Joint Research Centre

the European Commission's in-house science service

Serving society
Stimulating innovation
Supporting legislation

EXPERIENCES, ACHIEVEMENTS AND CHALLENGES OF JRC HOSTED EU REFERENCE LABORATORIES FOR CONTAMINANTS

Piotr Robuch, Joerg Stroka & Thomas Wenzl

European Commission

Outline

Who are we and how are things done?

EURL

European Union Reference Laboratory

Heavy Metals in Feed and Food

Polycyclic Aromatic Hydrocarbons (PAHs)

Mycotoxins

Examples

- Proficiency Tests
- Guidance Documents
- Training
- Analytical method development

Reality check

- PT evaluation
- Guidance document impact
- Who trains who?
- Standardisation of methods by CEN

European Commission

Networking between reference laboratories

European Commission

EURL Network Heavy Metals

EURL

European Union Reference Laboratory

Heavy Metals in Feed and Food

- 50 NRLs
- from 28 MS + NO + IS

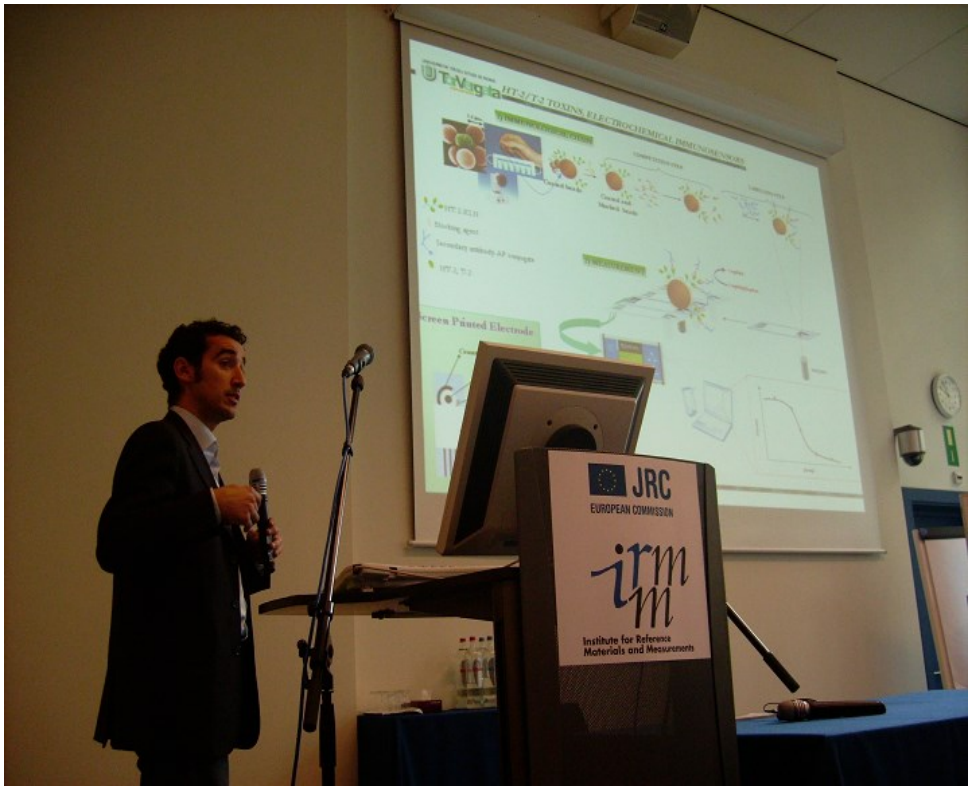
Mandate:

- Food of non-animal origin (incl. wild caught fish)
- Feed

European Commission

EURL mycotoxins workshop Geel 2009

BioCop Presentation (EC-sensor for T/HT 2 toxins)



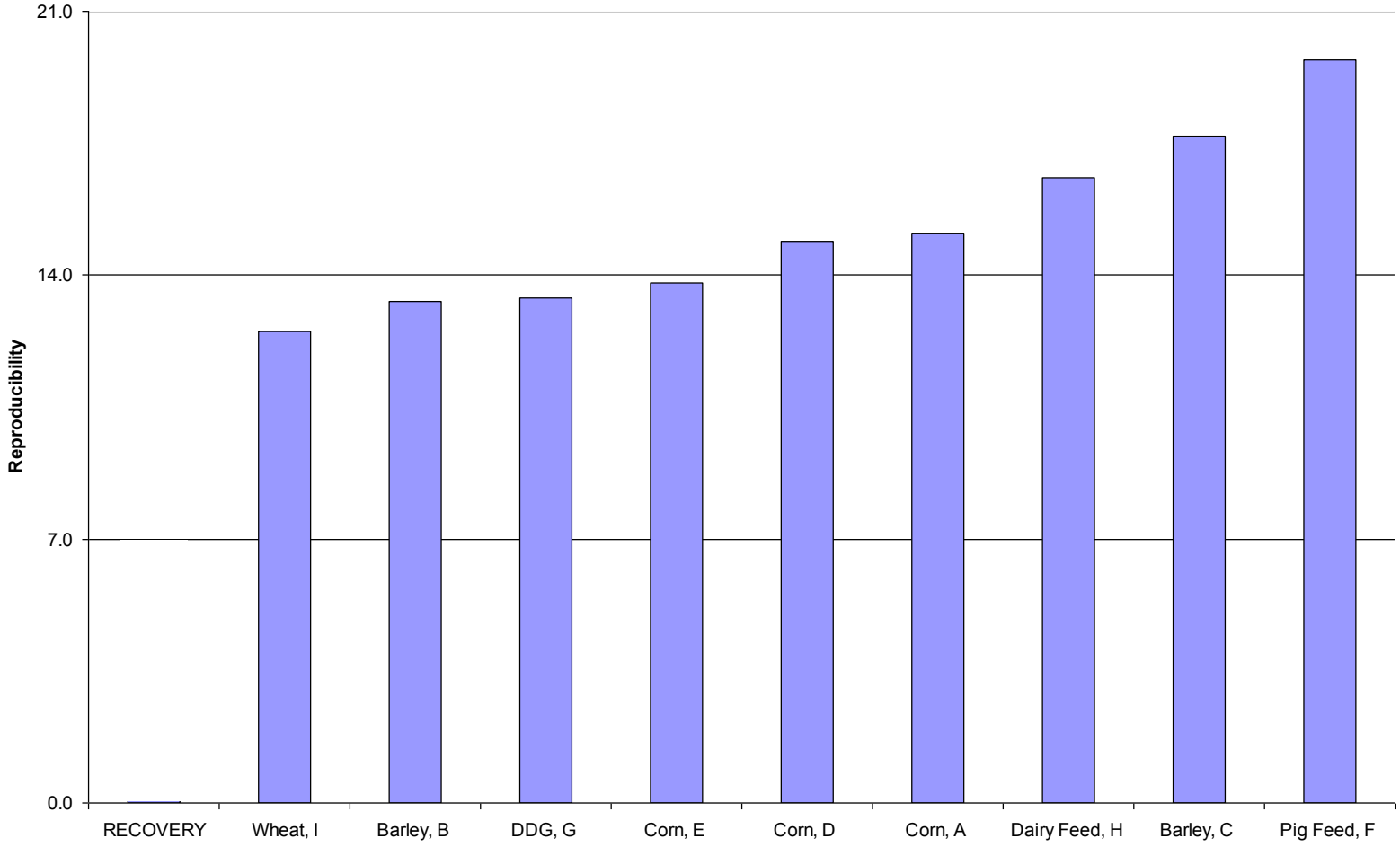
European Commission

European Commission

Conduct Proficiency Tests (Comparative testing) with NRLs (and OCLs)

European Commission

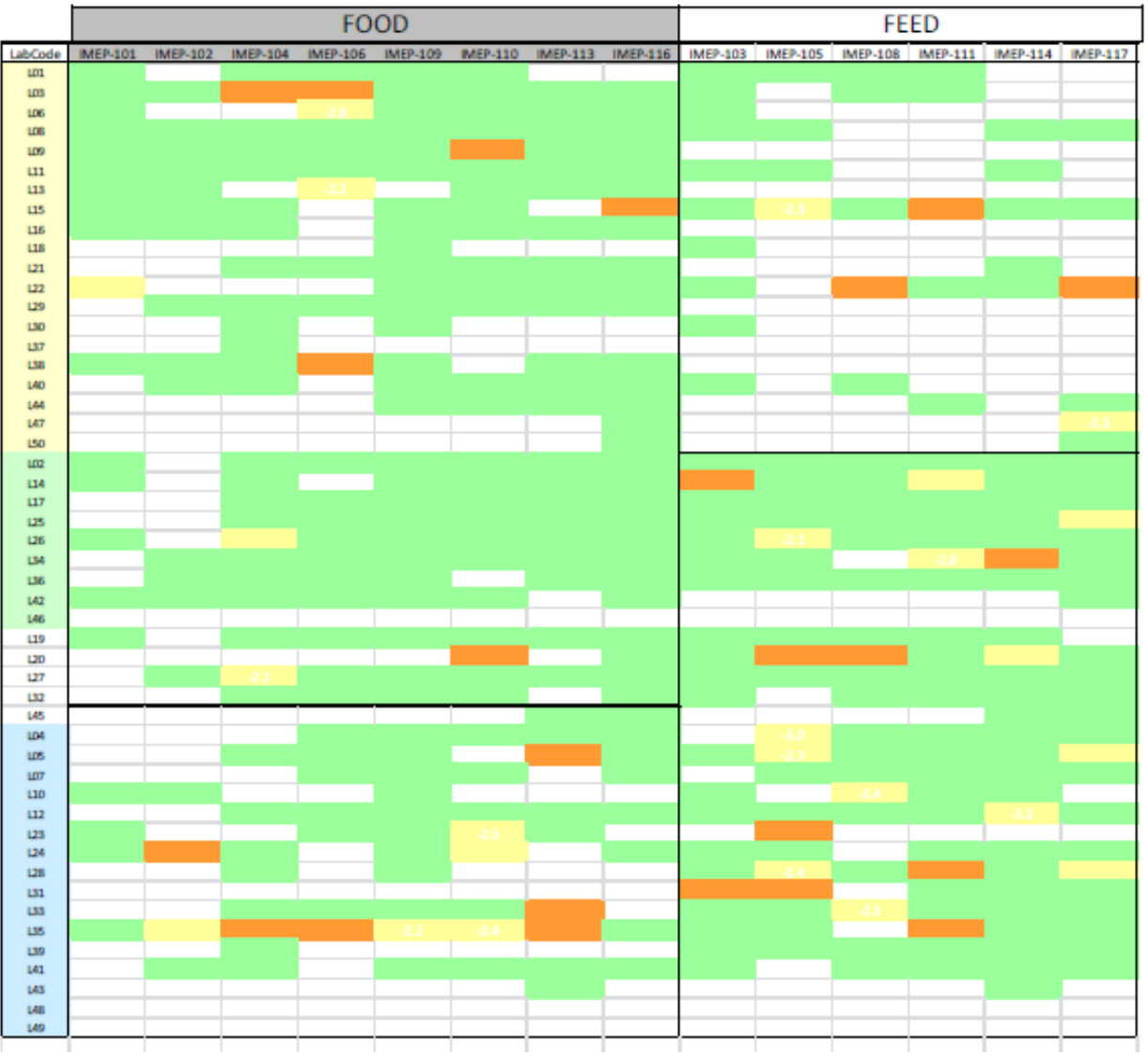
“Blind measured” vs “Known asked”



European Commission

Prciofinecy Tsets assrue reortping on basis what is osbreved, rahetr than what is asumsed shuold be obsvered.

