



# **Mycotoxins**

## **(Sampling - Sample Preparation - Methods)**

### **focused on Spices, Cereals,**

### **and European Union Legislation**

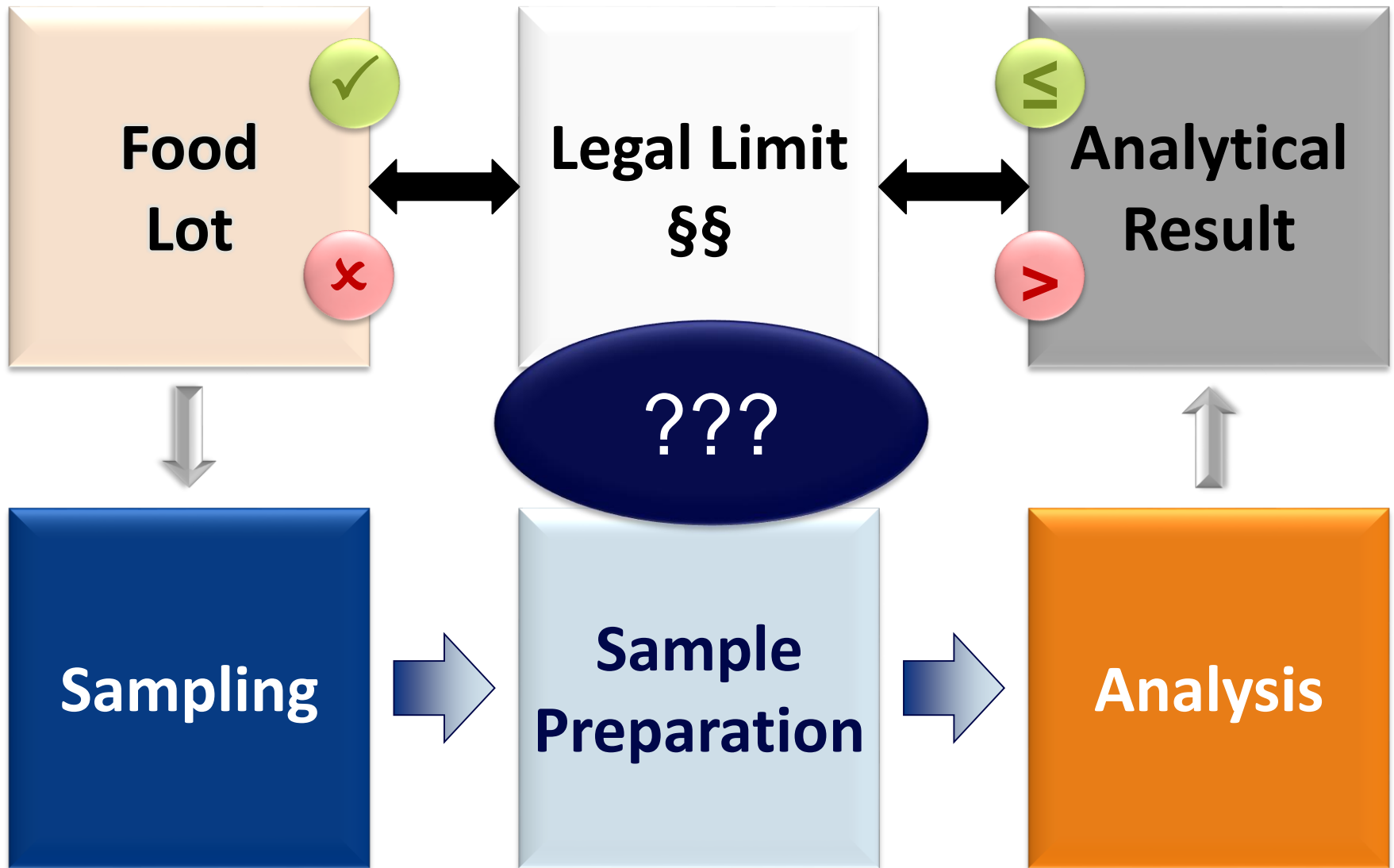
## **Micotoxinas**

**(Muestreo – Preparación de la muestra – métodos)**  
**enfocado a especias, cereales y la legislación Unión Europea**

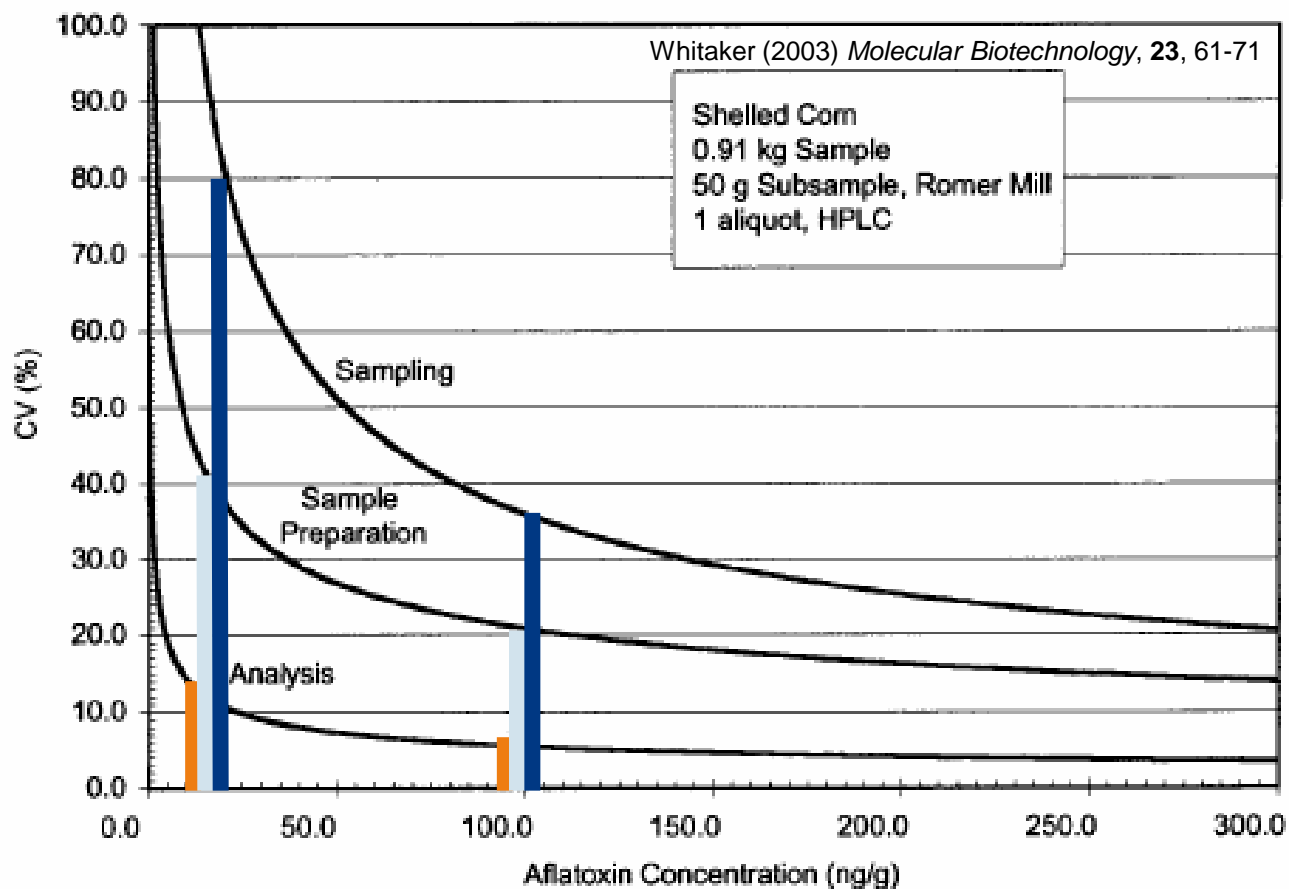
**INOFOOD,**

**23-24 October 2017, Santiago Chile**

**Mareike Reichel, Eurofins, Hamburg, Germany**



## Example Aflatoxins in Corn



← Depending on concentration

Sampling

Sample Preparation

Analysis

## Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs

**Legal  
Limit  
§§**

### *Article 1*

#### **General rules**

1. The foodstuffs listed in the Annex shall not be placed on the market where they contain a contaminant listed in the Annex at a level exceeding the maximum level set out in the Annex.

**Access to European Union Law: <http://eur-lex.europa.eu>**

## Commission Regulation (EC) No 1881/2006

**Legal  
Limit  
§§**

20.12.2006

EN

Official Journal of the European Union

ANNEX

Maximum levels for certain contaminants in foodstuffs <sup>(1)</sup>

### Section 2: Mycotoxins

Foodstuffs <sup>(1)</sup>		Maximum levels (µg/kg)		
2.1	<b>Aflatoxins</b>	B <sub>1</sub>	Sum of B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> and G <sub>2</sub>	M <sub>1</sub>
2.1.6	All cereals and all products derived from cereals, including processed cereal products, with the exception of foodstuffs listed in 2.1.7, 2.1.10 and 2.1.12	2,0	4,0	—
2.1.7	Maize to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs	5,0	10,0	—
