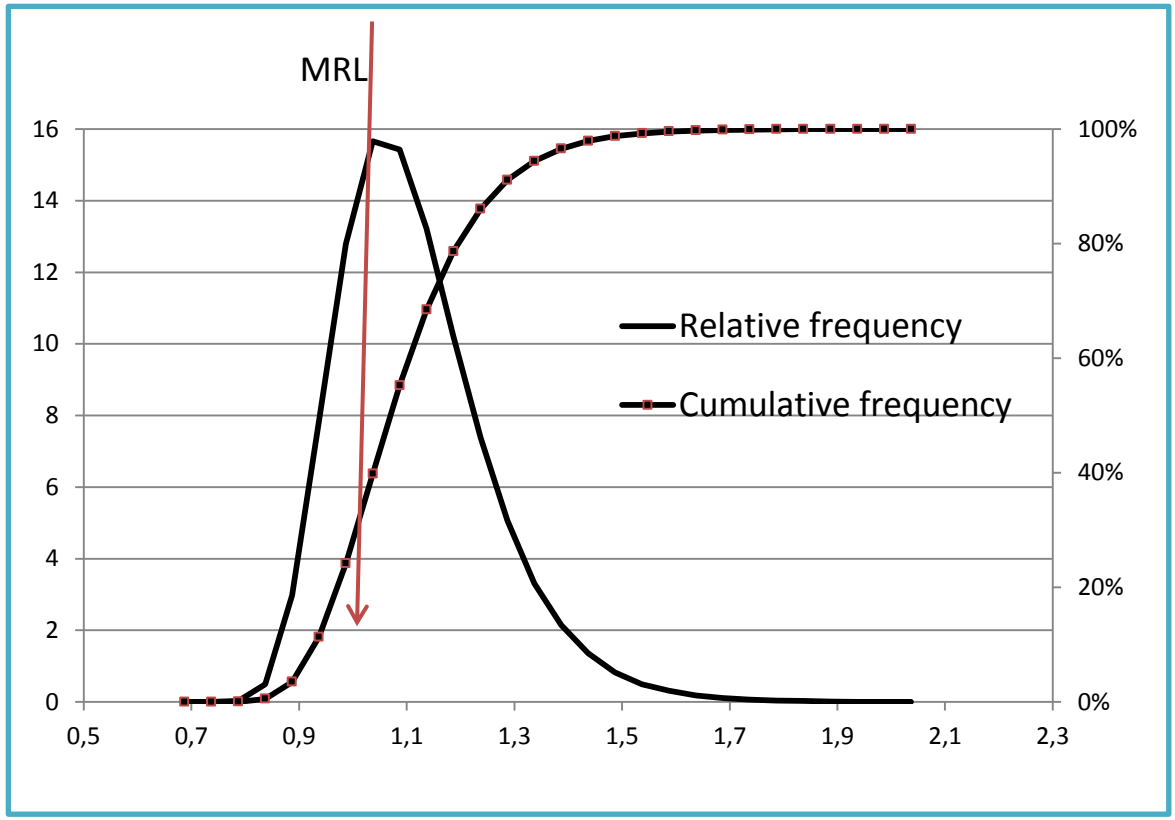
Relationship of AL and ML in case of determination of AFM1 in milk.



Where the product to be tested can be considered homogeneous such as bulk milk, the sampling uncertainty is practically zero. In this case only the uncertainty of the analytical measurement (CV_L) should be taken into account .

