Kockázat alapú mintavételezés statisztikai tervezése az aflatoxin M1 eredmények példáján

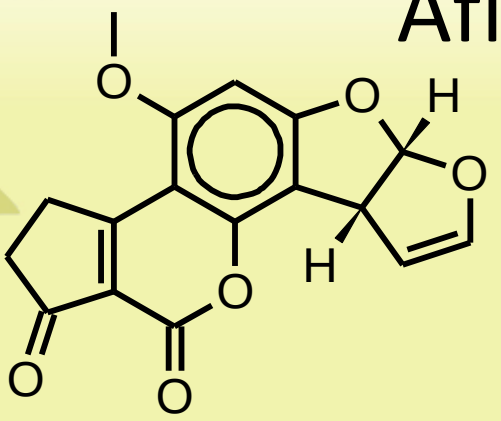
Statistical basis of risk-based planning - Aflatoxin M1 in milk

Prof. Dr. Ambrus Árpád, Farkas Zsuzsa, Kerekes Kata, Szabó István és Horváth Zsuzsanna,
NÉBIH ÉKI

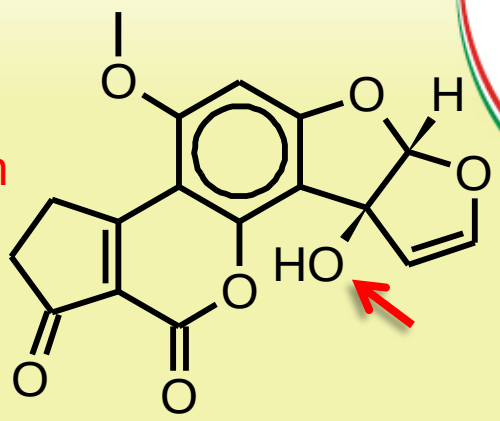
Outline

- Importance of control of aflatoxin contamination
- Italian data for developing risk-based early warning sampling plan
- Interpretation of compliance with ML, number of samples to be taken
- Principles of moving window,
- Principles of weighting of collection centres, establishment of reference values
- Performance and implementation of the sampling plan
- Preventive sampling plan

Aflatoxins



Metabolism in dairy animals
AFB1→AFM1



Milk kg/day	AFB1 µg/d	Carry over	AFM1 ng/kg
50	20	6%	270
25	20	2%	210

Mayor sources: maize, sunflower, silage (40- 400 µg/kg (formic acid trmt.) soybean, cotton seed, peanut, palm, copra (coconut)...

Milk average daily **consumption**: 35.5 to 285 g/person/day
High portion size: 712.6 g/day for 8-20 month toddlers and
1484.8 g/person/day for 14-80 yrs

AFM1 in milk should be regularly and strictly controlled!

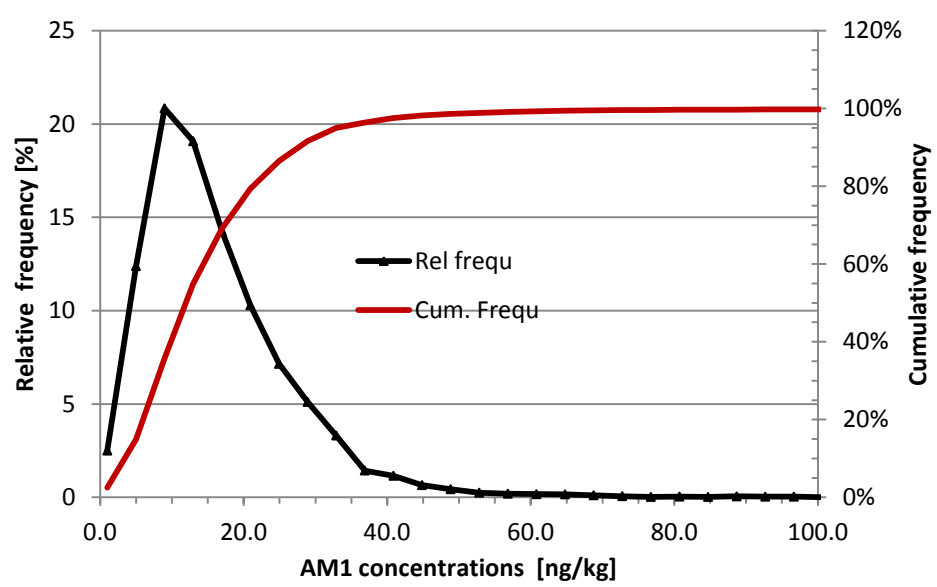
Self-control in Italy

Italian milk processing plants requested assistance in preparing statistically based economic sampling plan and provided the results of the analysis of 26113 samples from 2003-2010.