2.1.10	Processed cereal-based foods and baby foods for infants and young children <sup>(3)</sup> <sup>(7)</sup>	0,10	—	—

## Commission Regulation (EC) No 1881/2006

	Maximum levels (µg/kg)		
	Unprocessed	Direct consumption	Baby food
Aflatoxin B1	5,0*	2,0	0,10
Sum of B1, B2, G1 and G2	10,0	4,0	
Ochratoxin A	5,0	3,0	0,50
Deoxynivalenol	1,750*/1,250	750	200
Zearalenone	200*/100	200*/75	20
Fumonisin	2,000*	1,000*	200*

**Legal  
Limit  
§§**



## Commission Recommendation 2013/165/EU

Sum of T-2 and HT-2 Toxin	1,000**/200*/100	200**/100*/ 50	15
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\* Corn; \*\*Oats

## Commission Regulation (EC) No 1881/2006

Foodstuffs (1)		Maximum levels (µg/kg)	
2.1	<b>Aflatoxins</b>	B <sub>1</sub>	Sum of B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> and G <sub>2</sub>
2.1.9	Following species of spices: <i>Capsicum</i> spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne and paprika) <i>Piper</i> spp. (fruits thereof, including white and black pepper) <i>Myristica fragrans</i> (nutmeg) <i>Zingiber officinale</i> (ginger) <i>Curcuma longa</i> (turmeric)	5,0	10,0
2.2	<b>Ochratoxin A</b>		
"2.2.11.	Spices, including dried spices  <i>Piper</i> spp (fruits thereof, including white and black pepper) <i>Myristica fragrans</i> (nutmeg) <i>Zingiber officinale</i> (ginger) <i>Curcuma longa</i> (turmeric)  <i>Capsicum</i> spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne and paprika)  Mixtures of spices containing one of the abovementioned spices		15 µg/kg     30 µg/kg until 31.12.2014 15 µg/kg as from 1.1.2015  15 µg/kg"