Performance monitoring



Overview of NRL performances in EURL-HM PTs for the determination of CADMIUM in food and/or feed matrices. (2006-2014)

European Commission

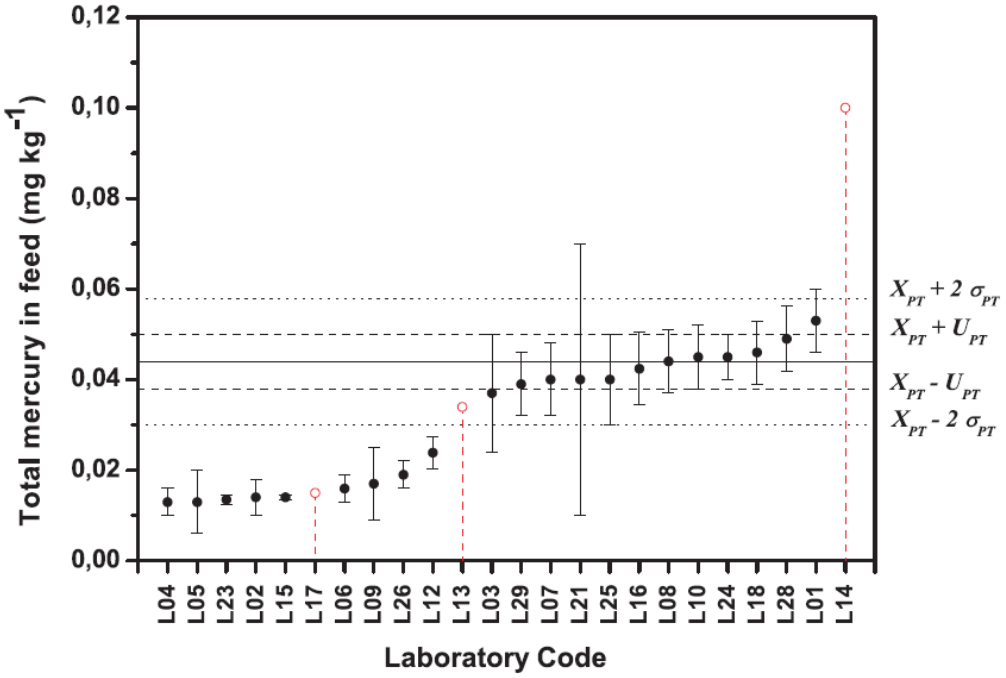


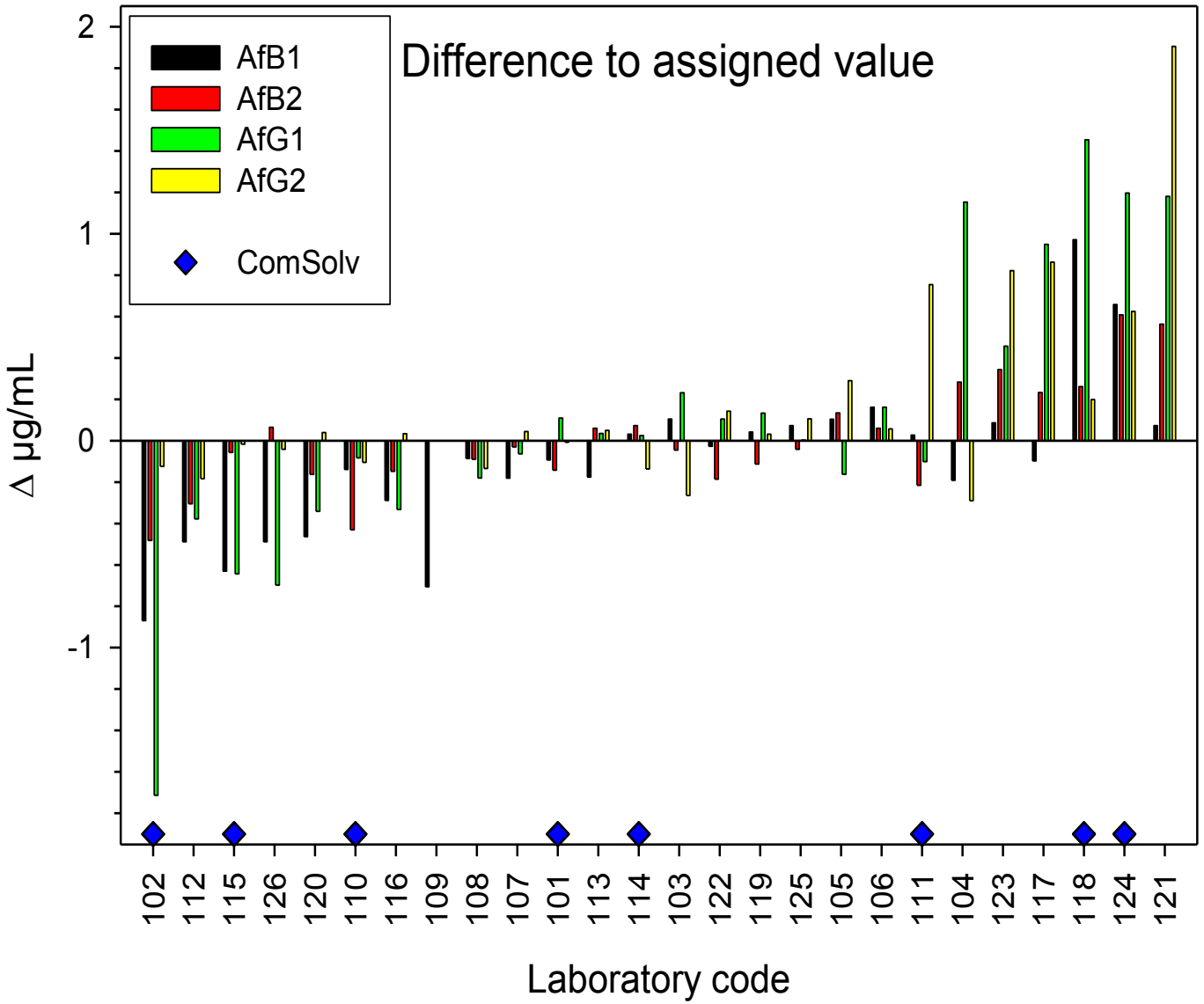
Figure E.5 — Participant results and uncertainties for results in IMEP 111 (data from Table E.6)

EURL-HM contribution to the ISO 13528:2015 standard

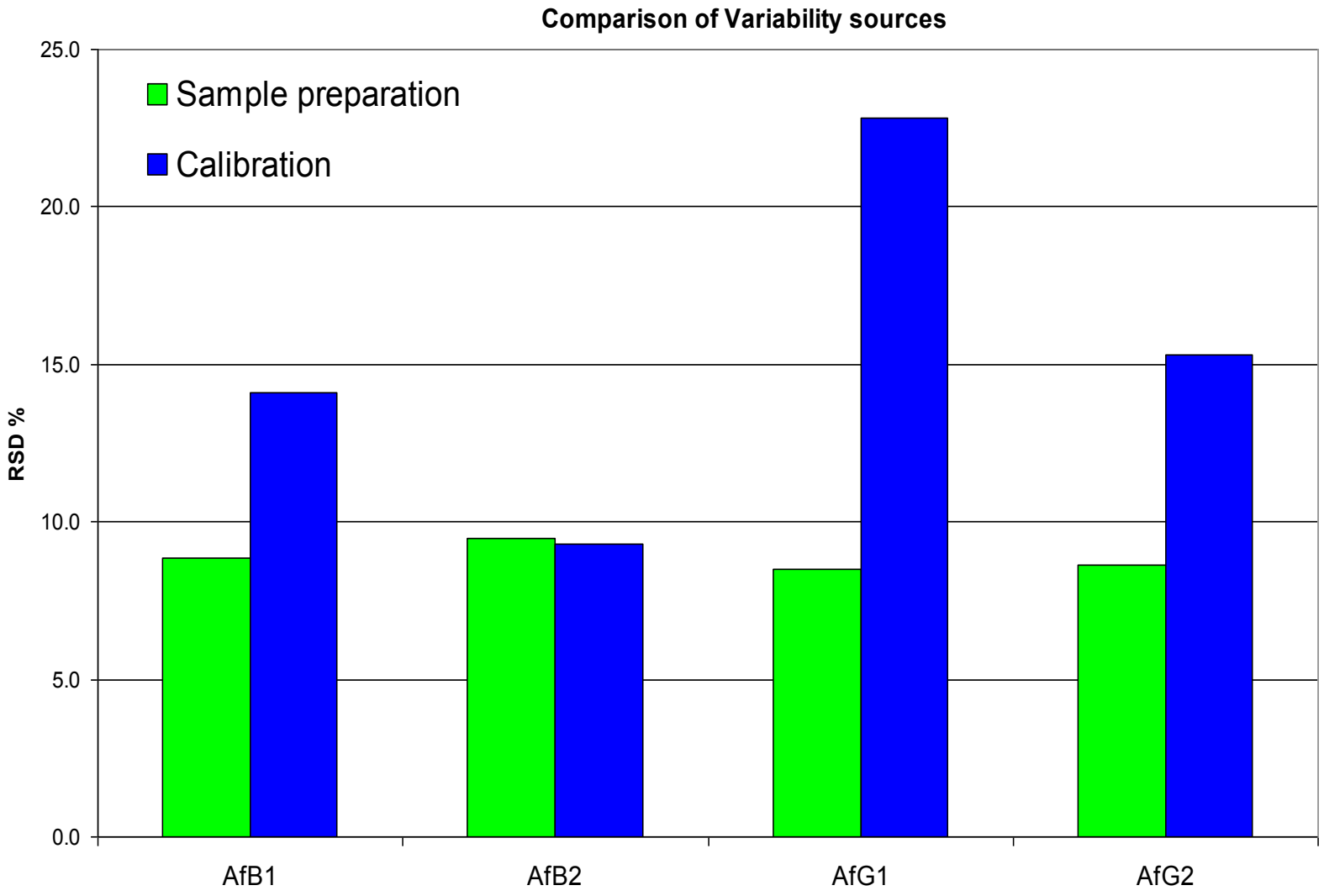
IMEP-111: Determination of […] and Hg in mineral feed - B. de la Calle et al. (2011)