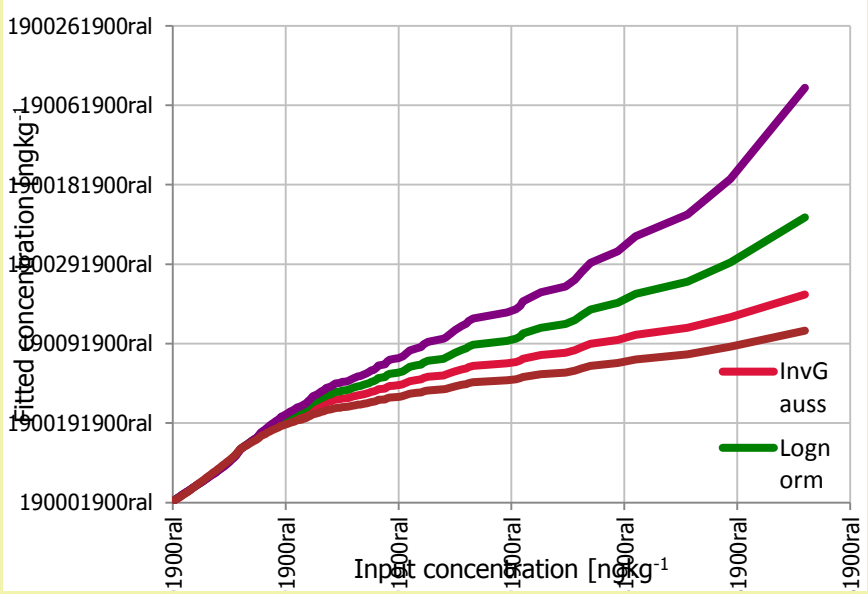
Base data from 2010

Processing plants	P 1	P 2	P 3	P4	P 5	Sum
No of Districts	48	17	5	17	34	121
No of samples	1653	572	169	447	975	3816
Milk sampled (t)	24050	12014	4300	6632	12521	59517
Ave.kg/sample	14549	21004	25443	14836	12842	
No of farms*	276	95	28	97	194	