**Legal  
Limit  
§§**

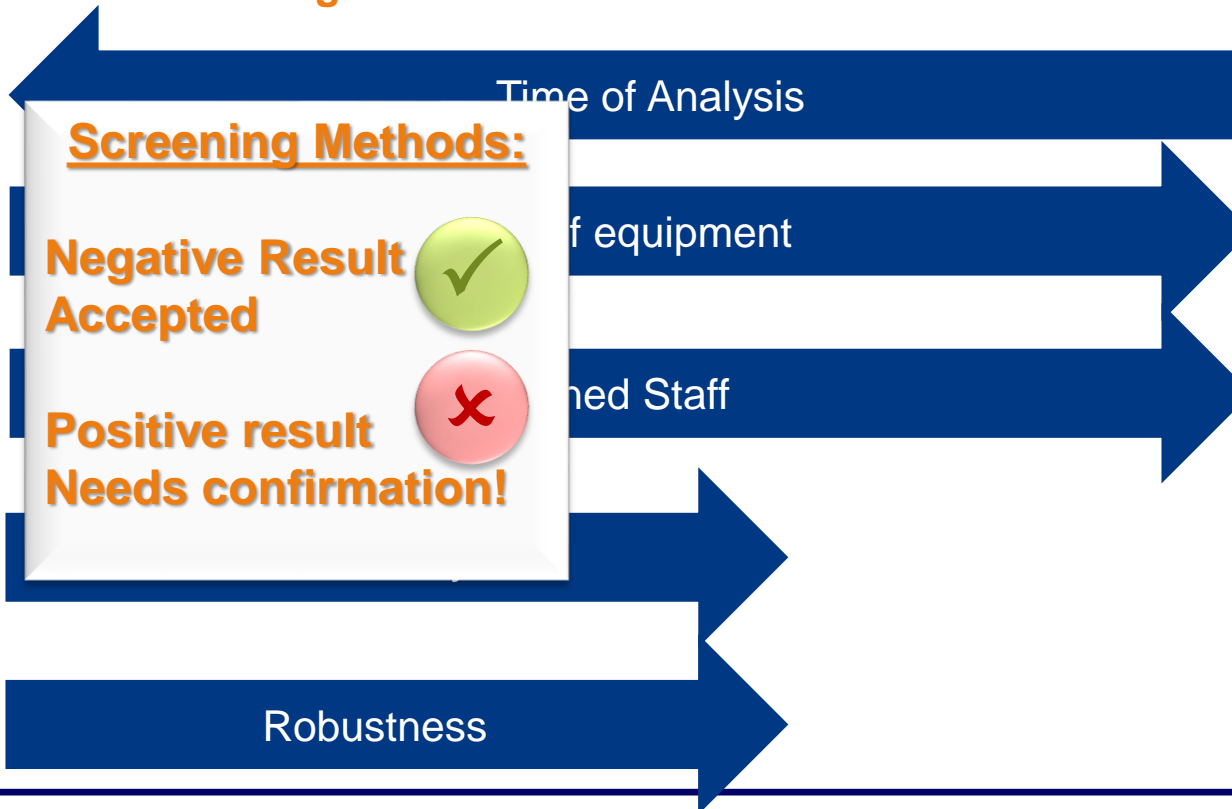


# Analytical Methods



On-site Testing

Reference Methods



## Screening Methods:

Negative Result Accepted



Positive result Needs confirmation!

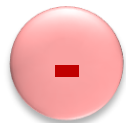




IAC  
HPLC  
FD



- Robust
- Cost-efficient equipment
- High sensitivity
- Wide linearity range

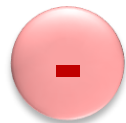


- Single toxin methods
- IAC clean-up costs
- Knowledge on matrix related interferences and proper detection techniques needed

HPLC  
MS/MS



- Multitoxin Analysis
- Fast, cost-efficient analytical preparation
- High selectivity
- Fast chromatography



- Strong Matrix effects
- Internal standard costs
- Limited linearity range
- Complex technique and instruments

Analysis

Lateral  
Flow  
Devices  
(LFD)

