Central and Eastern European Regional Training of Trainers' Workshop on the Identification and Documentation of Living Modified Organisms under the Cartagena Protocol on Biosafety“, Ljubljana, Slovenia, 11-15 April 2011

# Sampling and detection of living modified organisms:

# Sampling methodology

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# Outline

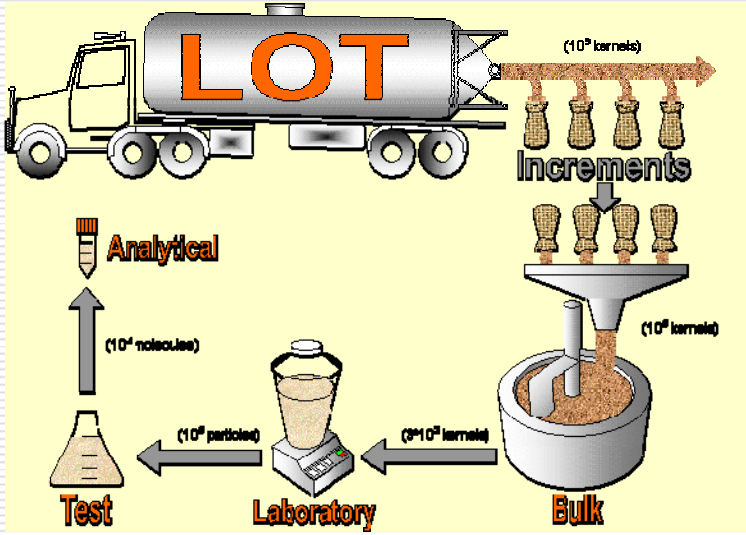
- **Introduction to sampling**
- EU legislative basis
- Sampling along the food and feed supply chain
- Sampling of seeds
- Sampling in the field

# Introduction

## The Concept of Sampling

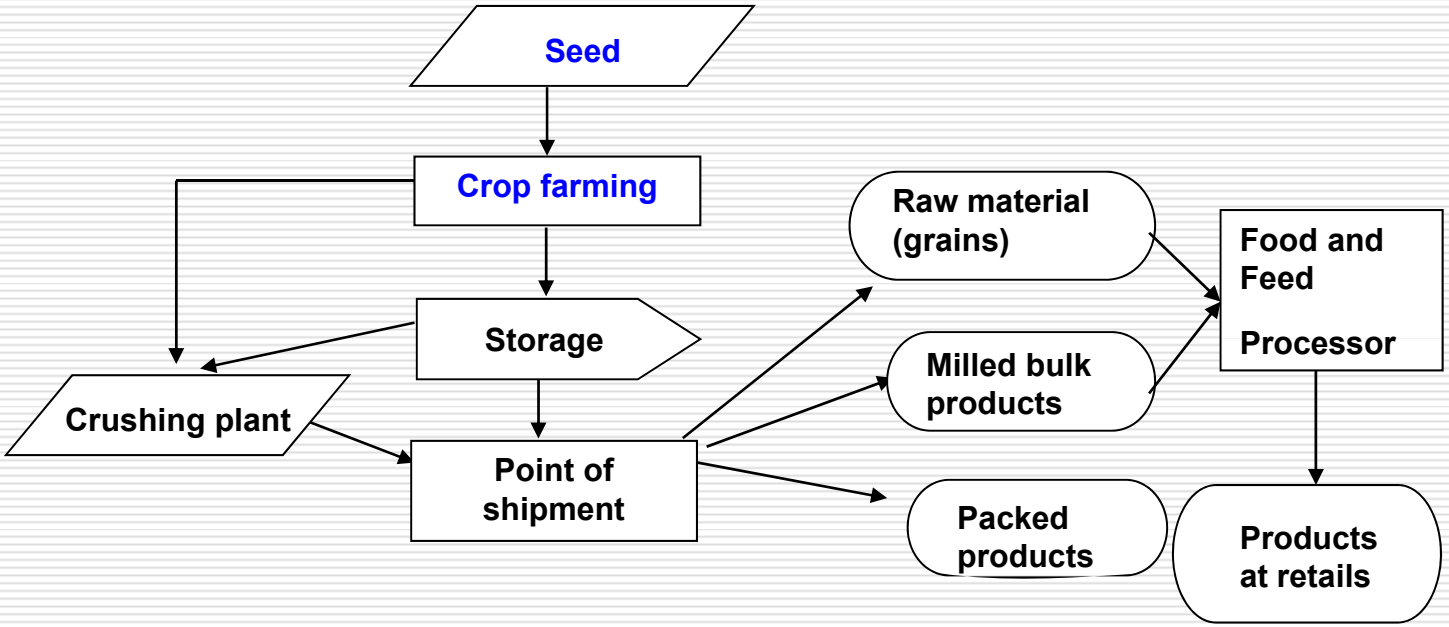
**Sample =** a collection of observations selected from a population according to a sampling procedure.

**Population =** the totality of observations about which we make inferences.




Sampling steps: general scheme

# Complexity of sampling in the supply chain

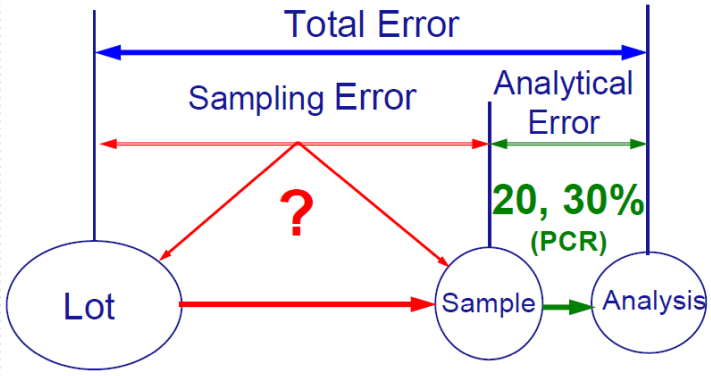






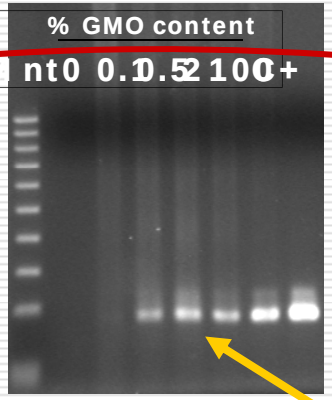

# Introduction cont.

- Sampling **is the most crucial first step in any analytical process** and is often the major source of error in the analysis of GMOs.
- The overall objective of a good sampling plan is to provide a representative sample for the analysis and to minimize this error.
- It is imperative that the sampling step is performed as accurately as possible so that the sample collected is representative of the batch under investigation (food, feed, seed, field) and to get **the most accurate "true value".**
- Obtaining representative samples deserves particular consideration since wrong sampling plan can greatly affect the reliability of the measured GMO levels.

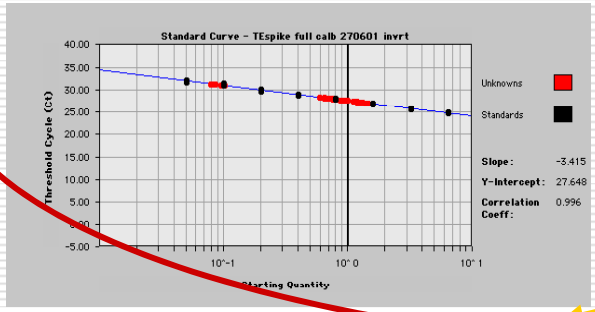


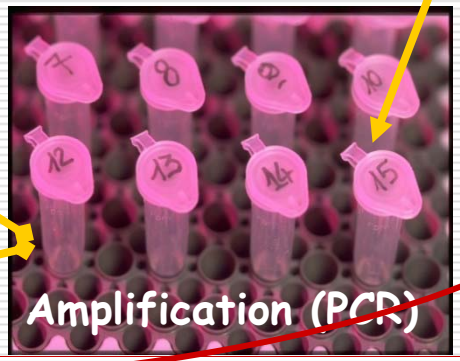
# Steps in GMO analysis & sources of errors

**Sampling error**


**Analytical error**



# Introduction cont.

- Sampling uncertainty greatly depends on the **content of the analyte** - the lower the GMO concentration is the higher is uncertainty. At GMO levels close to the threshold (i.e. 0.9%), uncertainty linked to sampling strongly affects the reliability of results and a rather complex sampling strategy is required.
- The reliability of the analytical result is affected not only by sampling, but also by **sub-sampling uncertainty**, defined as a secondary sampling stage aiming at homogenizing the laboratory sample and preparing the test portion.
- An important issue is also **statistical distribution of the population** from where the sample is taken (homogeneous versus heterogeneus distribution – the latest being characteristic for GMOs).

# Introduction cont.

Theory represents the basis for the development of reliable sampling methodologies but the challenge is to merge theory with:

- **Feasibility**
- **Costs**
- **Robustness**

Sampling of GMO in different commodities is performed by a variety of stakeholders with a wide spectrum of final goals, all of them implying different scenarios and consequently often needing different methodologies.