Nature of the distribution of AFM1 contamination in milk



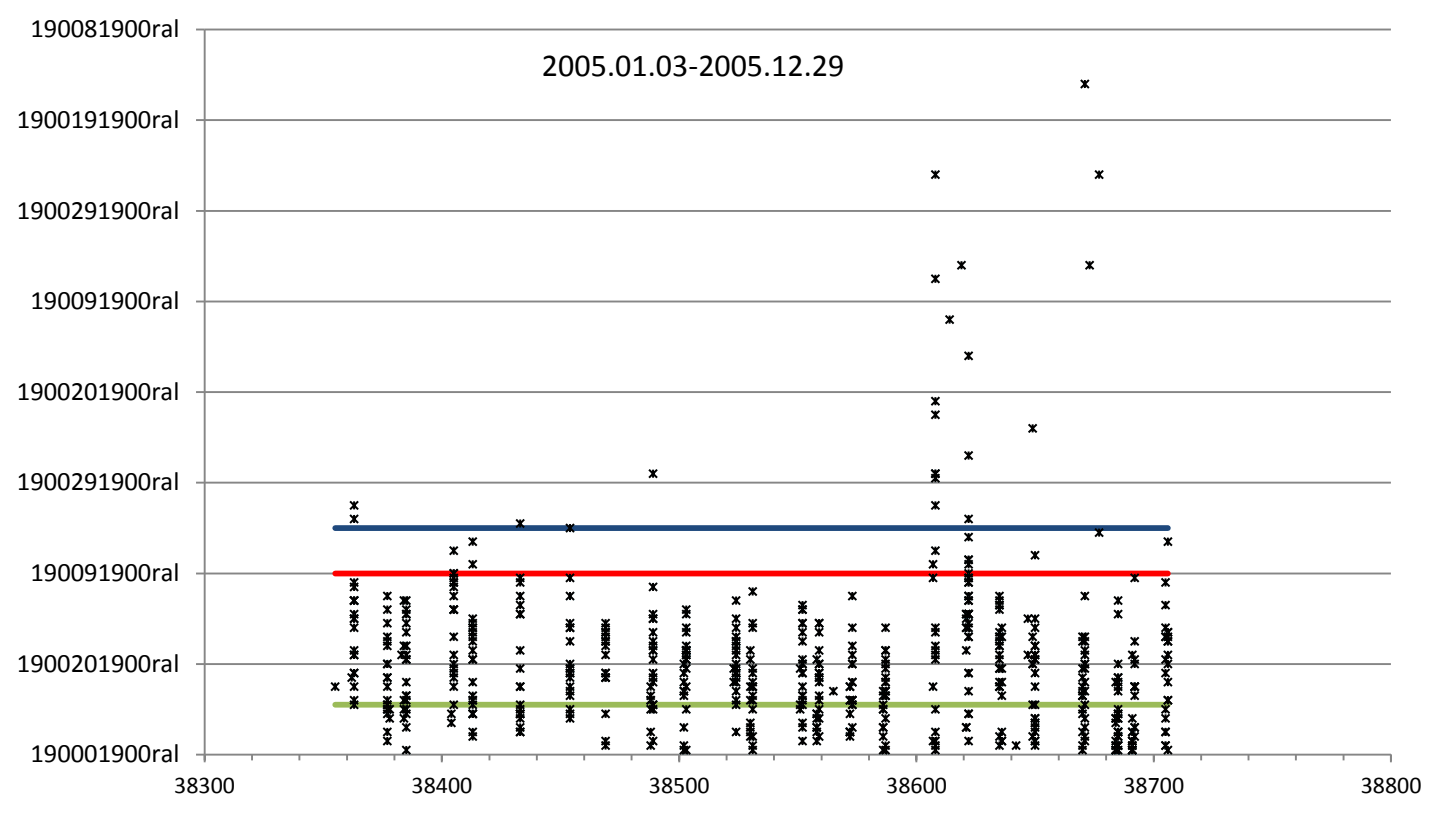
Relative and cumulative frequency distribution



Comparison of input and fitted distributions of the combined AFM1

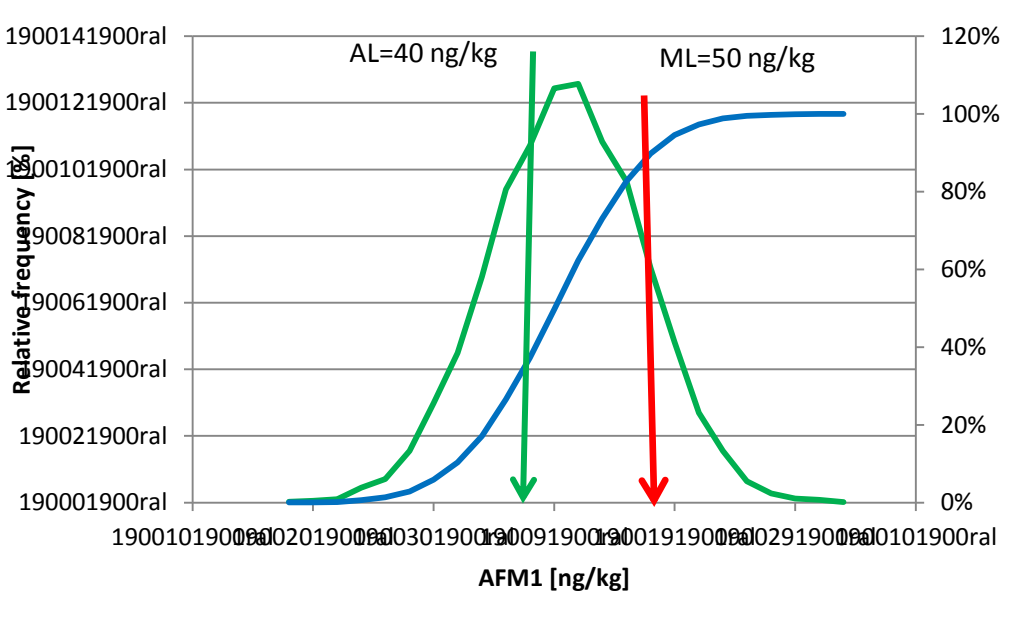
Distribution free statistical methods shall be used!