ELISA  
Tests

IAC  
HPLC  
FD

HPLC  
MS/MS

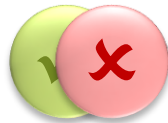
Analysis

On-site Testing

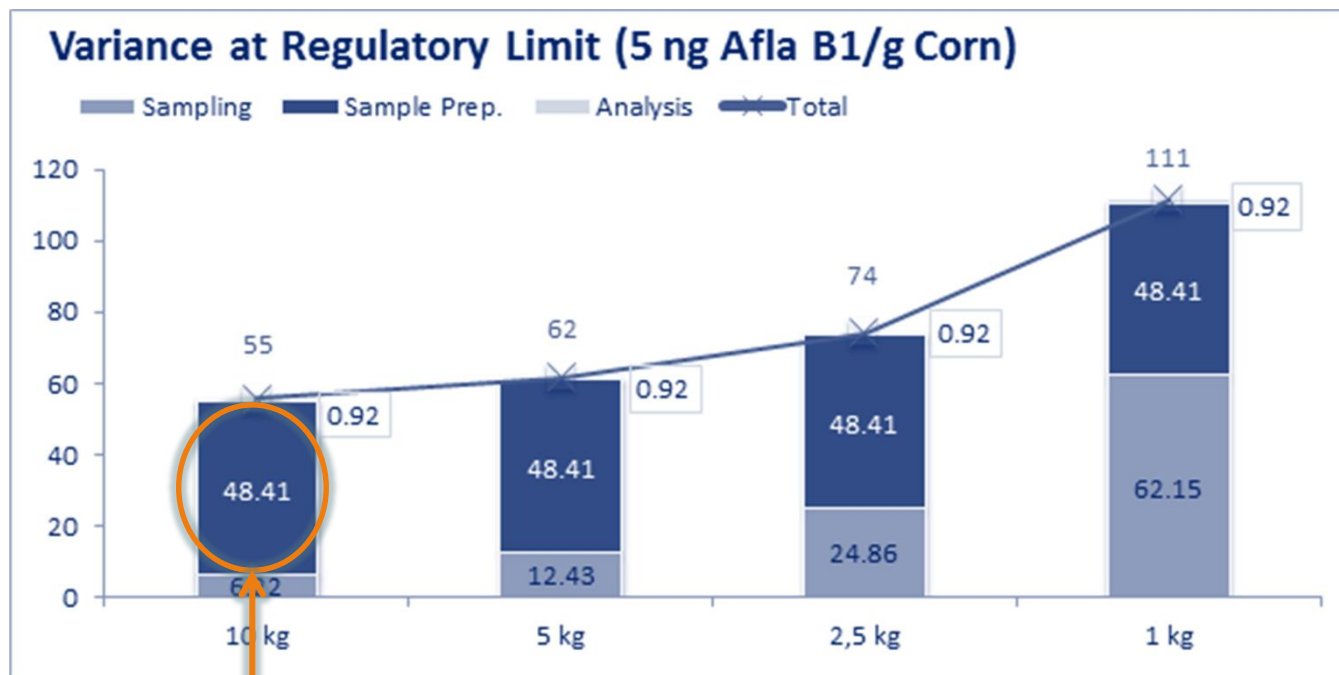
Reference Methods



Baby Food



## Example Aflatoxins in Corn



**Influence of sample preparation**

**Sampling**

**Sample Preparation**

**Analysis**

- Mycotoxin are distributed inhomogenously
- The complete sample has to be grinded
- Fine grinding  $<1$  mm of the whole sample
- Dry grinding with proper milling equipment and pre-grinding steps if necessary



~ 2 mm



0.7 mm

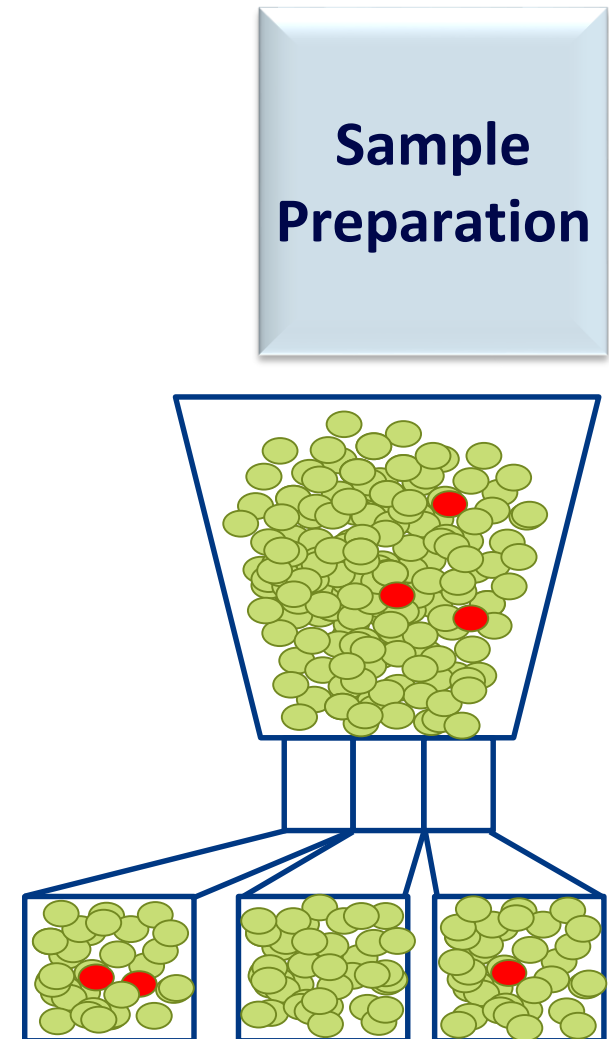
- For oily, fatty, and high sugar matrices wet-milling is necessary