A need for **"Fit for purpose sampling methodologies"**.

# “FIT FOR PURPOSE “ SAMPLING

**T R A C E A B I L I T Y along the Food and Feed Chain**

## Why , Where and When a reliable sampling is needed?

Official control

Legal disputes

Coexistence


Compliance with Legislation


Labelling

# Introduction cont.

**A 'good' sampling practice should:**

- minimize the unavoidable sampling error,
- ensure that the sample is representative of the entire population (statistical background, resources, facilities, skilled people) .

**"Primary" Sampling Programs**

- Sites (Where?)
- Types (Which?)
- Times (When)
- How Many ?

(control plans,monitoring, surveillance,HACCP, exposure assessment)

**"Secondary" Sampling**

Actual drawing of the sample for the analysis

# Introduction cont.

## Design of a sampling plan

Sequential Consideration of Factors in the design of a sampling programmes

- Definition of objectives
  Aims of the measurement
- Determine Sampling locations
- Fix Number of Increments and method of sampling
- Select method for sample (Protocol)
  Review in the light of experience

Validity?:

1.What use will the results obtained on the samplings following analysis be put to?

2. What decisions will be taken when the sampling and analytical work has been completed?

# Outline

- Introduction to sampling
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## Recommendation 2004/787/CE on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003

**General principles:**

- Official controls should be carried out without prior warning, except in cases where prior notification of an operator is necessary.
- Official controls **should be carried out at any stage of the production, processing, and storage,** distribution of products that contain or may contain GMOs or food and feed produced from GMOs, including at the point of import.
- Official controls should not differentiate between products intended for export outside the Community and products intended for placing on the market within the Community.
- Alternative sampling strategies to those recommended in this guidance may be applied.
- The sampling and detection should be carried out using sound scientific and statistical protocols in order to achieve an appropriate level of confidence for detection of GMOs or material produced from GMOs.

# Recommendation 2004/787/CE cont.

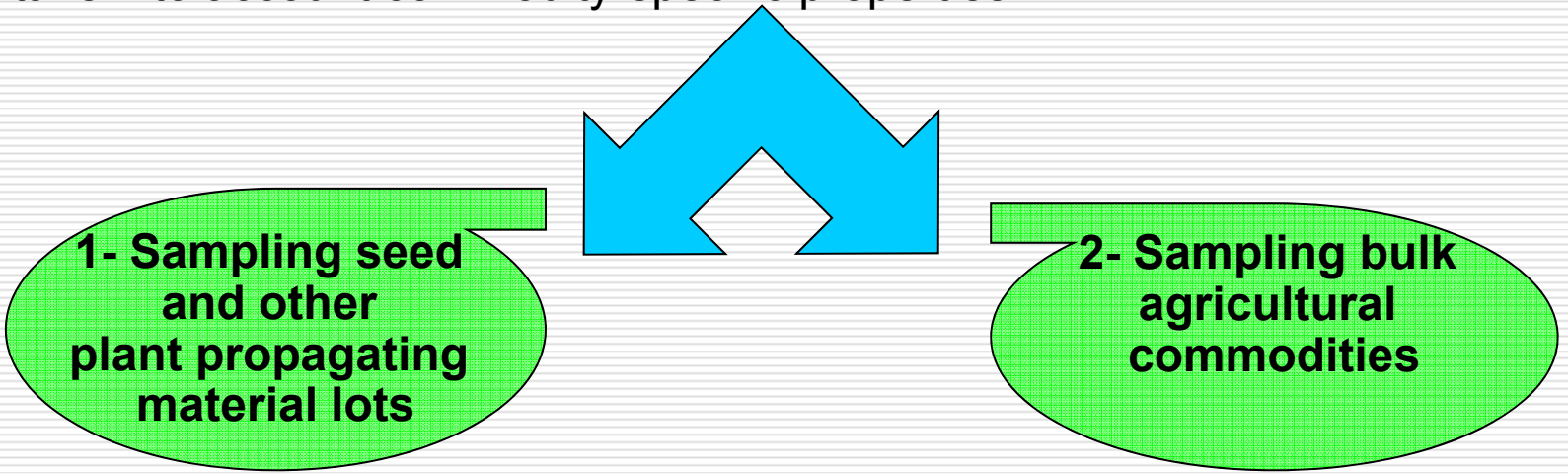
**Definitions** in line with ISTA, ISO standards 6644 and 13690 and FAO (International Standards for Phytosanitary Measures):

- **Lot:** is defined as a distinct and specified quantity of material (seed lot: a specified quantity of seed, physically identifiable and uniform, not exceeding the maximum lot size as defined in the seeds Directives and forming the total or a part of a consignment; other plant propagating material lot, food and feed products lot);
- **Increment sample**: small equal quantity of product taken from each individual sampling point in the lot through the full depth of the lot (static sampling), or taken from the product stream during a stated portion of time (flowing commodities sampling).
- **File increment sample**: an increment sample that is retained for a specific period of time for further analysis.
- **Bulk sample**: quantity of product obtained by combining and mixing the increments taken from a specific lot.
- **Laboratory sample**: quantity of product taken from the bulk sample intended for laboratory inspection and testing.
- **Analytical sample**: homogenised laboratory sample, consisting either of the whole laboratory sample or a representative portion thereof.
- **Counter sample:** a sample retained for a specific period of time for enforcement or referee purposes.
- **Percentage of GM DNA**: the percentage of GM-DNA copy numbers in relation to target taxon specific DNA copy numbers calculated in terms of haploid genomes.

# Recommendation 2004/787/CE cont.

**The sampling procedures** are defined in order to ensure that the samples collected and analysed are representative of the different types of commodities under investigation.

Whereas sampling protocols for the presence of GM seeds and other plant propagating material in seed lots should be developed according to the specific legislation on seeds or other propagating material, **sampling strategies for bulk commodities and food and feed products are addressed** in separate sections that take into account commodity-specific properties.



# Recommendation 2004/787/CE cont.

## Sampling seed and other plant propagating material lots

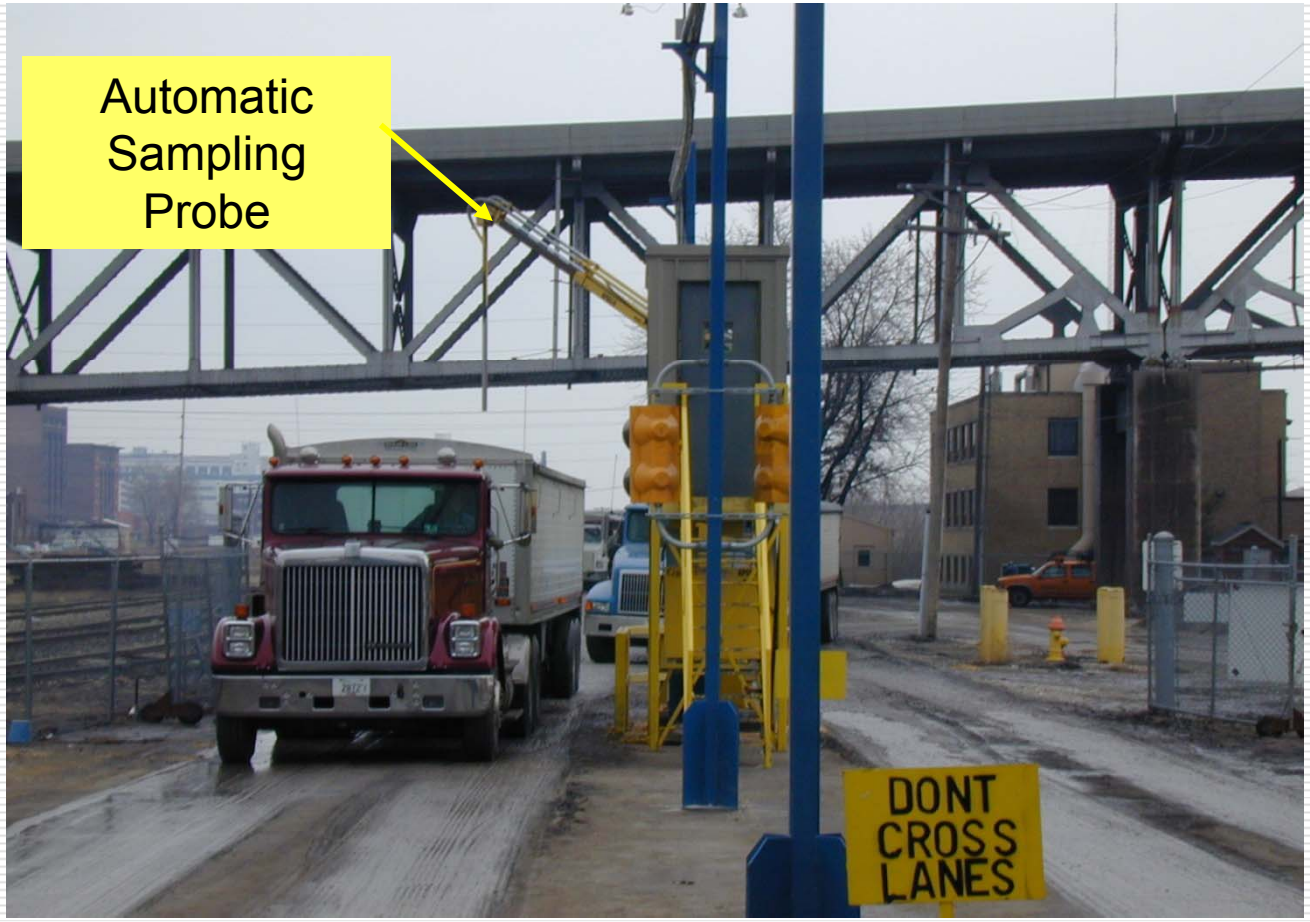
- The general principles and methods of sampling of seeds and other plant propagating material should be in accordance with the **International Seed Testing Association (ISTA) rules and the associated ISTA Handbook on Seed Sampling.**
- The sampling and testing schemes to be used for seeds or other plant propagating material should meet the requirements indicated in the specific legislation on seeds and other propagating material as regards statistical risks.