Variation of AFM1 along the year



Sampling plan should quickly react on changes of AFM1 concentration!

Considering the uncertainty of measured AFM1



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

$$AL = \frac{ML}{1 + kCV_R}$$

If we compare the measured AFM1 value to the ML, we make wrong decision in 50% of the cases. An AL<ML shall be chosen.

Number of samples (n) to be taken

to find with selected probability, β_t, at least one value above the selected percentile (β_p):

$$n = \frac{\lg(1-\beta_t)}{\lg \beta_p}$$

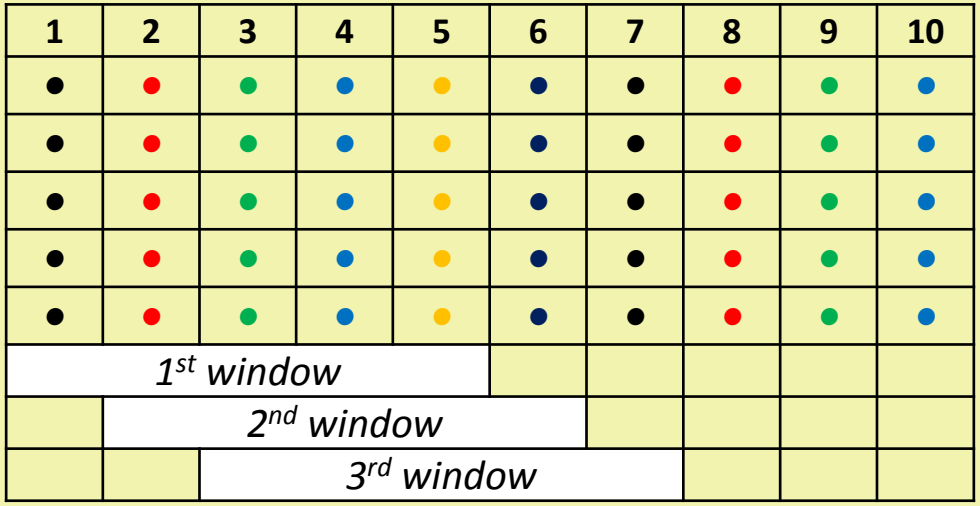
at least 2 values above β_p

$$\beta_t = 1 - \binom{n}{0} p^0 (1-p)^n - \binom{n}{1} p^1 (1-p)^{n-1}$$

Probability of finding at least one or two samples containing AM1 above the 40 ng/kg and 50 ng/kg limits based on ***n*** number of samples

n	40 ng/kg (P0.975)		50 ng/kg (P0.987)	
	1 sample	2 samples	1 sample	2 samples
20	0.40	0.09	0.23	0.03
25	0.47	0.13	0.28	0.04
29	0.52	0.16	0.32	0.05
35	0.59	0.22	0.37	0.08
49	0.71	0.35	0.47	0.13
85	0.88	0.63	0.67	0.30
142	0.973	0.87	0.84	0.55

Principle of moving window



1. Sufficient number of sample units (*k*) is collected for a defined period of time (the “window”).
2. The results of the latest *n* sample units are compared with the reference values, and it is added to the window while the oldest set is removed.
3. The window, always consists of *k* results, moves one set of results forward in time. The results are evaluated daily.
4. 25 samples represent 142 farms; β_p =0.98 β_t = 0.94

Accounting for the number of dairy farms/district

AFM1 [ngkg^{-1}] in milk produced by individual dairy farms						AFM1 Average, ngkg^{-1}
F1	F2	F3	F4	F5	F6	
40	12	12	12	12	12	**16.7**
40	12	12	12	12		**17.6**
40	12	12	12			**19.0**
40	12	12				**21.3**
40	12					**26.0**
40						**40.0**
20[a] 50%	12	12	12	12	12	13.3
18[b] 45%	12	12	12	12	12	13.0
16[c] 40%	12	12	12	12	12	12.7

Weighting factors for collection districts with 2-4, 5 and 6 farms are 1, 2 and 3, respectively. Districts collecting milk from 6 farms would be sampled 3 times more frequently than a district including 2 to 4 farms. Districts delivering organic milk: additional weighting factor of 2.