European Commission

PT 2007 (first PT of the EURL mycotoxins)



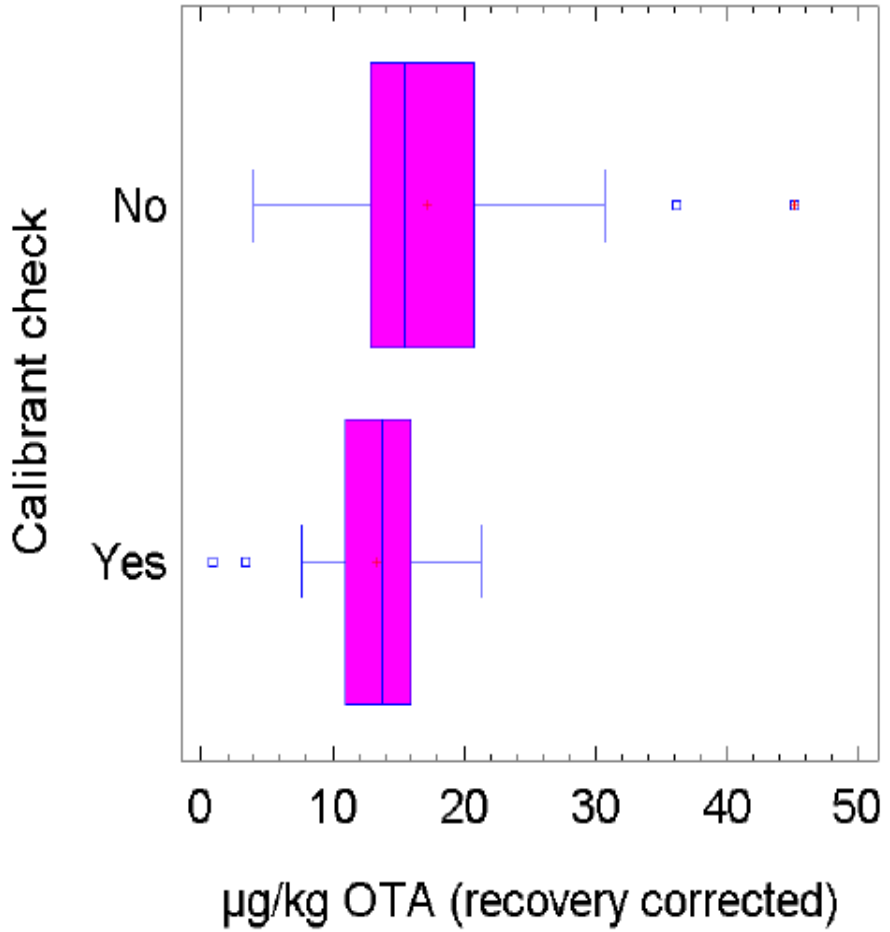
European Commission



European Commission

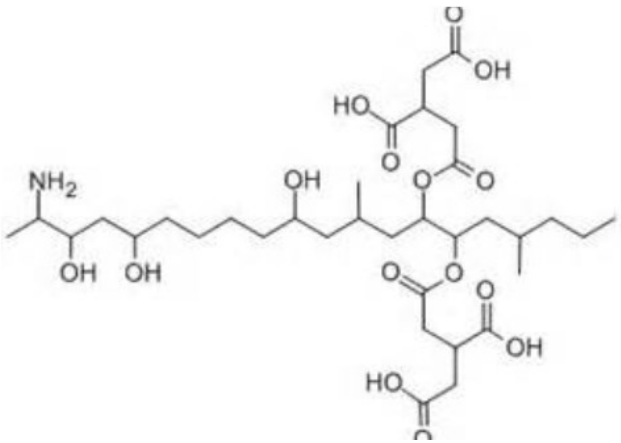
Calibrant issues with OTA

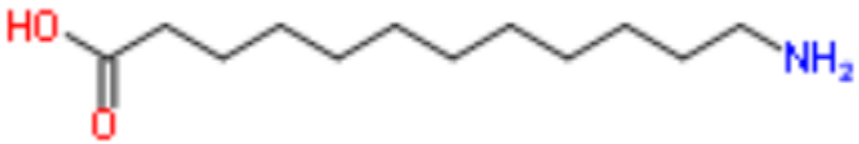
Figure 10: Influence of a photometric calibrant check on the result