**Sample  
Preparation**

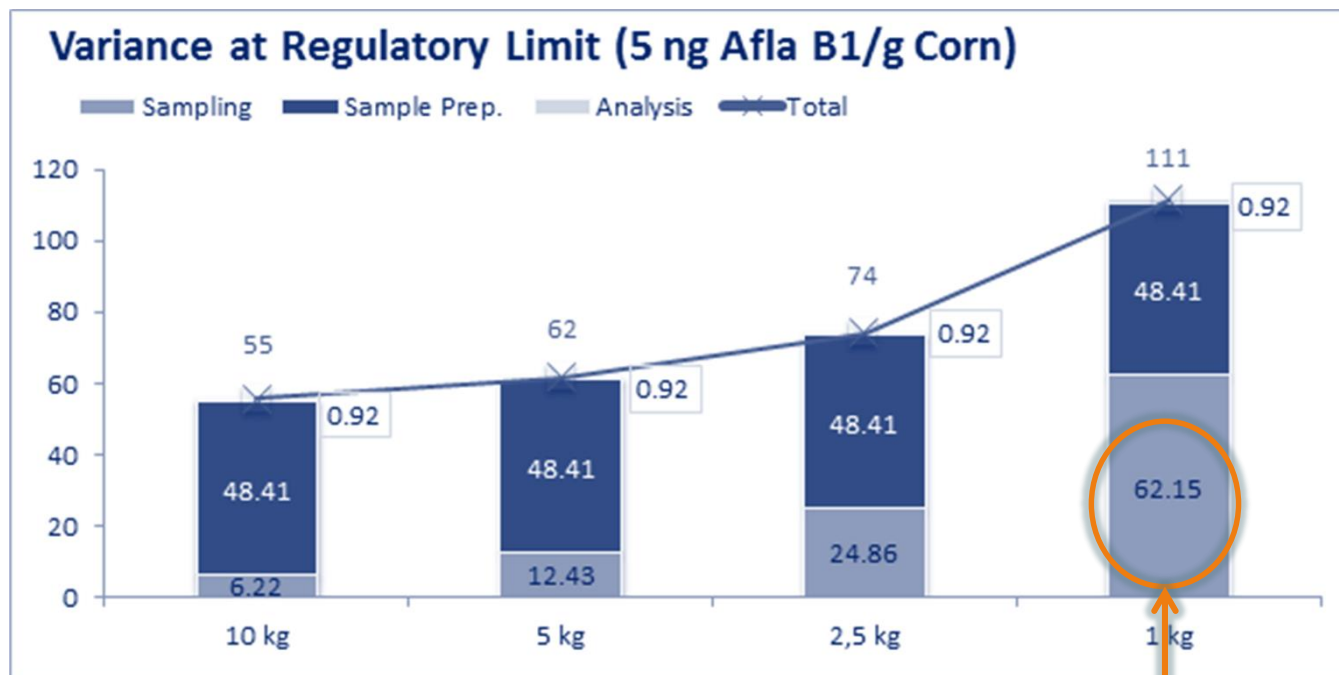


- Mycotoxin contamination of the whole lot could be caused by a few kernels
- Sample dividing before grinding increases the risk of incorrect decisions
- Inhomogeneous sample due to coarser grinding → Risk during sample dividing and weighing in the laboratory
- More reliable: Fine grinding <math>< 1\text{ mm}</math> of the whole sample
  - **For on-site control unrealistic in terms of needed workforce and time**



Sample divider

## Example Aflatoxins in Corn



Sampling

Sample Preparation

Influence of Sampling

Analysis

## Commission Regulation (EC) No 401/2006 Commission Regulation (EU) No 519/2014

### Sampling

“also of application for the official control of the maximum levels [...] in spices with a relatively large particle size....”

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.

## Commission Regulation (EC) No 401/2006

“...‘lot’ means an identifiable quantity of a food commodity **delivered at one time** and determined by the official to have **common characteristics, ...**”

Sampling

### B.2. General survey of the method of sampling for cereals and cereal products

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)
Cereals and cereal products	≥ 1 500	500 tonnes	100	10
	> 300 and < 1 500	3 sublots	100	10
	≥ 50 and ≤ 300	100 tonnes	100	10
	< 50	—	3-100 (*)	1-10

(\*) Depending on the lot weight — see Table 2.





## Commission Regulation (EC) No 401/2006

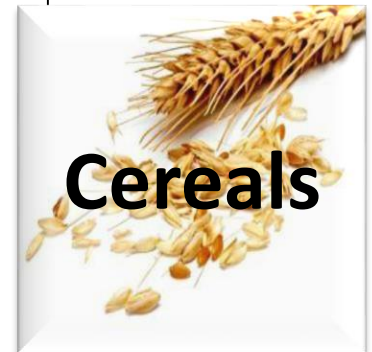
“...‘lot’ means an identifiable quantity of a food commodity **delivered at one time** and determined by the official to have common characteristics, ...”

# Sampling

Table 2

Number of incremental samples to be taken depending on the weight of the lot of cereals and cereal products

Lot weight (tonnes)	Number of incremental samples	Aggregate sample weight (kg)
≤ 0,05	3	1
> 0,05-≤ 0,5	5	1
> 0,5-≤ 1	10	1
> 1-≤ 3	20	2
> 3-≤ 10	40	4
> 10-≤ 20	60	6
> 20-≤ 50	100	10