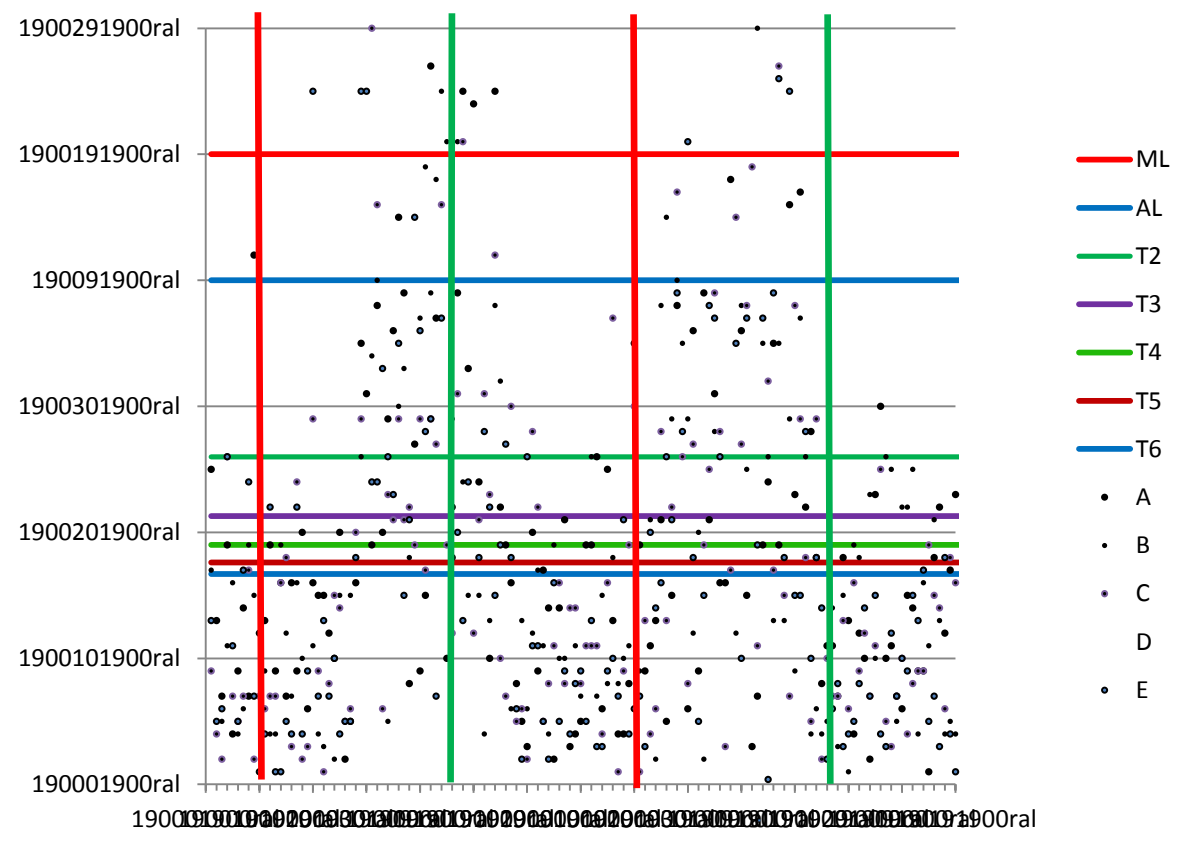
Allocation of number samples to be taken within one 28-day cycle

	P1	P2	P3	P4	P5	
Collection Centre	48	17	5	17	34	
Sampling unit	144	52	13	46	95	350
No of samples/cycle[a]	57	21	5	19	38	140
No of samples/cycle[b]	77	40	12[c]	36	61	225

Joint programme: Annual number of samples:
$12 \times 140 = 1680 \leftrightarrow 3816$; β_p 0.975, β_t 0.997

Independent control performed by the processing plant would require on an average 35%-160% larger number of samples per processing plants to achieve the same level of control.