# Recommendation 2004/787/CE cont.

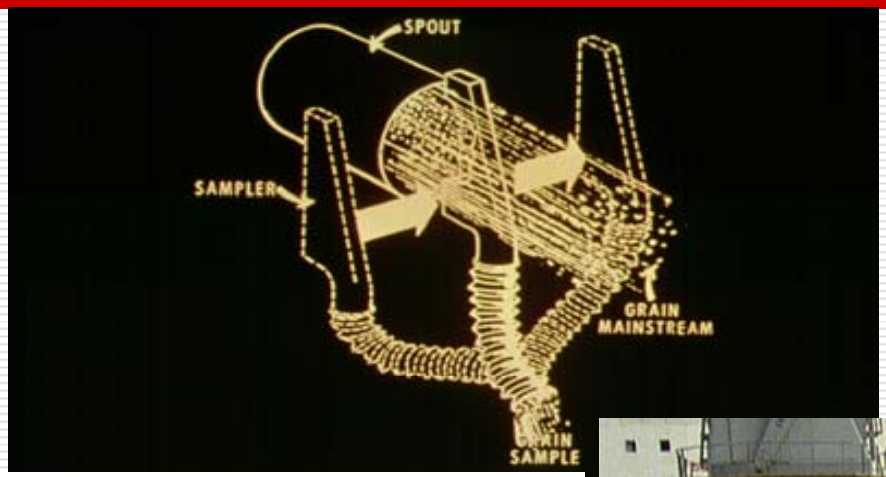
## Sampling bulk agricultural commodities

- The sampling protocol is based **on a two-step procedure** that allows to obtain estimates of GMO content and to obtain their associated uncertainty expressed as Standard Deviation (SD), without imposing any assumption on the possible heterogeneity of the GMOs.
- In order to allow the estimation of SD, a bulk sample should be produced and the derived analytical sample analysed for the presence of GM materials. Where the obtained analytical result is close to the established threshold (± 50 % of its value), the analysis of the individual file increment samples is recommended to provide a measure of the associated uncertainty.
- The following documents should be taken into account: ISO standard 6644 (dinamic sampling); ISO standard 13690 (static sampling); ISO standard 5725 (Accuracy trueness and precision) of measurement methods and results); ISO standard 2859 for sampling of packages;ISO standard 542 (Oilseeds, Sampling).

# Sampling bulk agricultural commodities: static lots (ISO 13690)



# Sampling bulk agricultural commodities: sampling from a grain stream: diverter (ISO 6644)




Diverter-type mechanical samplers used to sample grain moving through grain spouts or off of the end of conveyor belts. D/T's draw their sample by periodically moving a pelican-like device through the entire grain stream. The movement of this device is electrically timed and powered by an air cylinder or electric motor.

# Sampling bulk agricultural commodities cont.

The number of increments or sampling points (where the increment samples for creating the bulk sample and the file increment samples are taken) is defined according to lot size, as follows:

| Lot size (tonnes) | Size of the bulk sample (kg) | Number of incremental samples for the production of the bulk sample | Number of file increment sample to be retained by the laboratory |
|---|---|---|---|
| ≤ 50 | 5 | 10 | 10 |
| 75 | 7.5 | 15 | 15 |
| 100 | 10 | 20 | 20 |
| 200 | 20 | 40 | 40 |
| 250 | 25 | 50 | 50 |
| ≥ 500 | 50 | 100 | 100 |

In case of lots from 50 to 500 tonnes, the size of the bulk sample should be 0,01 % of the total lot size. In case of lots smaller than 50 tonnes, the size of the bulk sample should be 5 kg. In case of lots larger than 500 tonnes, the size of the bulk sample should be 50 kg.

# Recommendation 2004/787/CE: Protocol for the preparation of the analytical samples

1. Half of each increment samples are combined and mixed thoroughly to form a bulk sample.
2. The second half of each increment sample is stored individually and constitutes file increment samples.
3. From the bulk sample the analytical sample (the homogenized laboratory sample) and the counter sample are taken and analysed for the presence of GMOs according to 'analytical test protocols/testing methods.
4. The following cases are possible:
   - The result is above or under the threshold ±50% (therefore the lot will be respectively rejected or accepted)
   - The result is close to the established threshold (threshold ± 50 % of its value)

An estimation of the associated uncertainty may be necessary

# Sampling procedures for the detection of non-authorized GMO at low levels

**Since 2005 there have been 3 cases of EU unauthorised GMOs: maize Bt10, rice Bt63 and LL601 rice, Other unauthorised GMOs have been detected (maize E32, flax FP967, rice Kefeng 6 ) though did not lead to emergency measures because of effective actions taken by the exporting Country (sampling and confinement of suspect shipments).**

**The detection of UGMs on the EU market is reported by the EU National Reference Laboratories and the Member States via DG SANCO's Rapid Alert System for Feed and Food (RASFF).**

**COMMISSION DECISION**
**of 3 April 2008 on emergency measures regarding the unauthorised genetically modified organism 'Bt 63' in rice products 2008/289 (2008/289/EC)**

**Sampling and analysis guidelines for the detection of genetically modified flax (1) "Development of methods to identifying foodstuffs produced by means of genetic engineering techniques" of the Federal Office for Consumer Protection and Food Safety (BVL).**

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# The state of art on sampling prior to Co-Extra

**BULK** → EC Recommendation 787/2004
CEN Techincal Specification(prCEN/TS 15568)

- Systematic sampling: Increments + File increments (0.5 Kg each);
- Creation of a bulk sample mixing all the increments collected from a given lot;
- Evaluation of the bulk contamination level with respect to legal thresholds;
- If necessary analysis of individual increments to estimate confidence Interval around the estimate

**RETAIL** → **ISO 2859**

**SEED** → **ISTA**

# Bulk Lots Sampling - Adopted Protocols

- International Seed Testing Association (ISTA)
- US Dept. of Agric. Grain Inspection (USDA/GIPSA)
- EU Aflatoxin Directive 98/53
- WHO/FAO *Codex Alimentarius*
- Int. Organization for Standardization (ISO)