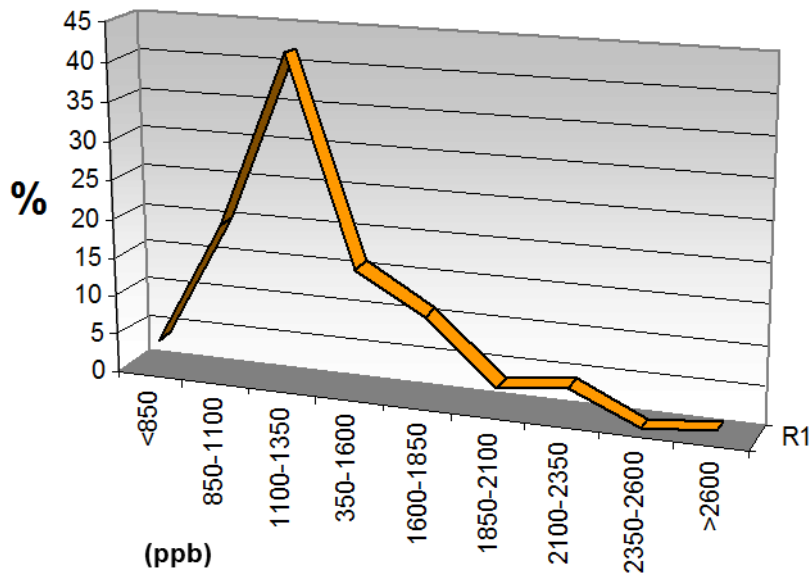


per truck

# 1 Truck – 100 Individual Samples

## Deoxynivalenol

Frequency Distribution



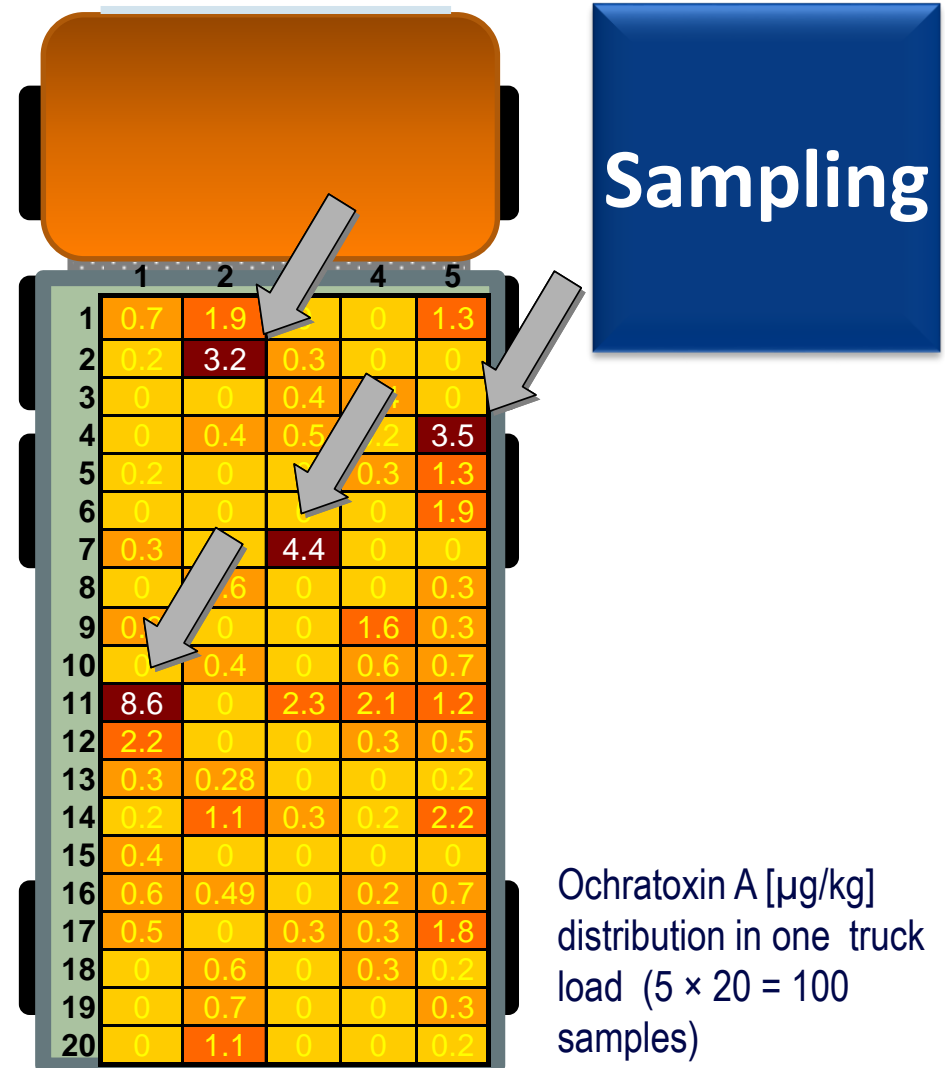
## Sampling

	1	2	3	4	5
1	1260	1440	1070	960	1075
2	1210	1370	1145	1185	1300
3	1360	1700	1160	860	930
4	1270	990	1025	1295	1160
5	1235	1285	1225	830	1195
6	850	950	845	1325	1032
7	1125	1170	1000	1010	1035
8	1045	1200	1515	850	1575
9	1790	1505	1245	1310	1475
10	1670	2245	2230	1765	1500
11	1885	1470	2655	1840	1570
12	1860	2130	1975	1700	1290
13	1620	1220	1460	1710	1580
14	2165	1270	1725	1520	1310
15	1280	1340	1540	1420	1730
16	950	1290	930	1100	1265
17	1290	1505	1275	1640	1220
18	1315	1110	1130	1260	1290
19	1210	1080	1240	1060	1075
20	1100	1180	1350	1125	1190

Deoxynivalenol [ $\mu\text{g}/\text{kg}$ ]  
distribution in one truck  
load ( $5 \times 20 = 100$   
samples)

According to Biselli, Persin, & Syben (2008) *Mycotoxin research*, **24**, 98-104

- Highly inhomogeneous distribution
- Few contaminated grains sufficient to cause the whole lot to violate maximum levels
- Sampling is the critical source of error
- Commission regulation for official controls [(EC) 401/2006] (100 × 100 g = 10 kg per truck)
  - **For on-site control unrealistic in terms of needed workforce and time**



According to Biselli, Persin, & Syben (2008) *Mycotoxin research*, **24**, 98-104