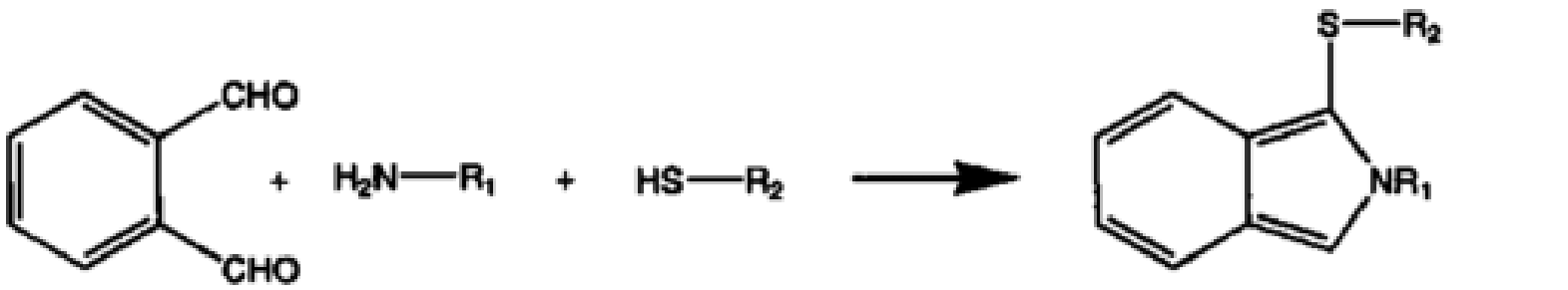


European Commission

Testing for calibrant content/stability

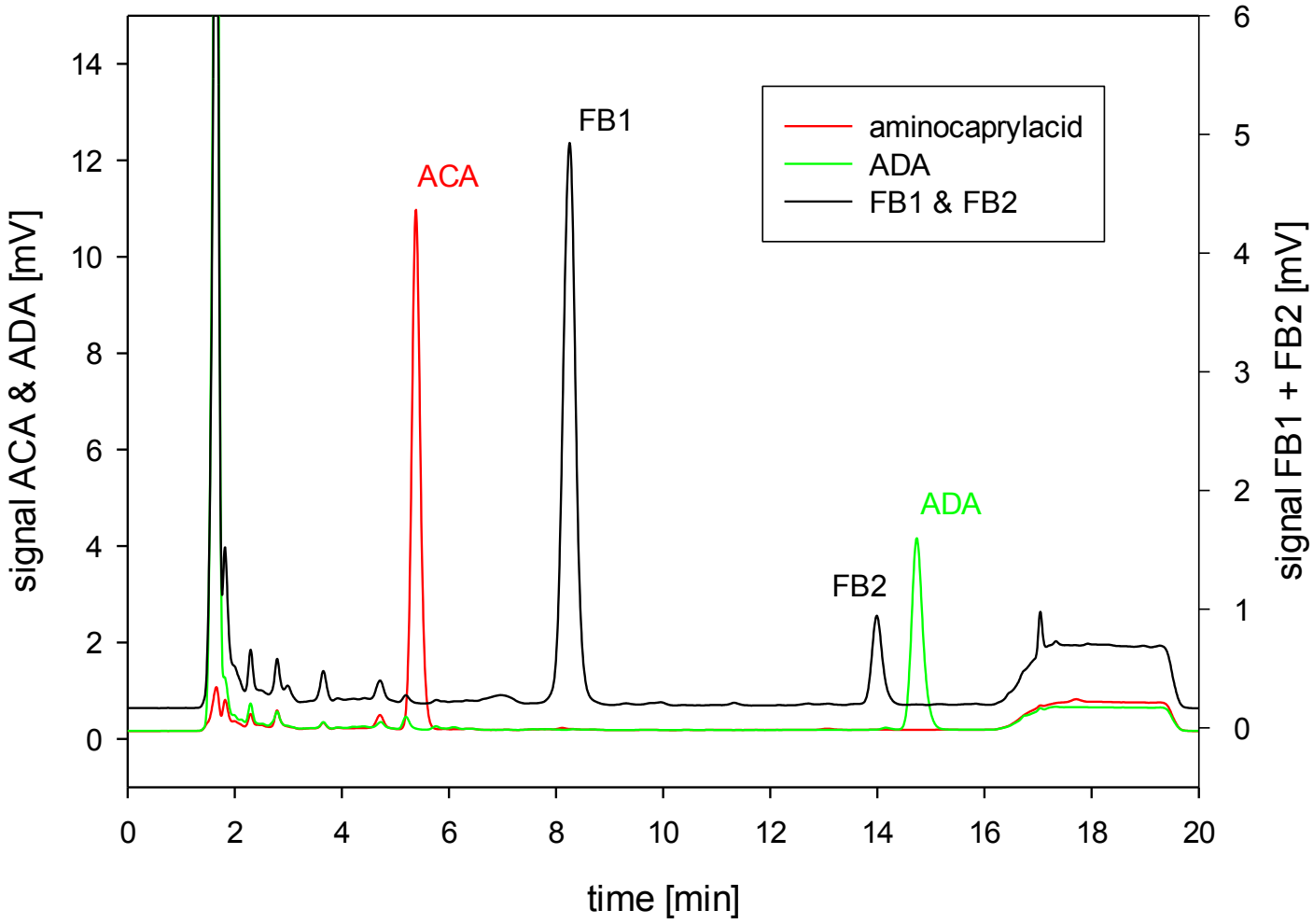






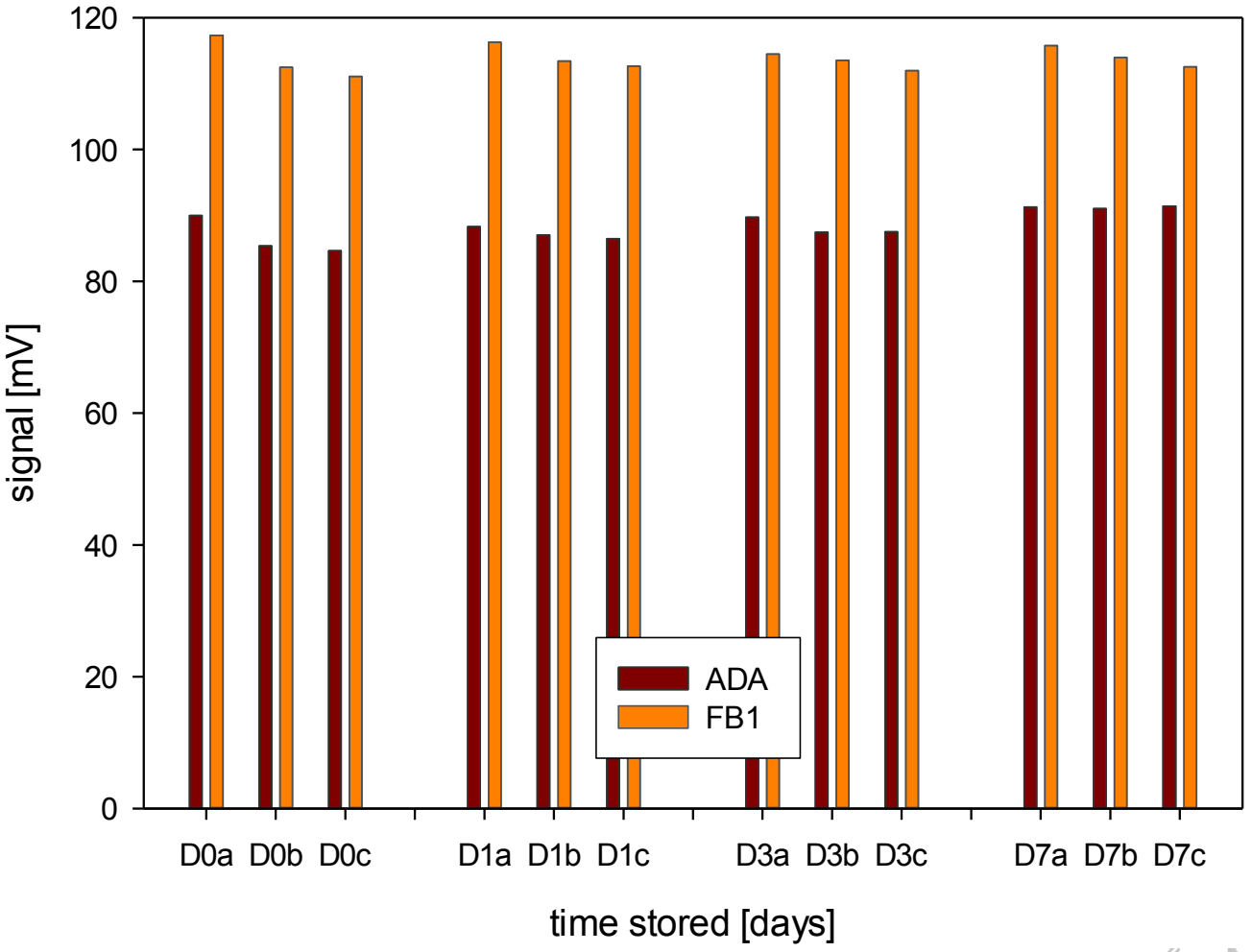
OPA Analyte (amine) Reaction partner Fluorescent derivative (Isoindole)

Testing for calibrant content/stability



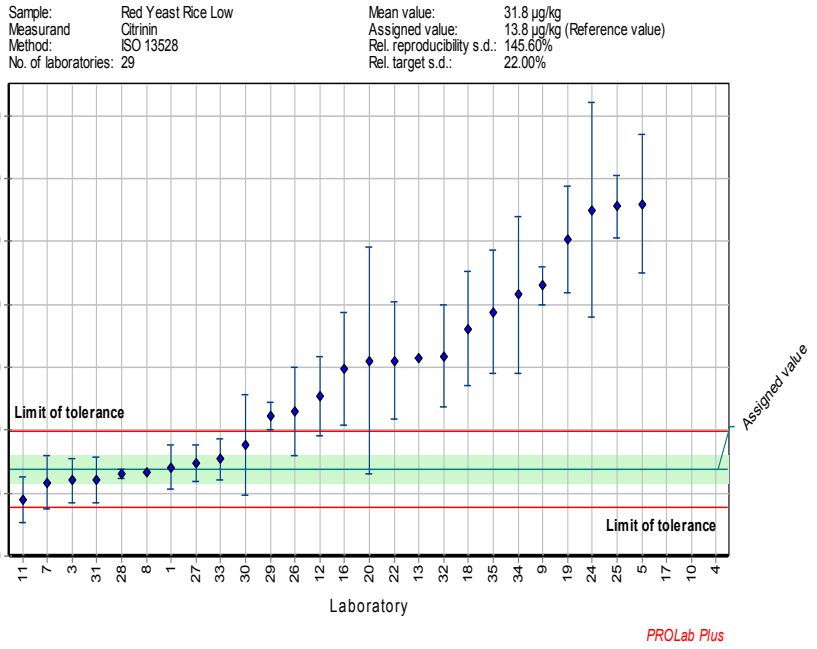
European Commission

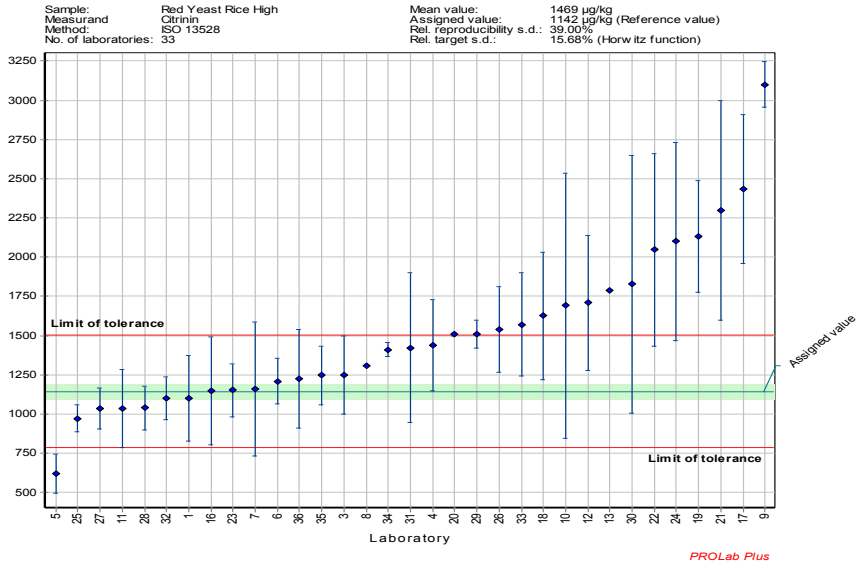
Testing for calibrant content/stability



European Commission

Calibrant content/stability solved?



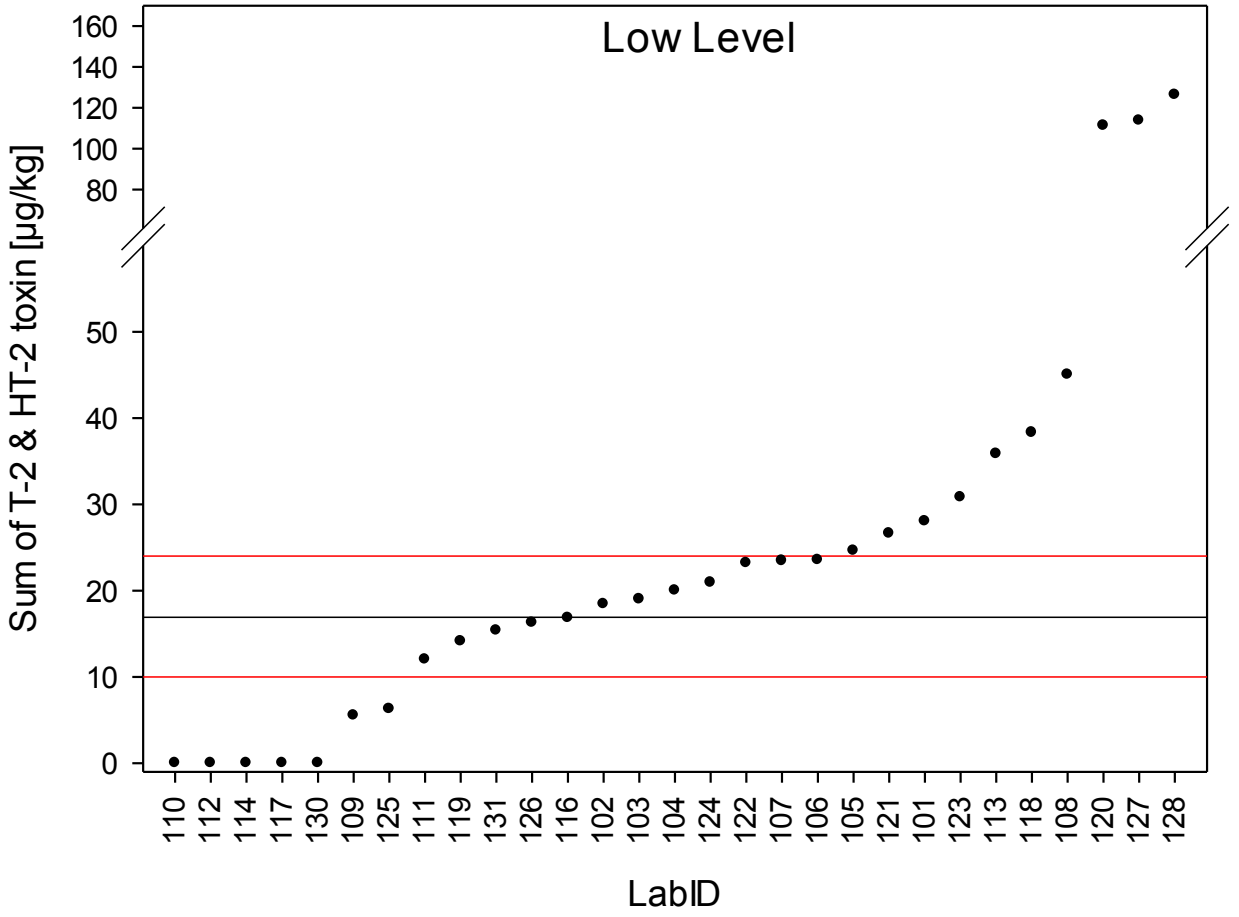


European Commission

Other pitfalls...