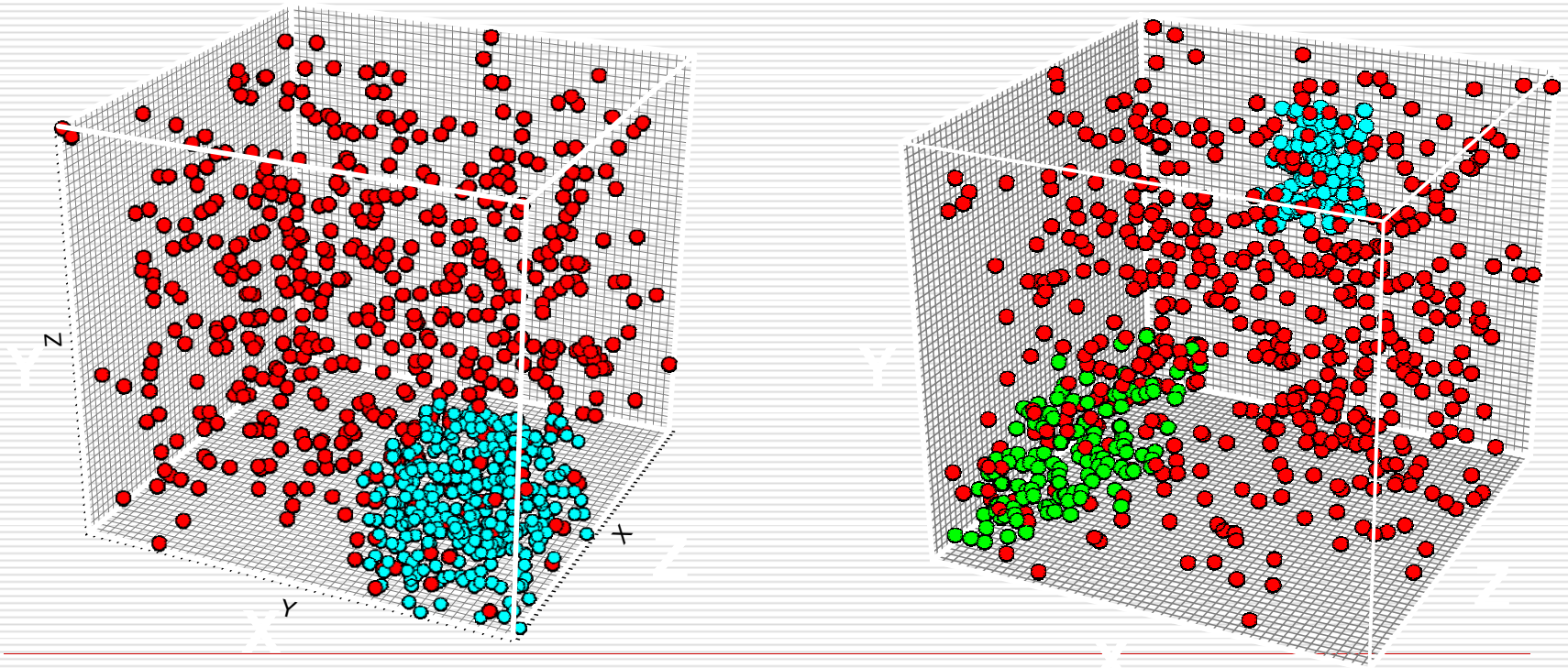
**Binomial or Poisson distribution**

**GMO distribution in the lot must be known before their application to GMO survey**

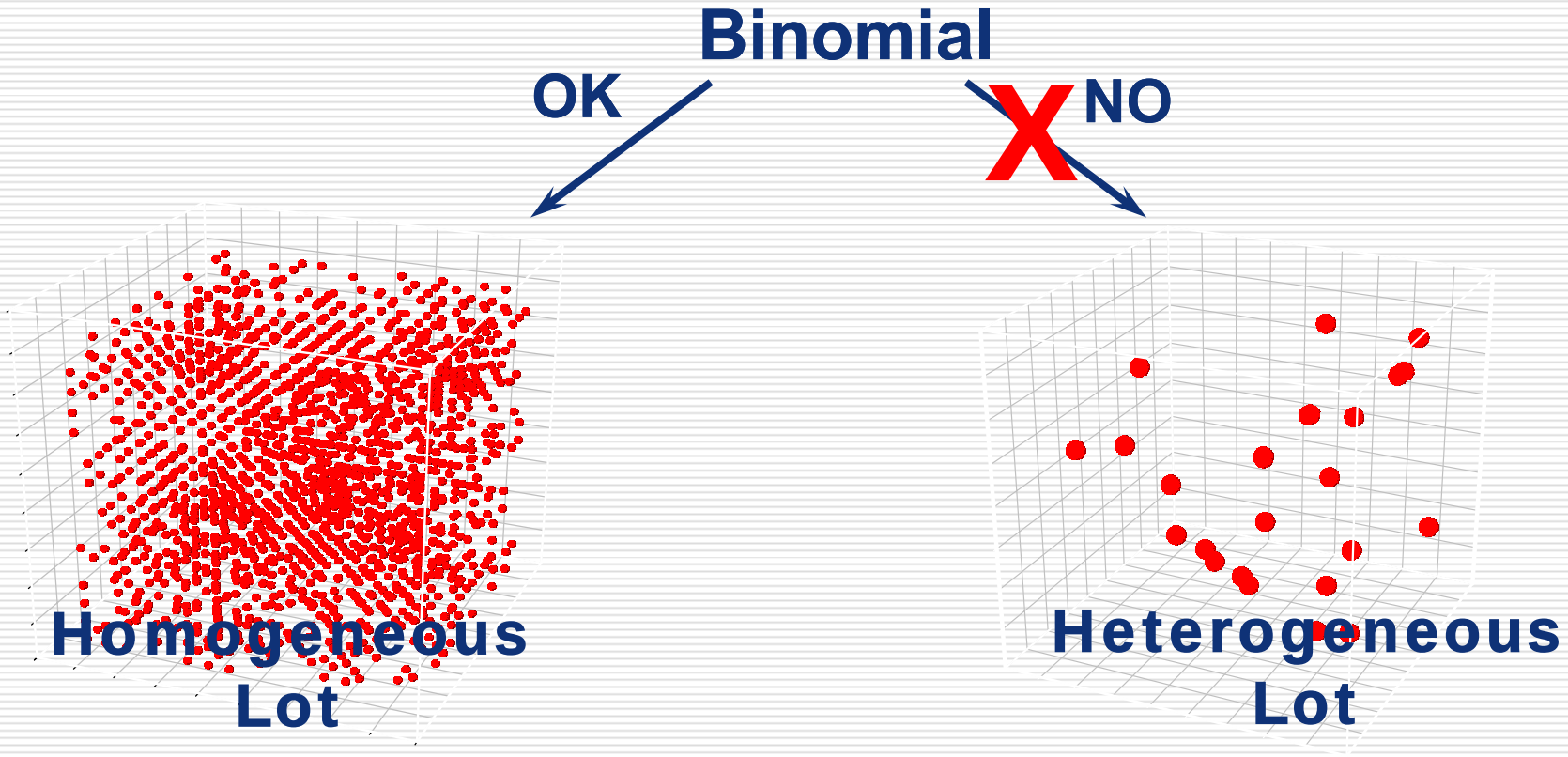
**Recommendation 2004/787/CE - Based on a general model that allows estimating GMO content of a lot without imposing any distribution**

# Distribution of GMOs in real lots (cont.)

## Other possible scenarios: different GMOs with different distributions



# Distribution of GMOs in real lots



| Source | Lot Size | Bulk sample | Laboratory sample |
|---|---|---|---|
| ISTA Intenational Seed Testing Association | Varies according to species: 10t to 40t max | 1kg | 1kg = 3000 kernels |
| USDA/GIPSA | Up to 10 bushels (=254.000 kg) or 10.000 sacks if the lot is not loose | Equivalent to Laboratory samples | 2.5 kg, but not less than 2 kg |
| ISO 13690 | Up to 500 t | Not indicated | > 1 kg (for kernels) |
| USDA/GIPSA StarLink | Follow general USDA/GIPSA guidelines | 2.5 kg | 2.400 kernels |
| EU Dir. 401/2006 | No limit if not separable, otherwhise up to 500 t | 1-10kg, Lot size < 50kg | 1-10 kg |
| CEN | Up to 500 t | 60 kg | 10.000 kernels |
| ISS | No limit | 24 kg | 24 kg |
| WHO/FAO (CX/MAS 01/3) | Discussed, but not specified | Not indicated | Not discussed |