- Particles from millions of kernels
- Distributed all over grain lots
- Easily to separate from the grains
- Small particles → No grinding
- Accumulation of mycotoxins
- **Correlation between the mycotoxin contaminations of dust and grains**



Sampling



rapidust   
mycotoxins

# Dust sampling

Build up data models by static sampling  
from samples in the lab



## Sampling



rapidust  
mycotoxins

- **Correlation is essential to re-calculate concentration from the dust to the concentration in the grain lot**
  - Shown for DON in wheat, rye, corn
  - Shown for ZON in wheat, rye, corn
  - Shown for Aflatoxins in corn
  - First promising results for OTA in wheat, rye, corn



Sampling



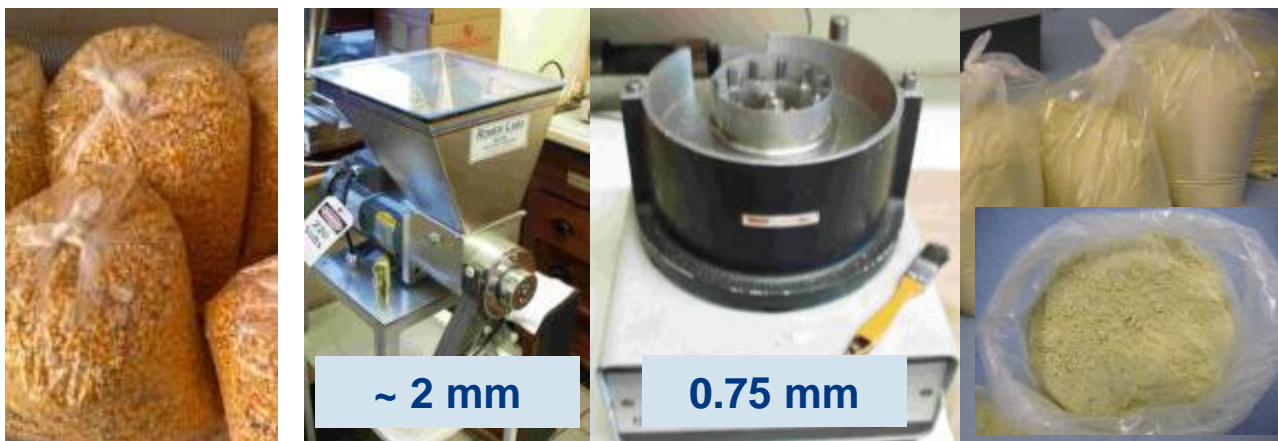
rapidust   
mycotoxins

Dust sampling < 2 min



Sampling

Kernel sampling, pre-grinding, grinding > 2 h



Sample Preparation



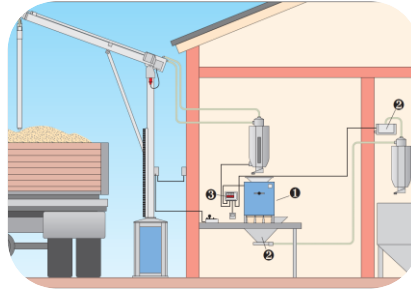
# Results DON in Corn

Truck	Origin/ Species/ Quality	DON [ $\mu\text{g}/\text{kg}$ ] direct (Kernel)		DON [ $\mu\text{g}/\text{kg}$ ] indirect (Dust)	
		Mean	Min-Max	Mean	Min - Max
1	Flint Corn 1	471 $\pm$ 15%	409	481 $\pm$ 3% 102 %	471
			572		491
2	Flint Corn 2-1	138 $\pm$ 14%	110	121 $\pm$ 3% 88 %	118
			152		123
5	Flint Corn 2-2	148 $\pm$ 18%	110	211 $\pm$ 4% 143 %	205
			172		218
7	Flint Corn 2-3	121 $\pm$ 45%	88	141 $\pm$ 4% 116 %	137
			203		145
3	Dent Corn 1-1	4372 $\pm$ 10%	3713	4302 $\pm$ 7% 98 %	4084
			4685		4521
4	Dent Corn 1-2	4415 $\pm$ 15%	3474	4306 $\pm$ 3% 98 %	4227
			4887		4386
6	Dent Corn 1-3	4428 $\pm$ 11%	3867	4647 $\pm$ 1% 105 %	4618
			5006		4677