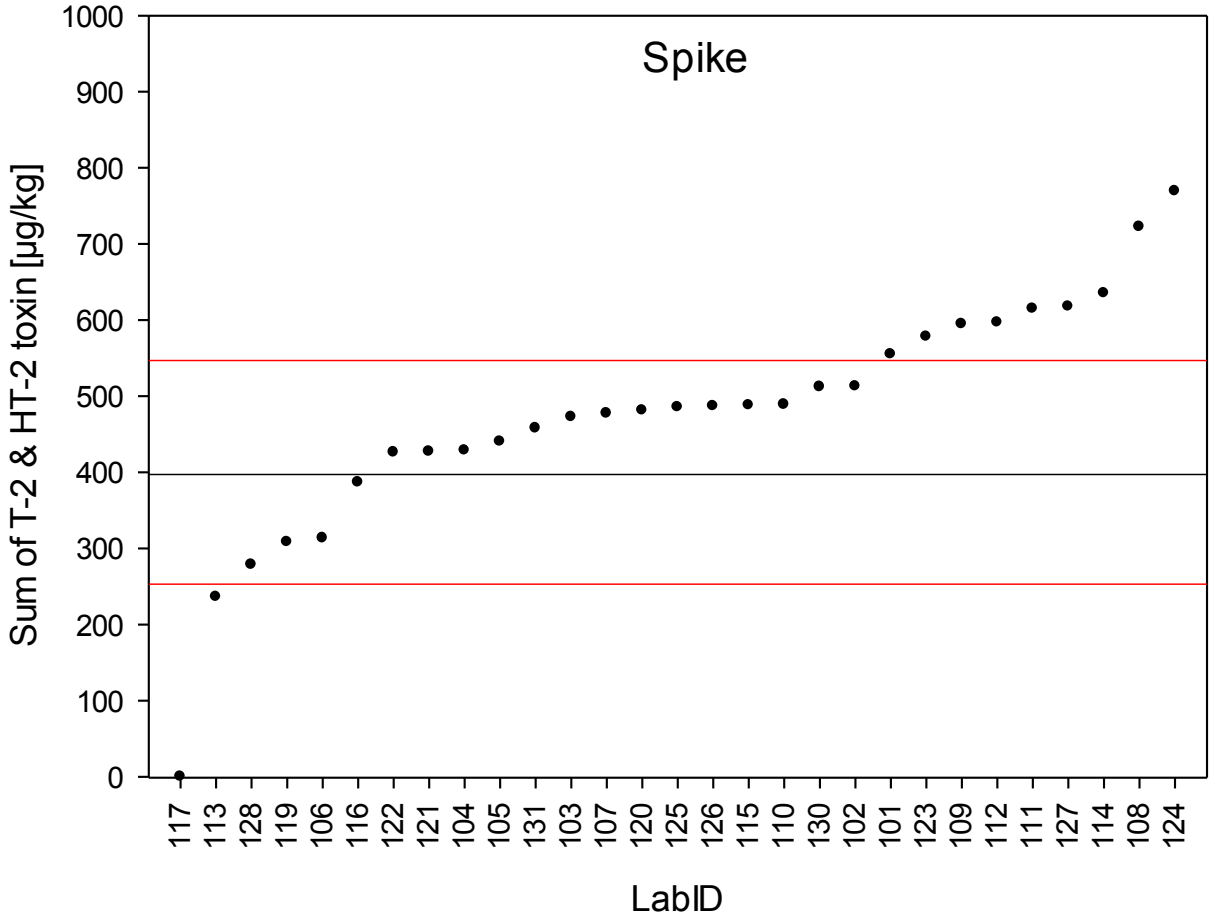
"what is your working range?"

First out of 3 samples in a PT (low level)



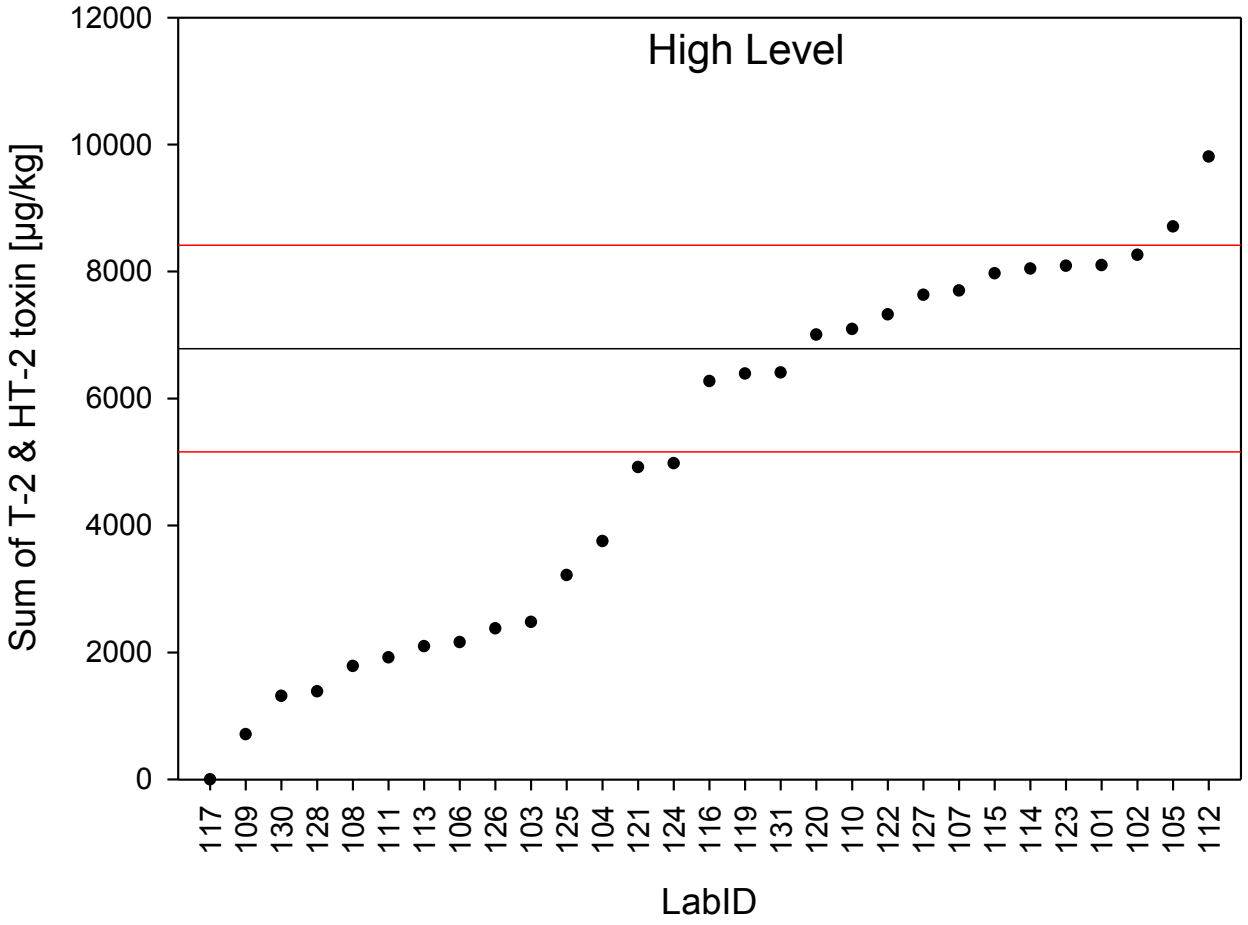
European Commission

Second out of 3 samples in a PT (mid level)

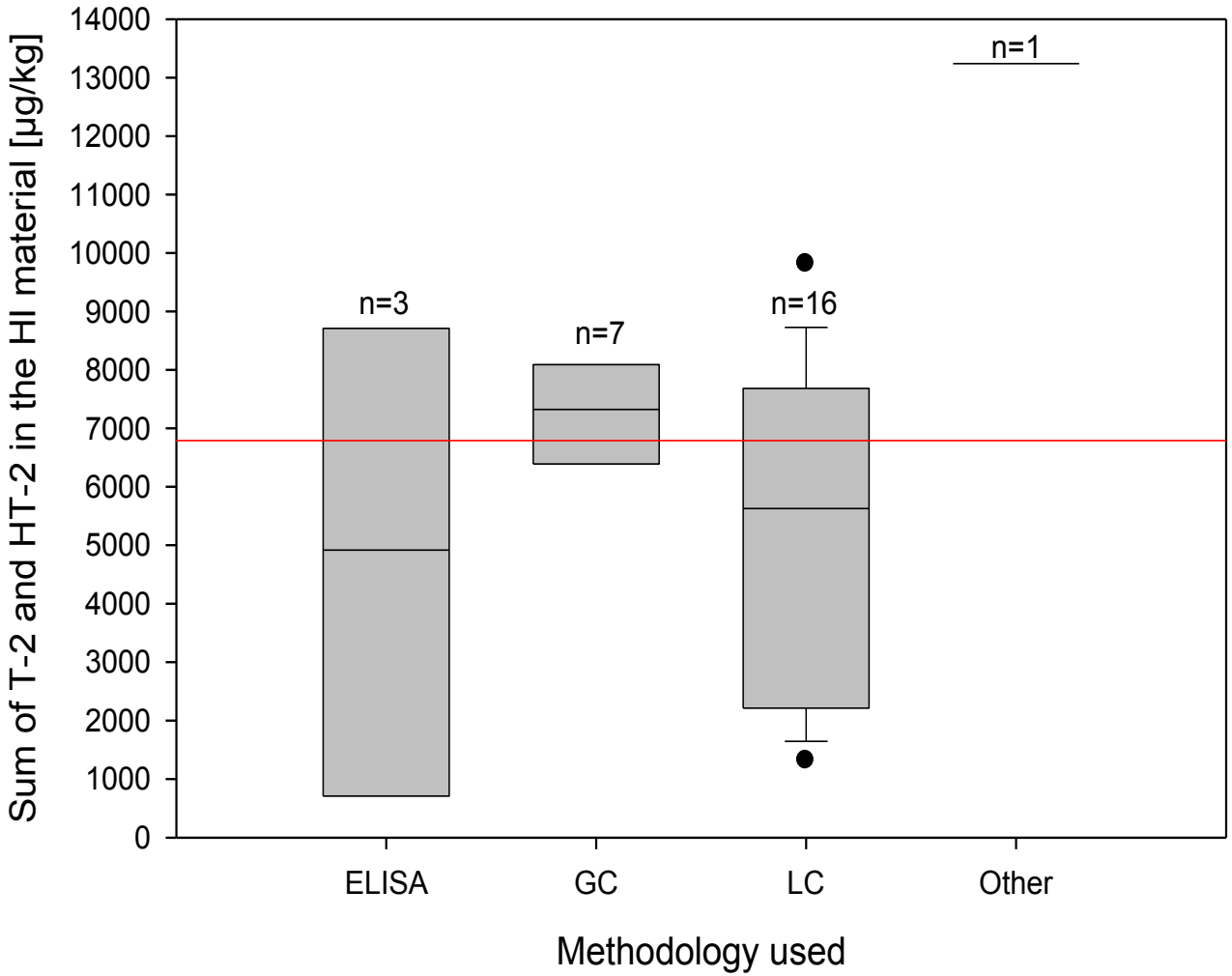


European Commission

Third out of 3 samples in a PT (high level)