Response rate



Normal Critical Normal Critical Normal

Principles of preparing and implementing stratified random sampling plan

- Processing plants prepare and implement a joint sampling plan, share the results of analysis immediately and carry out more rigorous sampling plans if needed.
- Sampling of randomly selected milk consignments continues every day over the whole year.
- The collection districts should be represented proportionally to their weights.
- The same collection district should not be sampled again within preferably 3 days (should not be present twice in the blocks of 15 samples), but at least within 2 days.
- Samples are collected at each dairy farms during transferring the milk to the tankers.
- The milk delivered by the tankers is sampled at processing plant according to the random sampling plan.

Action plan for each processing plant

Tanker milk	Dairy farms		
$M_C \geq$ ref. conc.	$50 > H_1 \geq 40$	$H_1 > 50$	Action
1d	0, 1	≤1	*Confirmatory test*
2d	0, ≥ 1	≤1	*Moderate level 1*
2d	0, ≥ 1	≤2	*Moderate level 2*
>2D	0, ≥ 1	≤ 3	*Moderate level 3*
>2D	0, ≥ 1	>3	***Crisis***

d: within one day; D: within 5 days

Action plan for different control levels

- Confirmatory test: analyse samples collected from individual farms, resample farms producing H_1 AFM1>40 $ngkg^{-1}$ next day, if contamination is confirmed stop collection of milk until depuration of cattle; Confirmatory tests shall be performed as a first step in every action level.
- Control at moderate **level 1**: take 10 samples from different districts/day for 3 consecutive days in the collection area of one processing plant where the contaminated milk was produced;
- Control at moderate **level 2**: take 10 samples from different districts/day for 3 consecutive days in the collection areas of all processing plant(s);
- Control at moderate **level 3**: take from each district or maximum 28 samples/day for 3 consecutive days in the collection areas of all processing plants;
- Control in case of **potential crisis level 4**: take samples from each incoming consignments until the AFM1 level decreases below reference values for 3 consecutive days

Follow up actions

i. In a case when one processing plant has to apply moderate level 2 or higher control all other processing plants have to implement moderate level 1 control, and depending on its outcome proceed either according to (ii) or (iii).

ii. Contamination level does not change or increases: apply the next level of control

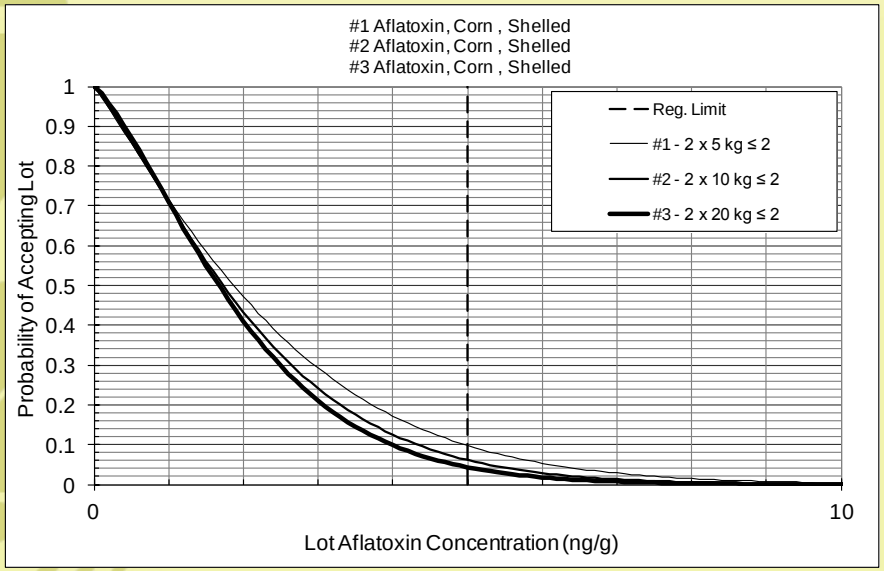
iii. Contamination level decreases after