# CoExtra: Development of Fit for Purpose sampling methods

Field

Bulk

Processing

Packages

# SAMPLING IN BULK – ISS (1)

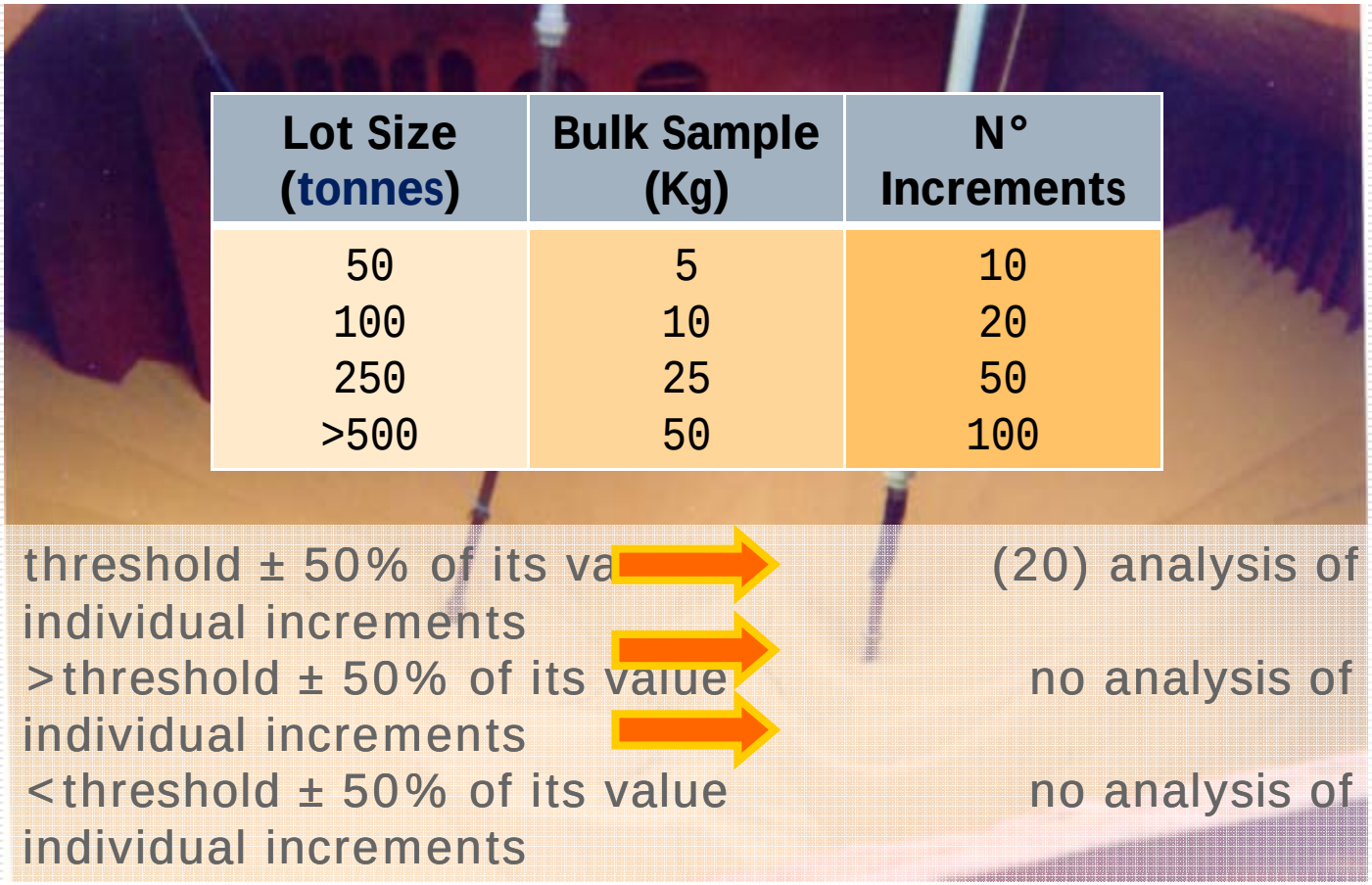
**Multifaceted study mainly aimed at:**

**testing a unified sampling methodology**

**suitable for control plan**

**of GMOs AND mycotoxins**

# BULK (787/2004)



| Lot Size (tonnes) | Bulk Sample (Kg) | N° Increments |
|---|---|---|
| 50 | 5 | 10 |
| 100 | 10 | 20 |
| 250 | 25 | 50 |
| >500 | 50 | 100 |

threshold ± 50% of its va → (20) analysis of individual increments

>threshold ± 50% of its value → no analysis of individual increments

<threshold ± 50% of its value → no analysis of individual increments

# Sampling in Bulk - ISS (2)

**The experimental design was based upon the implementation of sampling procedures on a number of lots of soybean grains (N=4) and of soybean flour (N=2) BY DYNAMIC SAMPLING**

**On each lot, three sampling procedures were simultaneously performed:**

- **the mycotoxin sampling plan according to Regulation 401/2006 (170 incremental samples, 100 g );**
- **the GMO sampling Recommendation 787/2004 (100 incremental samples, 500 g);**
- **a VERY INTENSIVE sampling to get the "true" GMO content of the lot (500 incremental samples, 500g);**

# SAMPLING SCHEME

| A 500 increments(500 g) every 6' | B 100 increments(500g) every 30' | B BIS | C 170 increments (100 g) every 18' | C BIS |
|---|---|---|---|---|
| 1 A (0') | 1 B (0') | | 1C (0') | |
| 2 A (6') | | 1 B BIS (6') | | 1 C BIS (6') |
| 3 A (12') | | | | |
| 4 A (18') | | | 2C ( 18') | |
| 5 A (24') | | | | 2 C BIS (24') |
| 6 A (30') | 2B (30') | | | |
| 7 A (36') | | 2 B BIS (36') | 3 C (36') | |
| 8 A (42') | | | | 3 C BIS (42') |
| 9 A (48') | | | | |
| 10 A (54') | | | 4 C (54') | |
| 11 A (60') | 3B (60') | | | 4 C BIS (60') |

**Legend:**
**A = very intensive sampling**
**B = sampling according to Rec. 787/2004**
**C = sampling according to Reg. 401/2006**

**5000 tons, 100 tons per hour, 50 hours of sampling**

**A: 500 samples gathered and analyzed**

**B: 100X2 gathered and analyzed**

**C:170X2 gathered and analyzed**

# BULK ANALYSIS - preliminary results

| Sampling Method | | Analysis | GMO% Mean | Stand. Dev. | CV% |
|---|---|---|---|---|---|
| Very intensive | A | 500 | 0.0078 | 0.0230 | 294.7 |
| OGM | B | 2 | 0.0061 | 0.0032 | 52.6 |
| | Bbis | 2 | 0.0024 | 0.0013 | 57.3 |
| Mycotoxin | C | 6 | 0.0008 | 0.0003 | 35.0 |
| | | | | 14 | 87.9 |

Precision of the two sampling method.

| Method | | Analysis | Mean | Mean of means | Stand. Dev. of means | CV% |
|---|---|---|---|---|---|---|
| GMO | B | 2 | 0.0061 | 0.00 | | |
| | Bbis | 2 | 0.0024 | | | |
| Mycotoxin | C | 6 | 0.0008 | 0.00 | | |
| | Cbis | 6 | 0.0016 | | | |

**Proc T TEST in SAS (version 9.1) was applied to test the hypothesis of the homogeneity between the mean concentration obtained with the two methods with the real "value". Only the difference between the mean of the mycotoxin method and the "real mean" is statistically significant.**

# BULK ANALYSIS- Conclusion

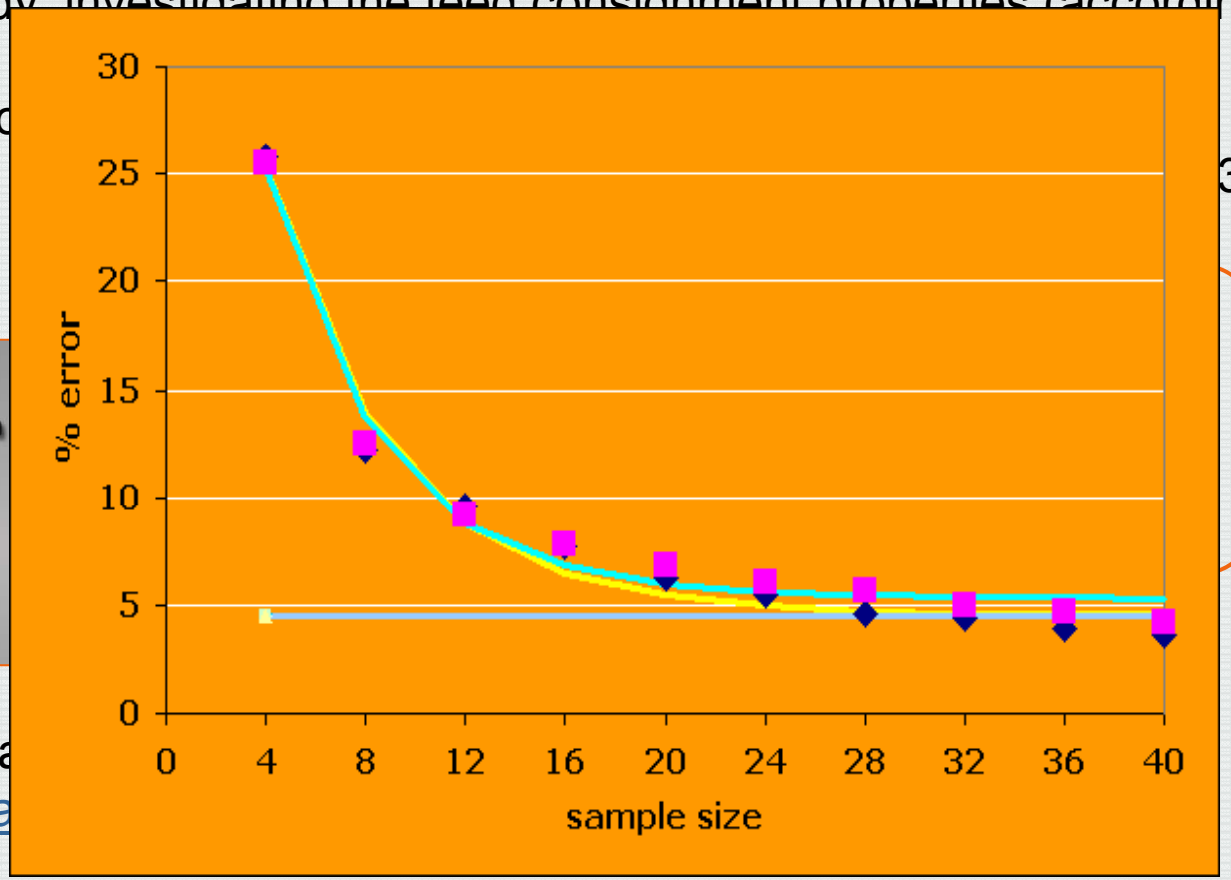
**The results are referred to a shipment with a very low level of GMO. At this level, sampling according to Rec 787/2004 is the most fit for purpose for GMO in a bulk. Since the number of increments in mycotoxins sampling scheme is higher than GMO sampling scheme, the reason for the failure of mycotoxins sampling scheme in providing a reliable value has to be attributed to the size of the increment (100g versus 500g).**