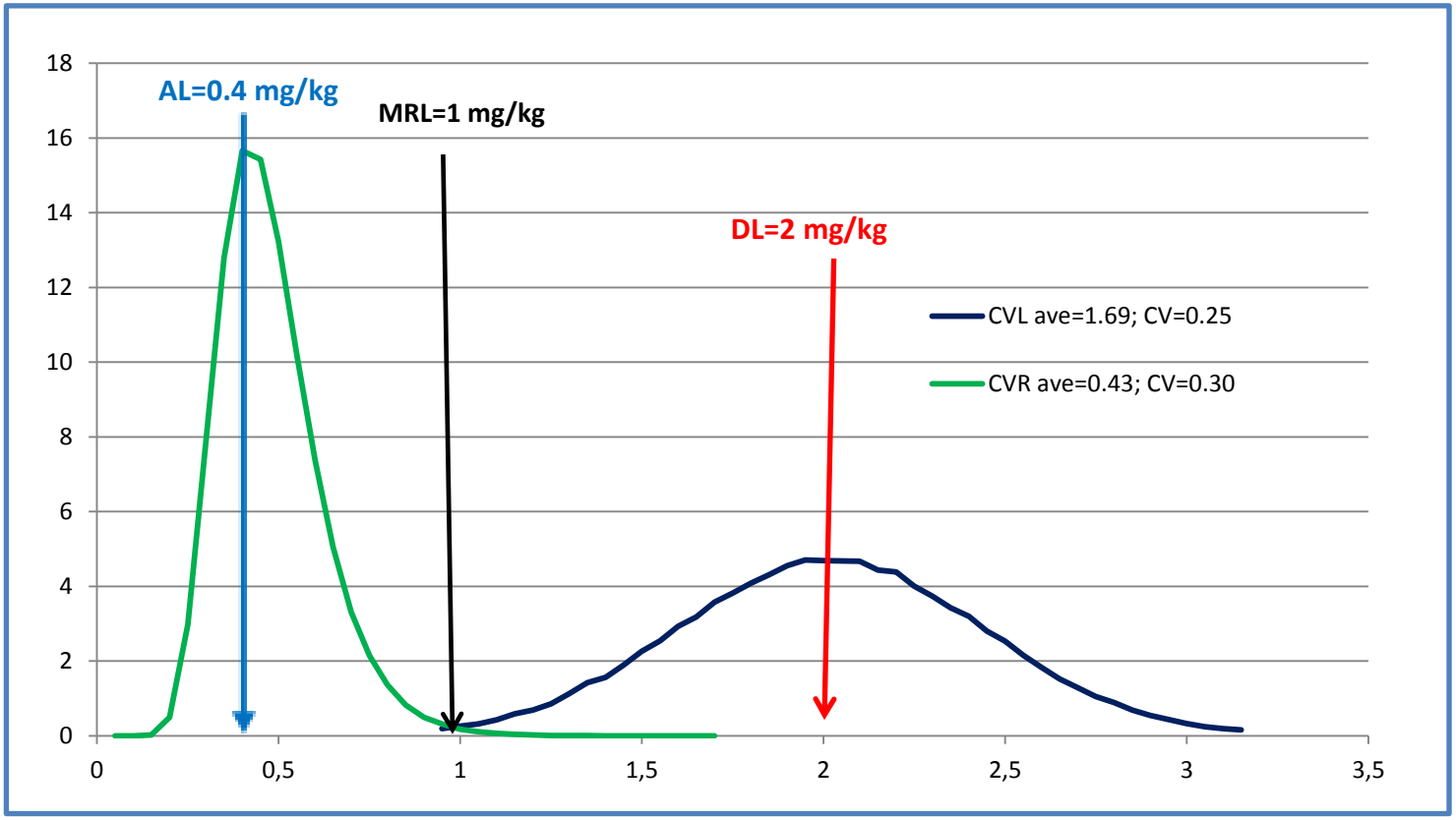
Comparison of mesured value to the MRL/ML

Distribution of residues in apple composite samples.



If we compare contaminants/residues in composite samples to the ML/MRL we would make wrong decision in over 50-70% of the cases depending on the measurand and sample.

Relationship of the Action limit (AL) for testing and the decision limit (DL) for verifying compliance with an MRL of 1 mg/kg of an apple lot.



Premarket control: Action limit

Post-market control: Decision limit (expanded uncertainty =2x SD, CV = 0.25)

Examples of estimated action limits for selected pesticide residues

	MRL mg/kg	AL mg/kg
acephate	0.02*	LD≤0.008
azoxystrobin	3	1.2
chlorpyrifos	0.05*	LD≤0.02
cyfluthrin	0.1	0.04
difenoconazole	1	0.4
indoxacarb	0.3	0.12
tetradifon	0.01*	LD≤0.004

Calculation of compliance of mycotoxin contamination taking into account the AL or targeted complinace level

Input data

Commodity - mycotoxin combination

Variability of measurand in composite samples.

Mass and number of laboratory sample

CV_L of the laboratory analysis

Test portion size

Web based tool has been developed:

http://www.fstools.org/mycotoxins/.