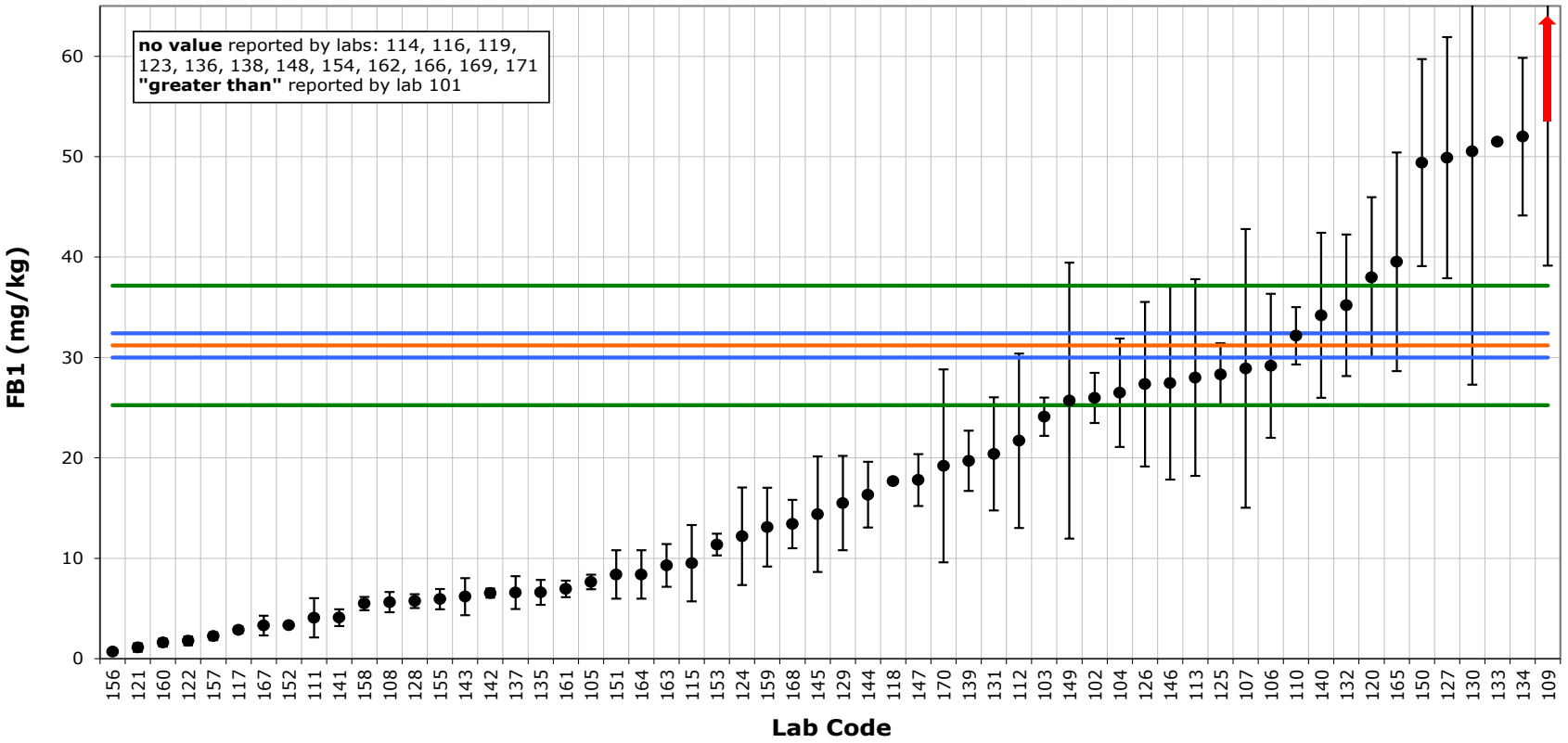
Methodologies…



European Commission

More on methodologies...

Figure 6: EU-RL Mycotoxins PT 2013: Fumonisin B1 in cereals - Sample B
Certified value: Xref = 31.2 mg/kg; Uref = 1.2 mg/kg (k=2); σ = 2.97 mg/kg

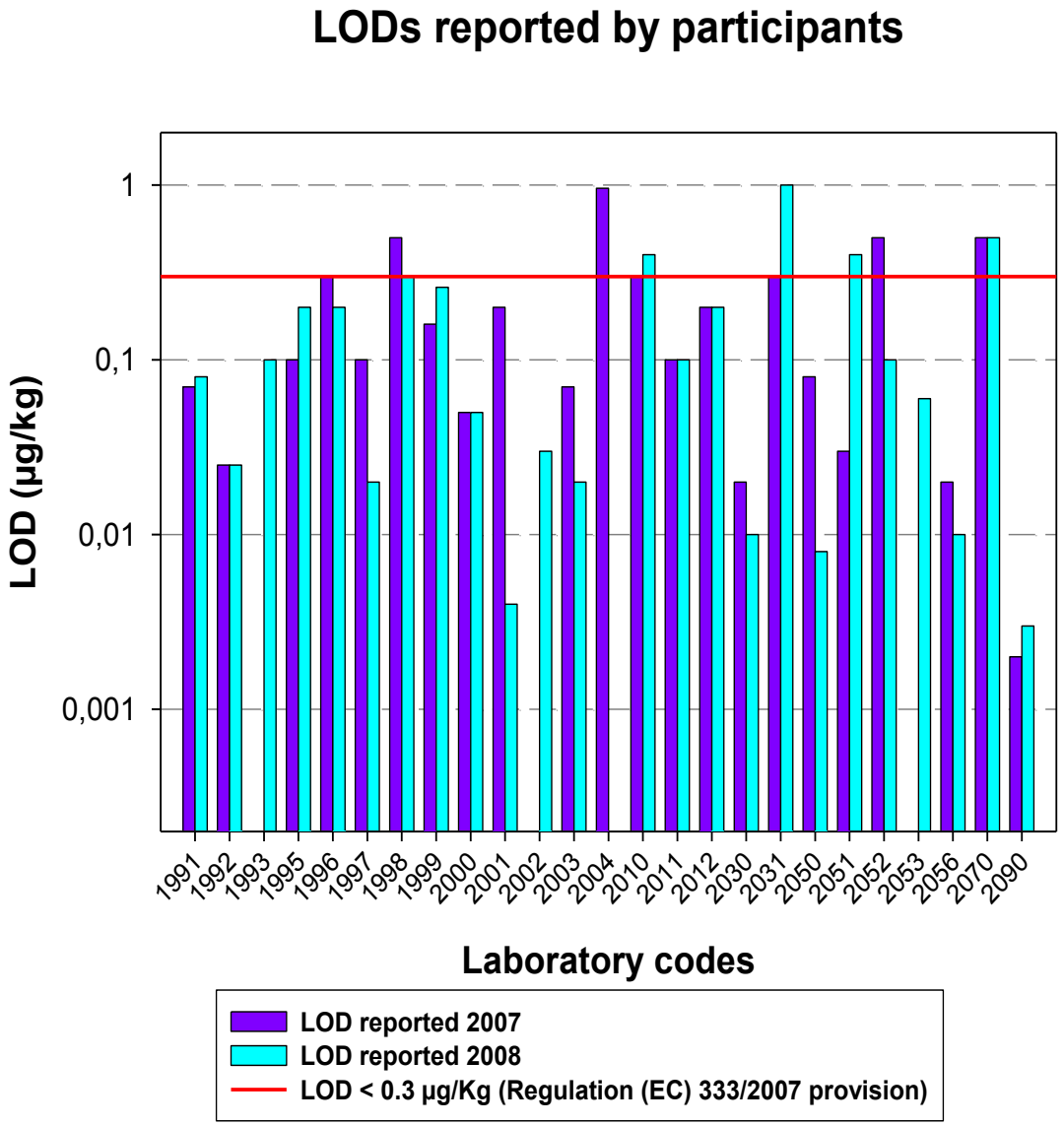


This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

European Commission

Drafting of Guidance documents (or more)…

European Commission



- Range = three orders of magnitude
- Discrepancies cannot be easily explained
- Provides challenges for harmonisaton

European Commission

Approaches for LOD estimation

Survey in 2008/2009

Limit of detection (LOD)

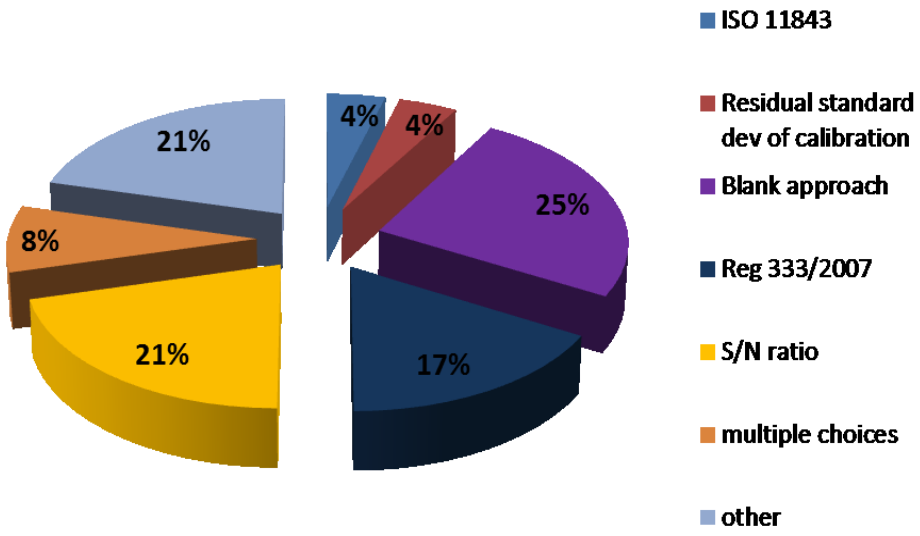
Please state on which basis you estimate the LOD of your analytical method (C_L is the corresponding concentration):

1. ISO standard 11843-2
2. German Standard DIN 32645
3. From the standard deviation of the intercept of the calibration line
4. C_L = the concentration where there is 5% of probability of a false positive
5. C_L is derived from the smallest measure (X_L) that can be detected with reasonable certainty. $x_L = \bar{x}_{bl} + ks_{bl}$

   Where: $\bar{x}_{bl}$ is the mean of the blank measures, S_{bl} is the standard deviation of the blank measures and k is a numerical factor chosen according to the confidence level desired *(source: IUPAC golden book)*.

   which value do you use for k? Enter your value:
6. S/N ratio S=signal; N=noise
7. Calculation from the residual standard deviation of the calibration line
8. EPA approach to method detection limit (MDL)
9. According to the LOD definition in Commission Regulation (EC) No 333/2007