# Sampling of packed products

**Experimental study on statistics for sampling GM soybean packed products**

- Purpose of the study: investigating the feed consignment properties (according to Moran's index)
  - identifying the c
- Sampling strategy: 3 tons in succession ,1 level (400 samples)
- Software tool


The installation packa
http://agrifish.jrc.it/ma



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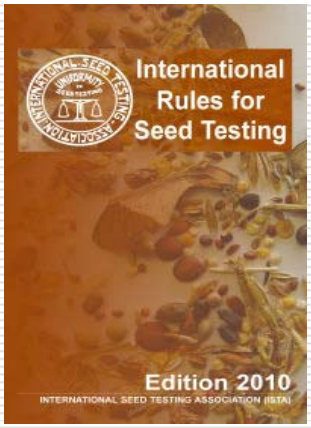
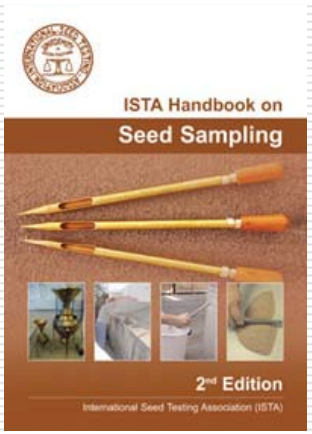
# Sampling of seeds

- Two organizations are aimed at ensuring uniformity in seed testing on the international level, the Association of Official Seed Analysts (AOSA) and the **International Seed Testing Association (ISTA).** Whereas AOSA is an organization of member laboratories across the United States and Canada, **ISTA is spread in 79 countries worldwide** and has around 120 accredited member laboratories. Both organizations develop, adopt and publish standard procedures for sampling and testing seeds and issue certificates of seed quality.

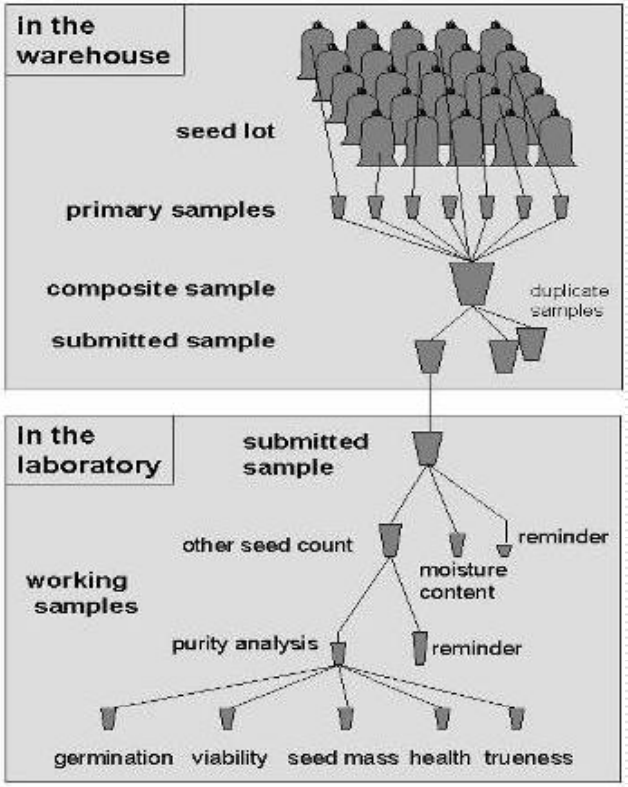
- In order to report accurate, representative and uniform test results on an ISTA certificate the **samples should be taken and prepared in accordance with the methods prescribed in ISTA Rules**. Technical aspects of sampling are elaborated in details in the **ISTA Handbook on Seed Sampling**.



# Sampling of seeds

- Seed sampling is the first substantial part of seed quality control, starting from drawing the primary samples from the seed lot in the warehouse, up to obtaining the representative working sample of a suitable size for the appropriate seed test .
- The test results are expected to reflect the average quality of the seed lot, therefore accuracy in sampling is of fundamental importance.
- Incorrect sampling may lead to misleading test results, discarding seed lots of high quality or to the approval of seed lots of low quality which may reduce crop yield or even result in complete failure.

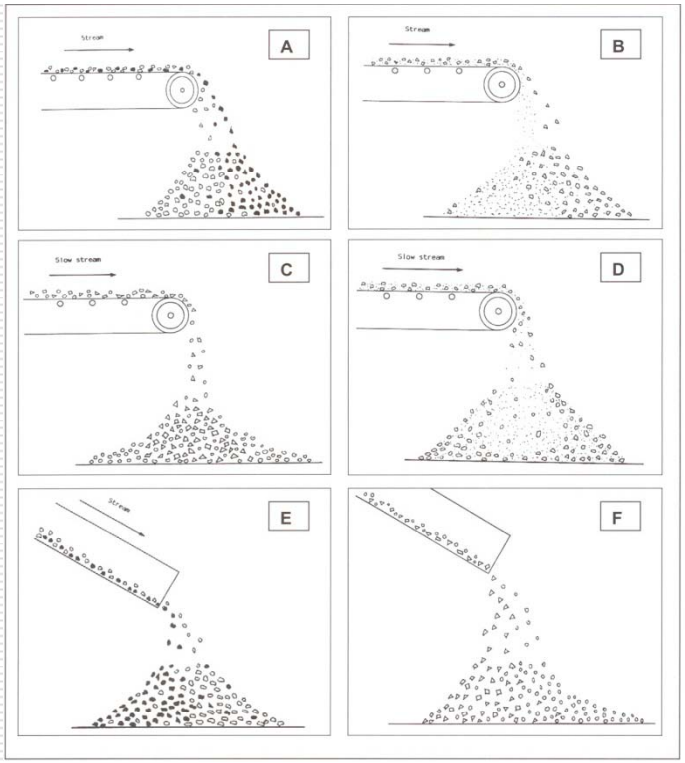



A schematic flow diagram of samples from the seed lot to the laboratory sample (Figure from Kruse et al. 2004).

# Sampling of seeds

## Basic requirements

- Prior to sampling the seed lot has to be checked whether it meets the basic requirements that are prescribed in the ISTA Rules regarding marking and sealing, **homogeneity,** maximum size and presentation of the seed lot.
- The seed lot itself must be physically identifiable so that the samples that are derived from it can be identified unambiguously, i.e. they can be related to the seed lot from which they originate.




Different examples for segregation of heterogeneous material at the discharge point of a conveyer belt or a chute (according to Pitard 1993, cited by Kruse 2004)

# Sampling of seeds – seed lots

Minimum sampling intensity for seed lots in containers

| Weight of individual container in the seed lot | Weight of lot (kg or number of containers) | Number of primary samples |
|---|---|---|
| >100 kg | up to 500 kg | at least 5 |
| | 501 – 3000 kg | 1 for each 300 kg, but not less than 5 |
| | 3001 – 20.000 kg | 1 for each 500 kg, but not less than 10 |
| | 20.001 kg and more | 1 for each 700 kg, but not less than 40 |
| 15 - 100 kg inclusive | 1 – 4 containers | 3 from each container |
| | 5 – 8 containers | 2 from each container |
| | 9 – 15 containers | 1 from each container |
| | 16 – 30 containers | 15 from the seed lot |
| | 31 – 59 containers | 20 from the seed lot |
| | 60 or more containers | 30 from the seed lot |
| containers < 15 kg | - Containers shall be combined into smaller units not exceeding 100 kg (e.g. 20 containers of 5 kg, 33 containers of 3 kg or 100 containers of 1kg).<br>- For seed mats and tapes, small packets or reels may be combined to sample units that not exceeding 2.000.000 seeds.<br>- The sampling units shall be regarded as containers.<br>- Intensity is performed as defined for containers of 15-100 kg. | |