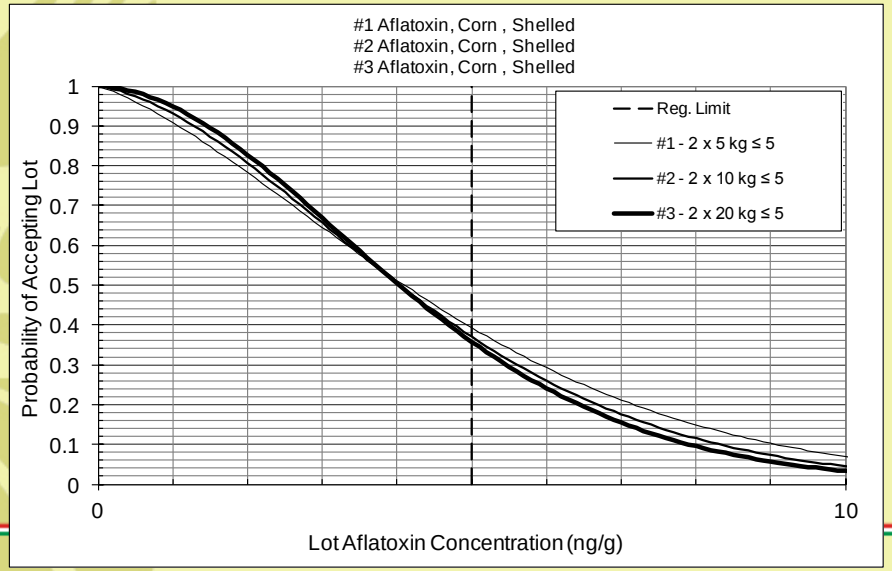
- Confirmatory test: continue normal sampling programme according to pre-defined sampling plan
- Moderate levels 1 and 2: if no noncompliance was observed in 3 consecutive days continue normal sampling programme according to pre-defined sampling plan
- Moderate level 3: if no noncompliance was observed in 3 consecutive days reduce sampling frequency to moderate level 1
- Crisis level: if no noncompliance was observed in 3 consecutive days reduce sampling frequency to moderate level 1.

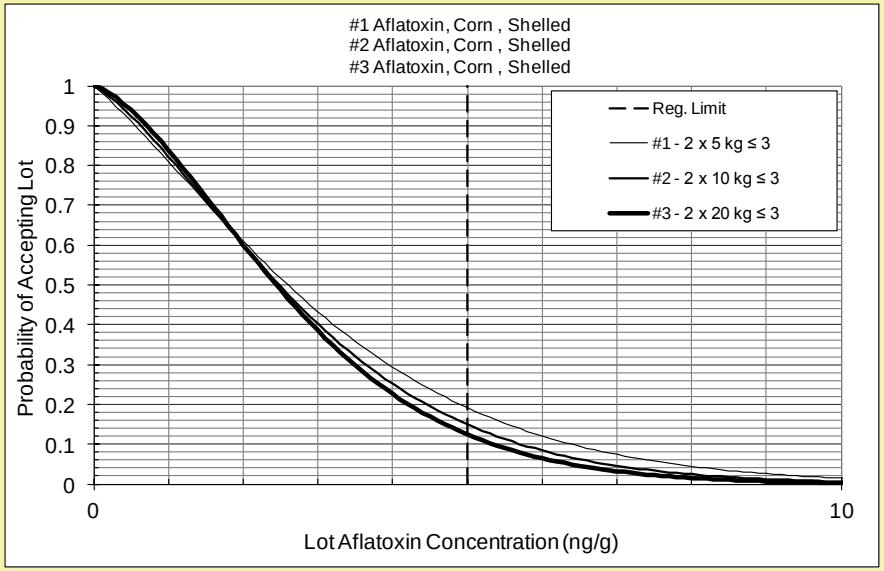
Preventive sampling plan by the dairy farms

- A higher level of compliance and reduced economical loss could be achieved if the dairy farms would get their feed accompanied by analytical certificate from reliable sources, or getting their own feed production analysed.
- Average daily milk production of 1767 kg per farm and an average price of 0.3€/litre, the loss for not selling milk during an average 6-day depuration period is 3180€.
- ELISA detection–based analyses of 3 kinds of feeds with duplicate samples would require ~ 600 €
- Action limit for AFB1 in corn: 2 µg/kg!

Acceptability of AFB1 in feed







Take representative sample!!!
DO NOT COMPARE AFB1 TO 5 µg/kg LEGAL LIMIT!

Final remark

The sampling and action plan developed

- provides the desired compliance level according to the performance objectives of milk processors;
- is economic and enables quick identification of deffective lots with high probaility
- can be easily adapted to different production situations.

Thank you for your attention