Template for mycotoxins

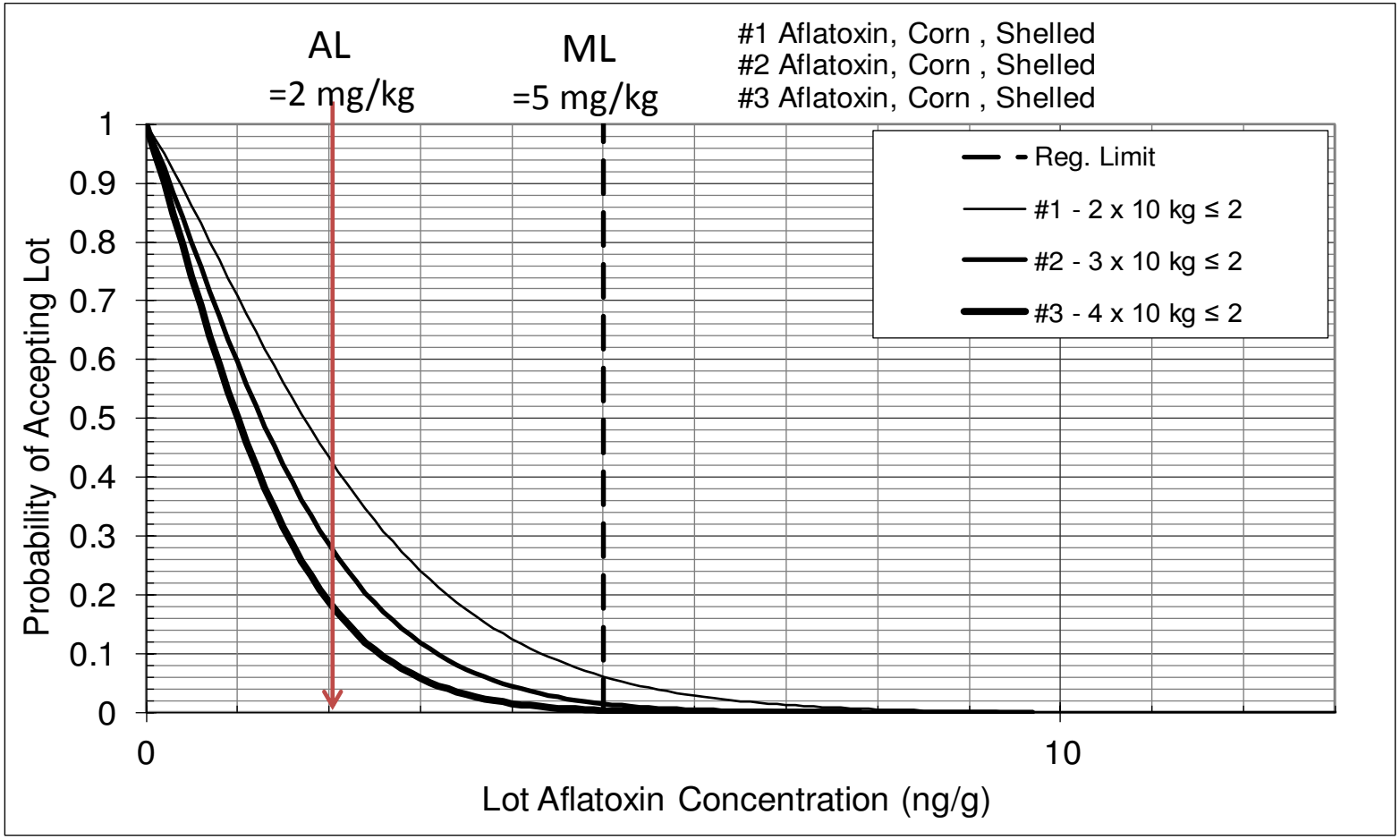
Mycotoxin, Commodity	Aflatoxin, Corn , Shelled	Aflatoxin, Corn , Shelled	Aflatoxin, Corn , Shelled
Laboratory Sample Size - ns (kg) =	10.00	10.00	10.00
Number Laboratory Samples - scnt *(#)* =	2	3	4
Test Portion - nss (g) =	50	50	50
Number of aliquots - na =	1	1	1
Accept/Reject Limit (ng/g) =	2	2	2
Regulatory Limit (ng/g) =	5.0		

The Mycotoxin Sampling Tool can be accessed at the following website address: **http://www.fstools.org/mycotoxins/**.

FAO encourages Codex members to use the tool. Feedback on the tool can be sent at food-quality@fao.org

Additional references on related topics can be found on the web at http://www.bae.ncsu.edu/usda/www/whitaker1.htm

Practical examples



Conclusions and recommendations

- The concept of the action limit can be applied for the verification of the compliance of a particular lot, or can be used within an early warning control programme.
- AL depends on CV_R (n, p, ap, CV_L) acceptable violation rate
- The sample size (number of primary samples, total mass) should correspond to that specified in relevant legislation.
- Producers should define suitable control points when appropriate action levels (Performance Criterion) can be applied.
- The sampling programme should be based on the precise definition of the sampling frame, weighting the potential risk associated with the production of a given product and the random sampling of the products all over the production cycle.
- Under such conditions the analytical results can be used to verify that the production is under control.



Close collaboration of all stakeholders is required for limiting rejection of lots and disputes in food trading

Thank you for your attention.