# Available Dust Sampling Types

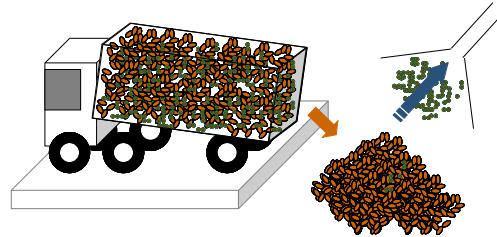
Before unloading



Sampling of kernels  
+ Sieving

**Sampling**

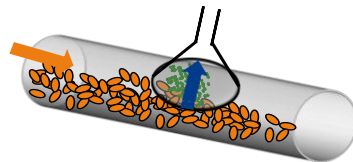
At unloading



Tailgate and side  
dumping

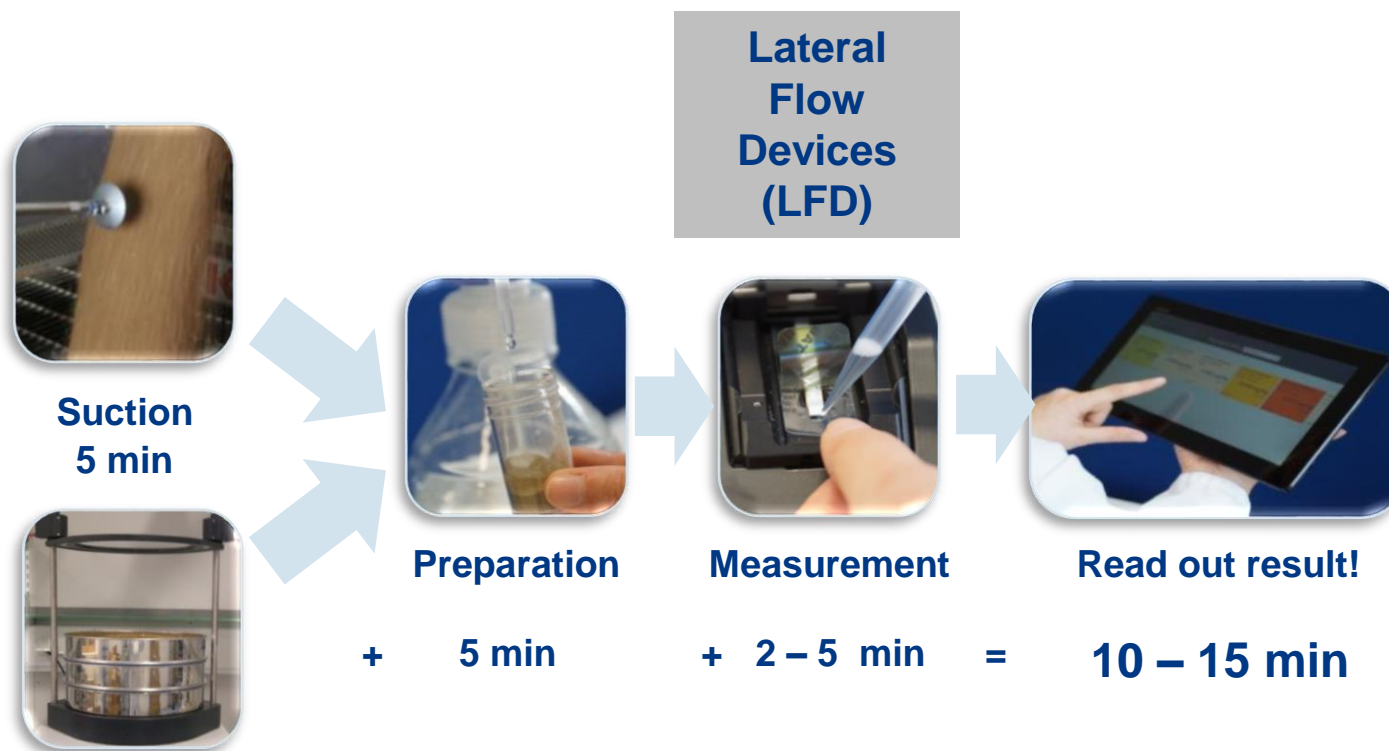


In-process



Conveyor, elevators,  
grain cleaning,  
loading

**rapidust**  
mycotoxins




**Sampling**

**Sample Preparation**

**Analysis**

→ Result is based on the whole lot  
or on 5 – 10 kg sample

→ Result after 10 – 15 min

	Reference method			Rapid tests
<b>Sampling</b>	8 – 100 insertions	5 – 8 insertions	Suction	1 – 8 insertions
<b>Sample size</b>	5 – 10 kg Kernels	5 – 10 kg Kernels	Dust	1 – 5 kg Kernels
<b>Sub-sampling</b>	No (complete grinding)	No; all kernels are sieved at once	No	Sub-sample ~ 100 g
<b>Grinding</b>	0.7 mm	<b>Unnecessary</b>		1 – 2 mm
<b>Analysis</b>	Reference method (HPLC-MS/MS)	Strip test		Strip test
<b>Execution</b>	Mostly external	On-site		On-site
<b>Sensitivity</b>	Very high	High	Very high	Low
<b>Execution Time</b>	≥ 10 h	<b>10 – 15 min</b>		≤ 30 min
<b>Evaluation</b>	Very secure	Secure	Very high	Insecure

**Sampling**

**Sample Preparation**

**Analysis**

→ The rapidust procedure is fast, simple & secure

- **Sampling is the most important step in mycotoxin analysis**
- **Innovative solutions like dust sampling can overcome laborious sampling procedures**
- **Good methods for sample preparation are at least as important as good analytical procedures**
- **Wet milling is to be preferred for analysis of aflatoxins and ochratoxin A in fat and sugar rich products**
- **Fast on-site testing good as pre-screening tool**
- **HPLC-MS/MS suitable for multimycotoxin analysis in simple matrices such as cereals**
- **IAC HPLC-FD still best for aflatoxins and ochratoxin A analysis at low levels and in complex matrices as spices**

**Sampling**

**Sample  
Preparation**

**Analysis**

**Thank you!**

**Thank you for your attention!**

**The global Eurofins Operational Best Practices Programme  
CCT Contaminants and Residues**

**Dr. Robert Gatermann**

**The Eurofins Competence Center for Biotoxins  
at Eurofins WEJ Contaminants, Hamburg:**

**Simone Staiger, Dr. Jan Sebastian Mänz**

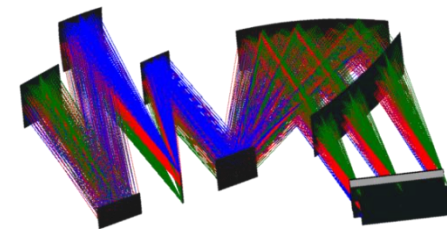
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