Only 2 laboratories out of 24 apply "calibration" based approaches



LOD/LOQ Guide Challenges

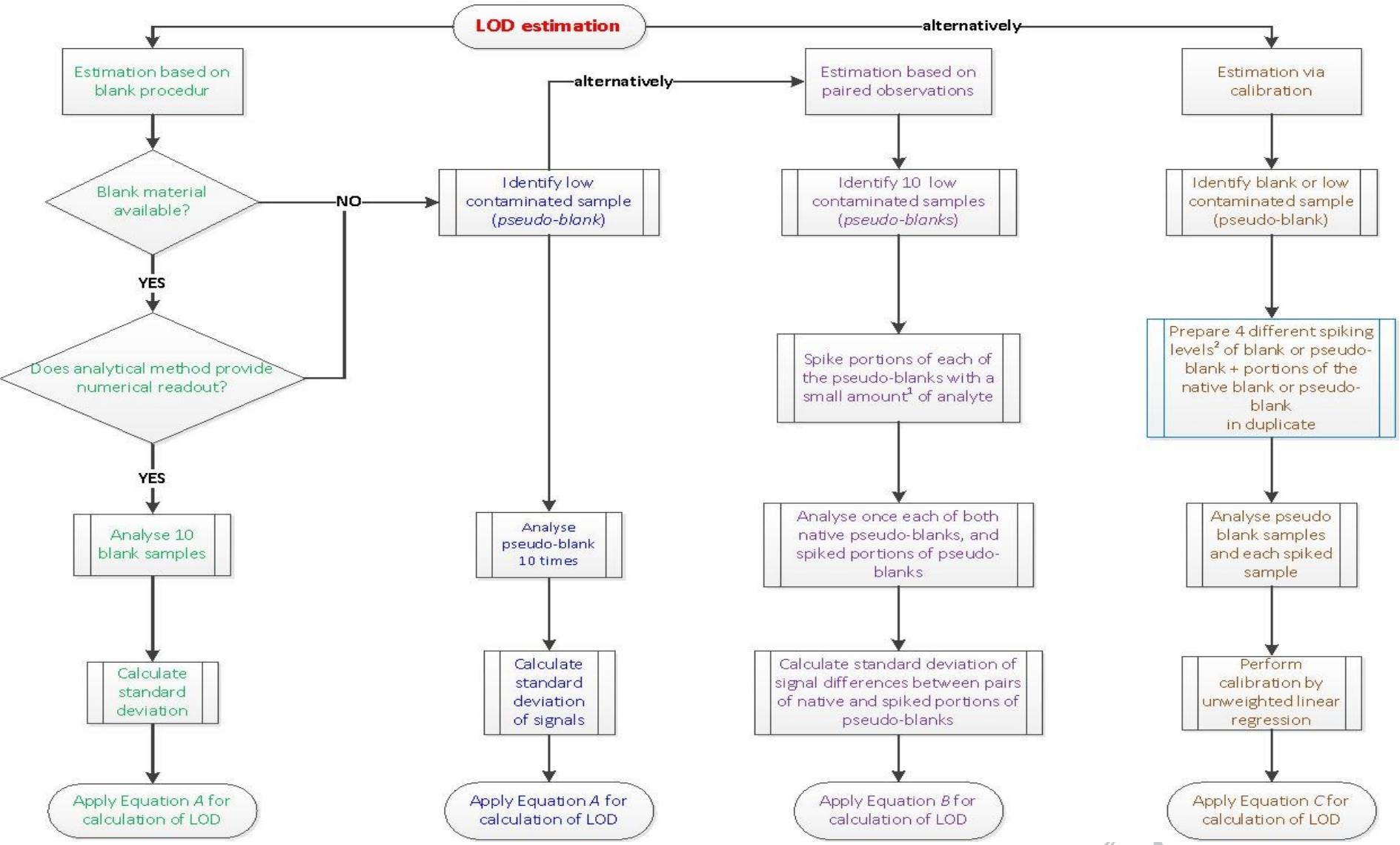
HM, PAHs, mycotoxins:
Find scientifically correct, practical approach

- *Implemented during method validation*
- *Major step: estimation of LOD*

Dioxins:
Find scientifically correct solution that

- *is compatible with established practices*
- *does not impact current exposure models*
- *abstains from LOD*
- *is partially implemented for each sample*

Experimental approaches



European Commission

Take-home messages LOD/LOQ guide

- Different options for estimating LOD/LOQ
- Estimation in matrix
- Application of entire analytical method - identification criteria
- Limited experimental burden
- At level close to LOD (both calibration and matrix samples)
- Simple mathematical models

COMMISSION REGULATION (EU) No 519/2014

of 16 May 2014

amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis

For screening methods with a response proportional with the mycotoxin concentration the following applies:

$$Cut\text{-}off = R_{STC} - t\text{-}value_{0,05} * SD_{STC}$$

R_{STC} = mean response of the positive control samples (at STC)

t-value: one tailed t-value for a rate of false negative results of 5 % (see table B)

SD_{STC} = standard deviationScreening methods with a response inversely proportional with the mycotoxin concentration

Mycotoxin "electronic working groups" for guidance documents relevant for mycotoxins

Identification criteria for mycotoxins (NRL, NL)

Best practise in mycotoxin laboratories (NRL, IE)

Laboratory subsampling (NRL, SE)

Future planning:

Handling of calibrants

PT and "working group" conclusions

Proficiency tests remain the main tool to identify individual and methodological aspects to improve measurement capacity in the EU and beyond.

Guidance documents (or more):

Screening methods

Identification Criteria

Establishing LOD/LOQ estimates

Best practise in the mycotoxin laboratory

Laboratory sub-sampling

Provide Training…

European Commission

Training activities

European Commission

EURL website

- https://ec.europa.eu/jrc/en/eurl/mycotoxins
- or search for the string "**EURL mycotoxins**"

9 Presentations related to analysis of food and feed contaminants

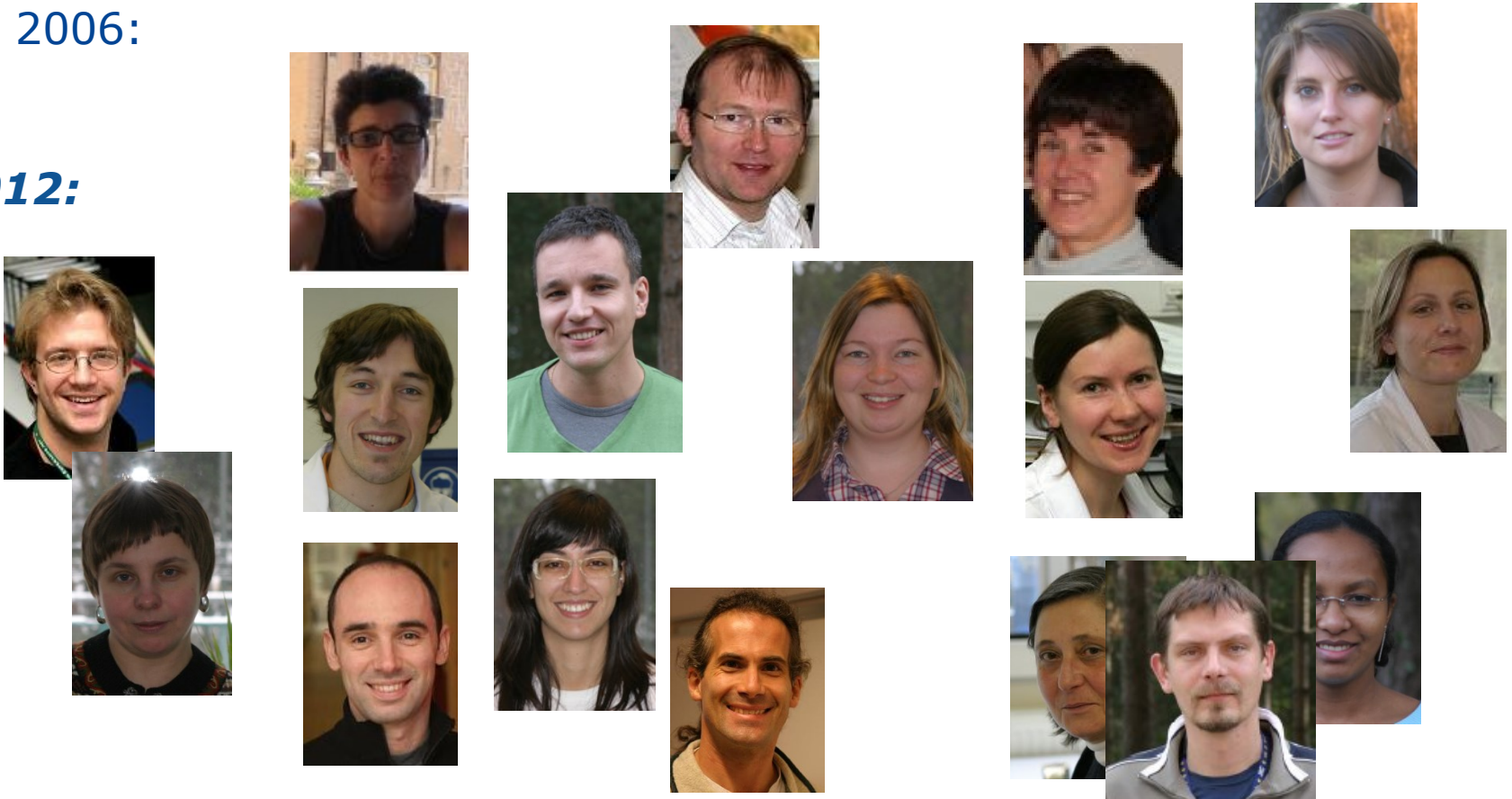
Plus "Identification Criteria for Mycotoxins: Workgroup leader Hans Mol From the EURL (residues) and NRL (mycotoxins), RIKILT, Wageningen

European Commission

Mycotoxin Group @ IRMM

Start 2006:

2012:



European Commission

Analytical method development…

European Commission

The cascade Approach

Standardization in Europe (Feed)

Mandate M/522 - publication of 12 standardized methods of analysis for animal feed (project number 5 will produce 2 standards):

- Determination of ergot alkaloids in feed materials and compound feed by LC-MS
- Determination of tropane alkaloids in feed materials and compound feed by LC-MS
- Multimethod for mycotoxins in feed materials and compound feed by LC-MS
- Determination of organic acids in premixtures, feed materials and compound feed
- Criteria approach for methods of analysis for mycotoxins in feed
- Criteria approach for methods of analysis for heavy metals in feed
- Determination of authorised coccidiostats at additive and 1 and 3 % carry-over level in compound feed with LC-MS

Standardization in Europe (Food)