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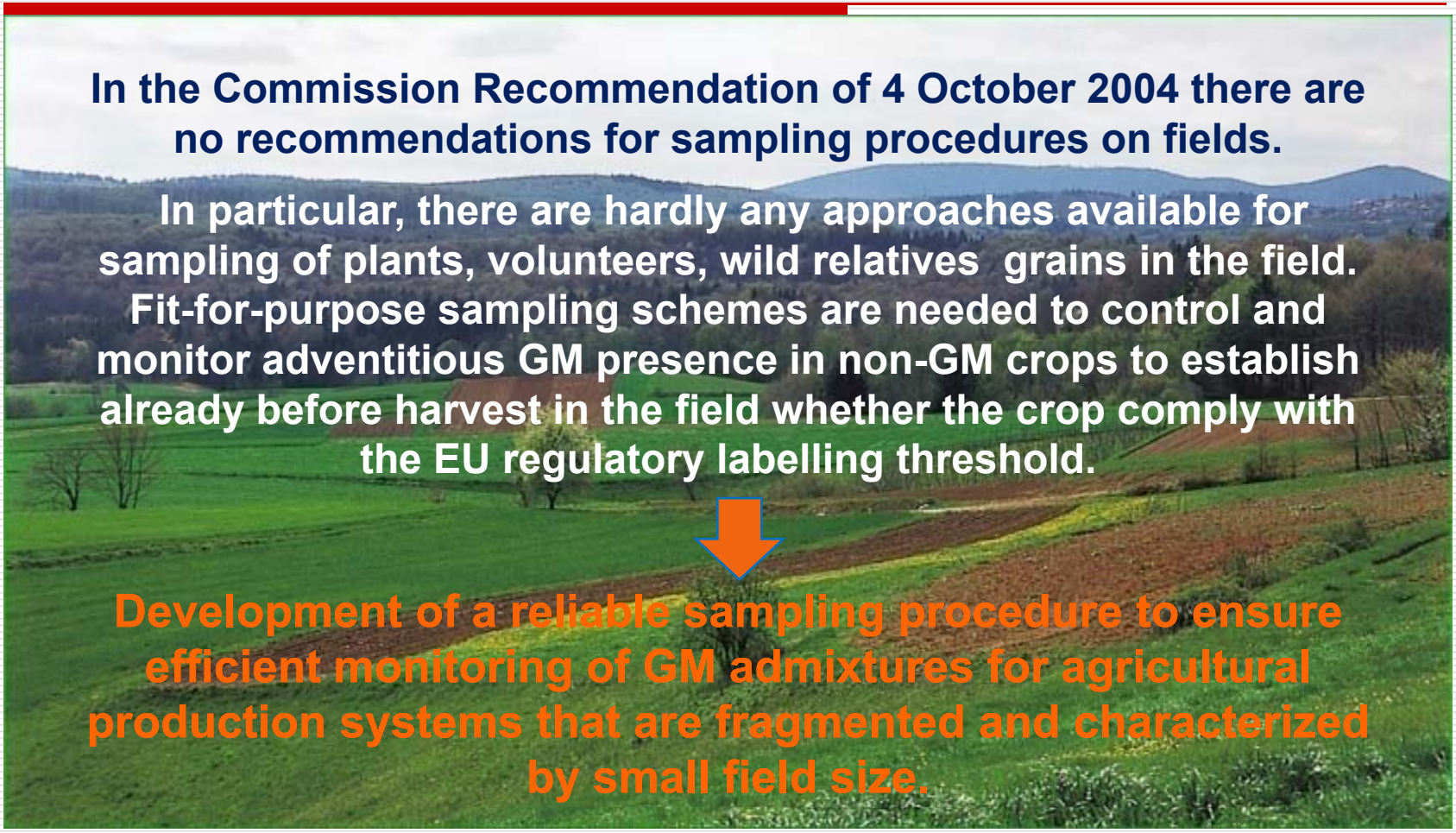
# Sampling in the field- KIS



**In the Commission Recommendation of 4 October 2004 there are no recommendations for sampling procedures on fields.**

**In particular, there are hardly any approaches available for sampling of plants, volunteers, wild relatives  grains in the field. Fit-for-purpose sampling schemes are needed to control and monitor adventitious GM presence in non-GM crops to establish already before harvest in the field whether the crop comply with the EU regulatory labelling threshold.**

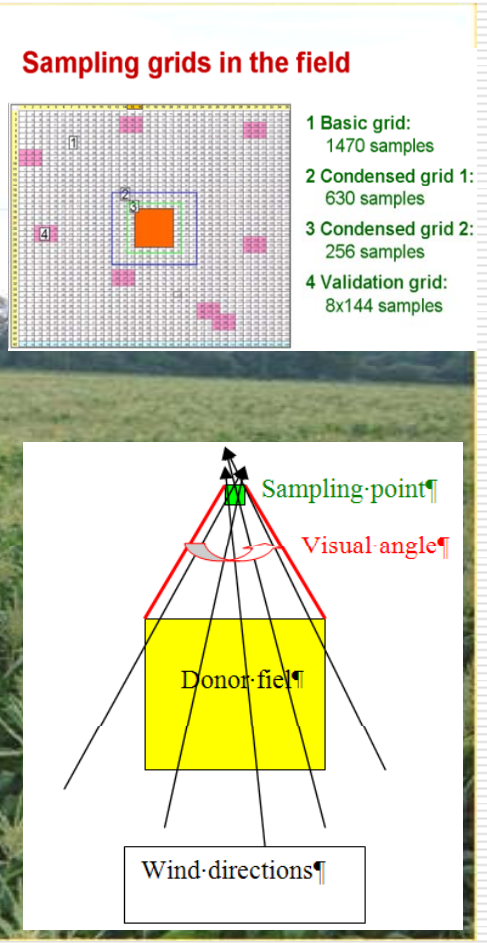
**Development of a reliable sampling procedure to ensure efficient monitoring of GM admixtures for agricultural production systems that are fragmented and characterized by small field size.**

# Sampling in the field- KIS

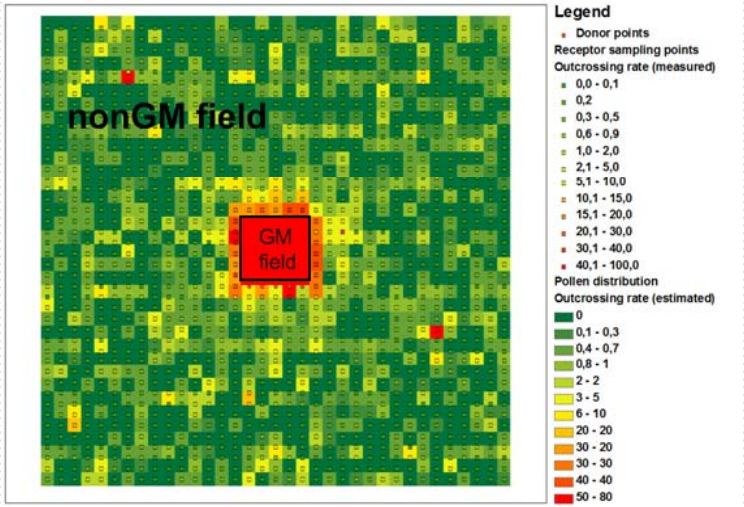
- A two year field trial was conducted.The phenotypic method with two conventional maize varieties was used in order to determine the outcrossing rate (OCR).

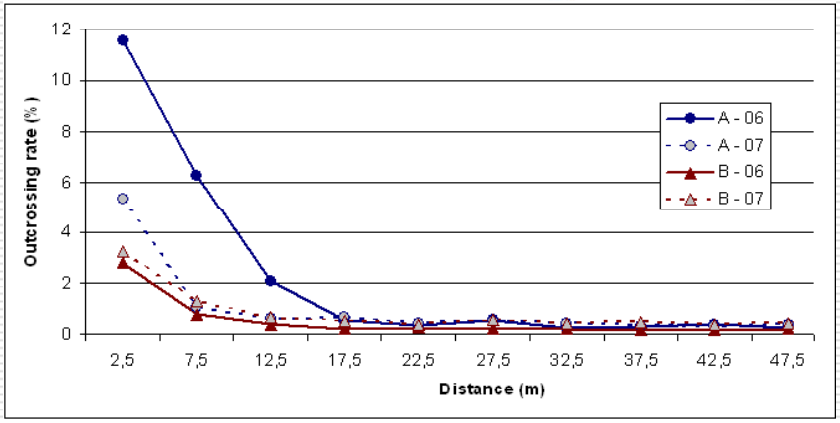
- Four different sampling grids were defined within the recipient maize field before collecting samples in autumn; altogether 3600 samples were collected every year.

- Based on the attributes describing the properties of boundary layer that were measured during the growing period and collected data on outcrossing rate a prediction model for the spatial variability of outcrossing rate was developed using data mining techniques.



# Sampling in the field- KIS




**Gene flow in the field:**

-Higher rates near the GM fields.

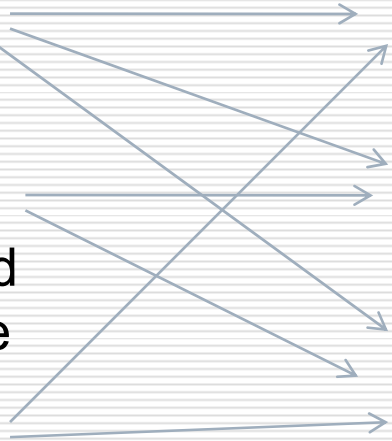
**Gene flow - OCR as a function of distance:**

-OCR decreases rapidly with the distance from the GM source.

-Long tail distribution (lower rates in more distant positions (in the centre) of the field)

# Sampling in the field- KIS

- economic aspect: cost effectiveness
- technical aspect: walking distance and weight of the sample
- statistical aspect: good estimation



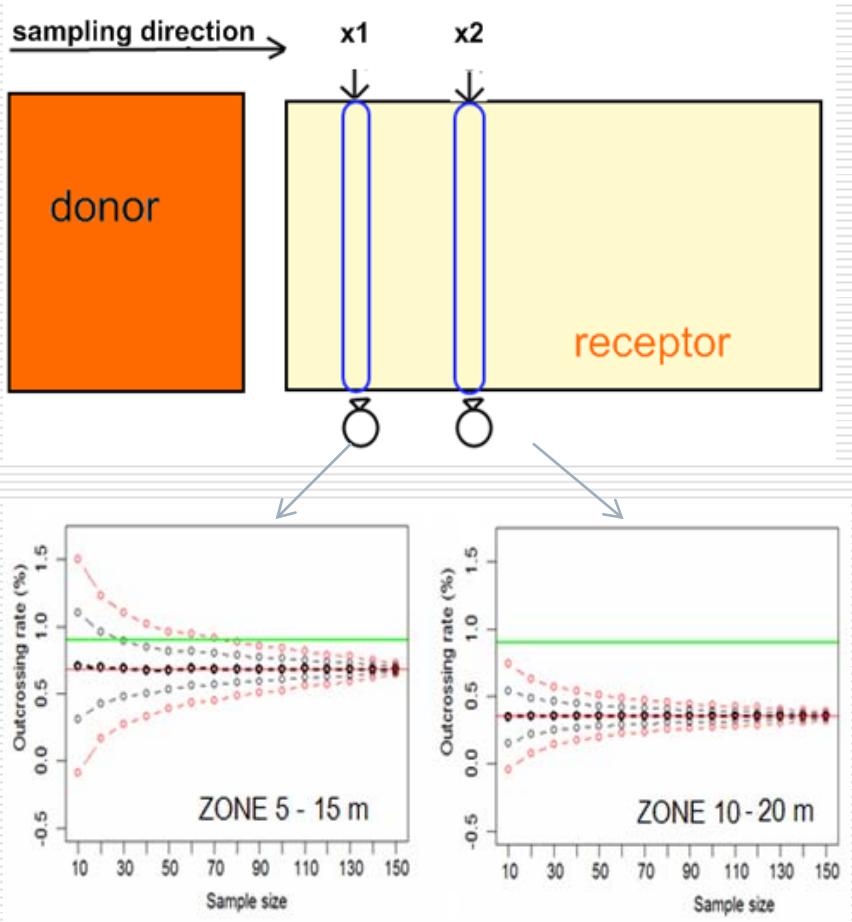
Sampling zones (e.g.: 10 - 20 m)

Number of samples (acceptable from 50 to 100)

sampling sheme (e.g.: random sampling, sistematic sampling)

SIMULATIONS: R statistical programme (R Development Core Team, 2007).

# Sampling zone, number of samples




Sampling in the entire field is technically too demanding, the estimates obtained are less accurate → **systematic sampling in the two rows** is technically feasible and cost effective … i**n order to later use of a fitting function (function of distance)** to estimate the percentage of OCR in the whole field according to the size (length) of the field.

Sampling zone: 10m and 25m from the GM field

Number of samples:
2 times: 30 to 50 cobs

# Estimation of outcrossing rate (OCR)

Estimation of the outcrossing rate in the field by integrating the appropriate fitting function - **Two-point fitting function approach**

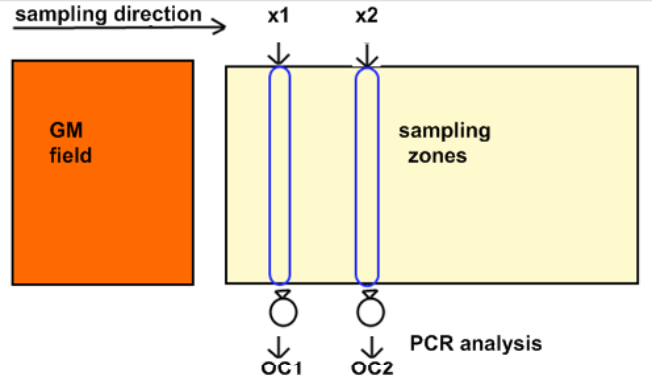
| Field length | OCR with NO buffer zone | OCR with buffer zone 5m | OCR with buffer zone 10m |
|---|---|---|---|
| 25 m | 2.74 % | 1.06 % | 0.67 % |
| 50 m | 1.5 % | 0.67 % | 0.45 % |
| 75 m | 1.06 % | 0.49 % | 0.35 % |
| 100 m | 0.82 % | 0.40 % | 0.28 % |
| 150 m | 0.57 % | 0.28 % | 0.21 % |
| 200 m | 0.43 % | 0.23 % | 0.17 % |

Legend: red: %GMO > 0.9% - labelling required;
yellow: %GMO ± 0.9% - sampling required for precise determination;
green: %GMO < 0.9% - labelling and sampling not required

# Sampling approach

Two-point fitting function approach – six steps approach:

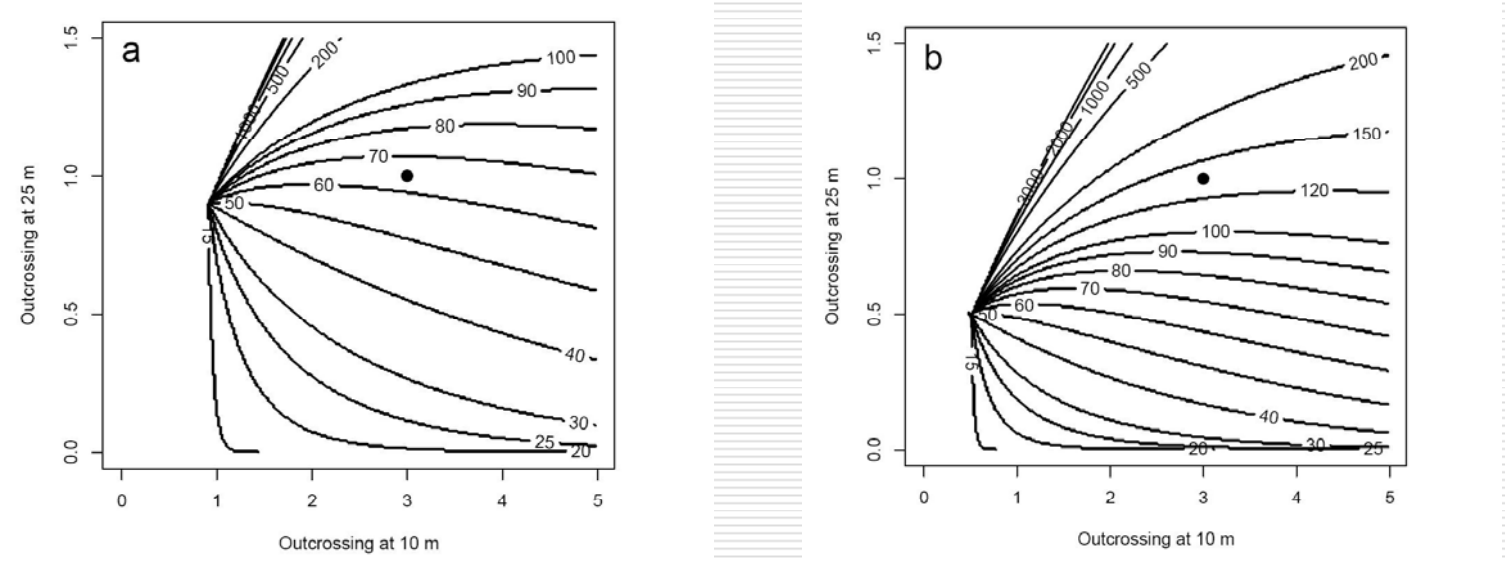
- **Step I:** Sampling in the field in distances $x_1$ and $x_2$ (30-50 cobs)




- **Step II:** Determine the outcrossing rates $oc_1$ and $oc_2$ (PCR analysis)
- **Step III:** Calculate the fitting function parameters a and K ($x_1$, $x_2$ , $oc_1$ and $oc_2$ )
- **Step IV:** Determine the field length (A and B)
- **Step V:** Determine the OCR in the field
- **Step VI:** Labelling decision

$$\overline{OC} = \frac{K}{1-a}\,\frac{(B^{1-a} - A^{1-a})}{B-A}$$

# Determination of critical field lengths




Contour plot of critical field lengths (in meters) for measured outcrossing rates at 10 m and 25 m from the donor field (field type A). Lines represent minimal distances for mean OCRs in the field below 0.9 % (a) or 0.5 % (b). Filled circle in position of outcrossing pair ($oc_1$=3, $oc_2$=1) shows the minimal field length to achieve given OCR levels.

Šuštar-Vozlič, Rostohar, Blejec, Kozjak, Čergan, Meglič, 2010. *Anal Bioanal Chem* 396 (6): 2031-2041

# Sampling in the field- conclusion

The developed approach allows a simple three step decision support system for monitoring the adventitious presence of GMO in the field, which is as follows:

- **i) step one:** using the prediction model an estimation of mean outcrossing rate in the field is calculated from the geometric data of GM donor and non-GM receptor;
- **ii) step two:** if the adventitious presence of GMOs near the threshold level in the field is expected, two distances are determined within the receptor field and a sampling scheme set up, samples are then collected and analysed;
- **iii) step three:** if the results of the analyses show the level of GMO being above the threshold (including the standard deviation) then the yield is labelled as GMO, if the results are below threshold then the yield is not labelled as GMO and if the results are in between those two values then additional analyses shall be recommended (sampling in the field and/or silo).

# The contribution of the following people is greatly acknowledged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