METHODS OF ANALYSIS TO BE MANDATED

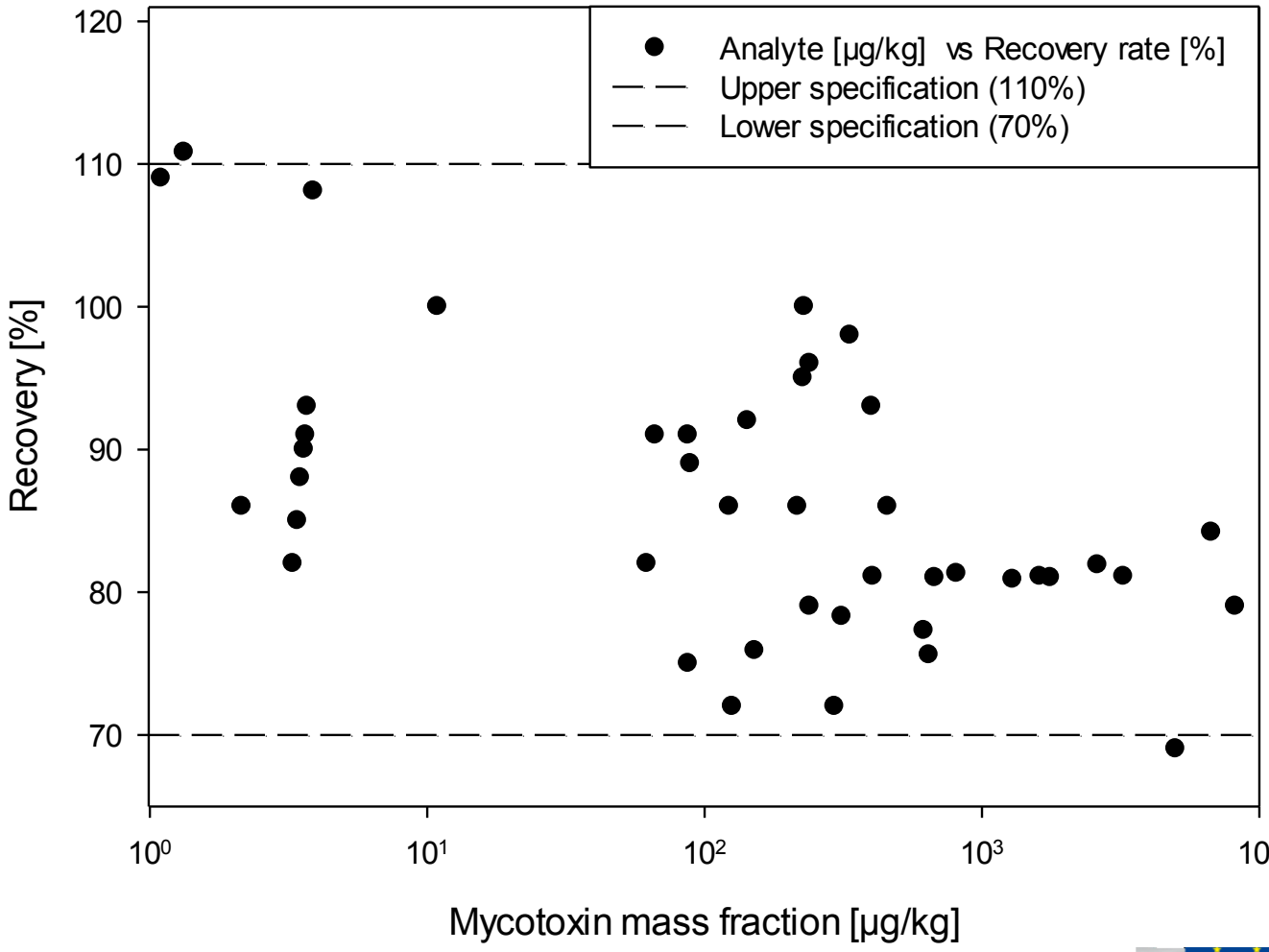
N.	Title	Deadline	Requested deliverable
(1)	(2)	(3)	(4)
1	Determination of ergot alkaloids (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and their epimers) in cereals and cereal products	31/12/2016	EN
2	Determination of T-2 and HT-2 toxin in cereal based foods for infants and young children by LC-MS/MS	31/12/2016	EN
3	Determination of zearalenone in vegetable oils including refined maize oil	31/12/2016	EN
4	Multimethod for determination of zearalenone and trichothecenes at least including deoxynivalenol (DON) and its acetylated derivatives (3-acetyl-DON and 15-acetyl-DON), nivalenol and T2 and HT-2 in cereals and cereal products by LC-MS/MS	31/12/2016	EN
5	Multimethod for the screening of ochratoxin A, aflatoxin B1, deoxynivalenol, zearalenone and fumonisin B1 and B2 in foodstuffs, excluding foods for infants and young children, by LC-MS/MS	31/12/2016	EN

6	Determination of aflatoxins in spices (for which an EU maximum level has been established) other than paprika	31/12/2016	EN
7	Determination of ochratoxin A in cocoa and cocoa products, spices (for which an EU maximum level has been established) and liquorice.	31/12/2016	EN
8	Determination of ochratoxin A in meat, meat products and edible offal	31/12/2016	EN
9	Determination of Alternaria toxins (at least including alternariol, Alternariol monomethyl ether, tenuazonic acid, tentoxin and altenuene).by LC-MS/MS	31/12/2016	EN
10	Determination of phomopsin A in lupins and lupin derived products by HPLC-MS/MS	31/12/2017	EN
11	Determination of citrinin in food by LC-MS/MS	31/12/2017	EN

Secretariat: Annemieke.Venemans@nen.nl

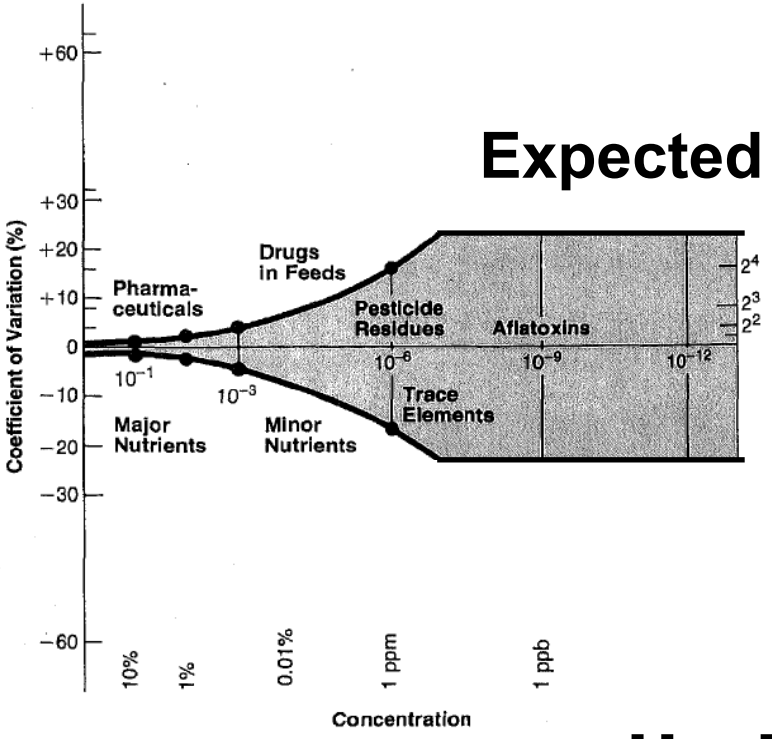
European Commission

Performance criteria for mycotoxin method



European Commission

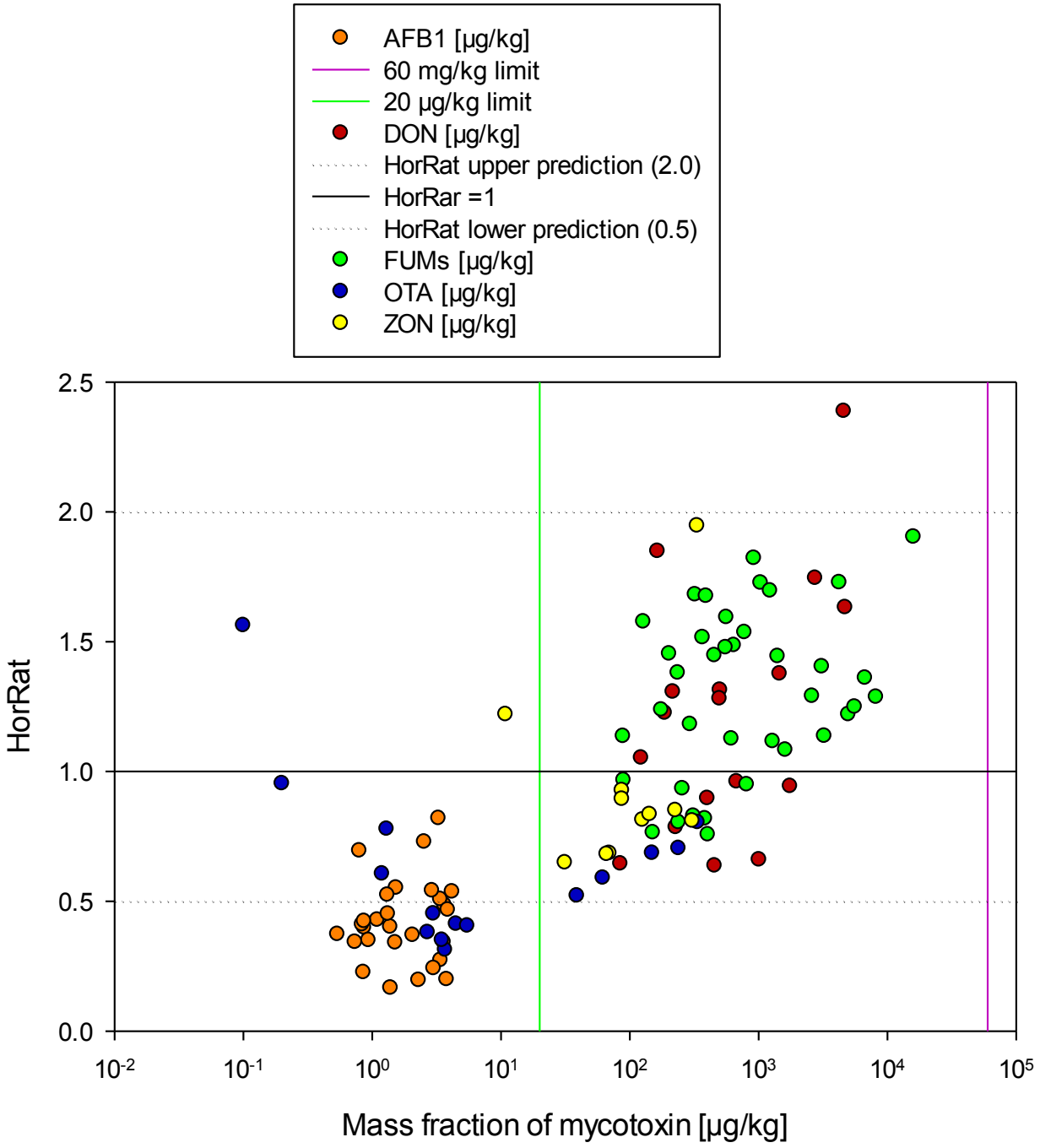
$(\sigma_p) = 0.02^{c \times 0.8495}$; where c is the mass fraction of the analyte in the sample



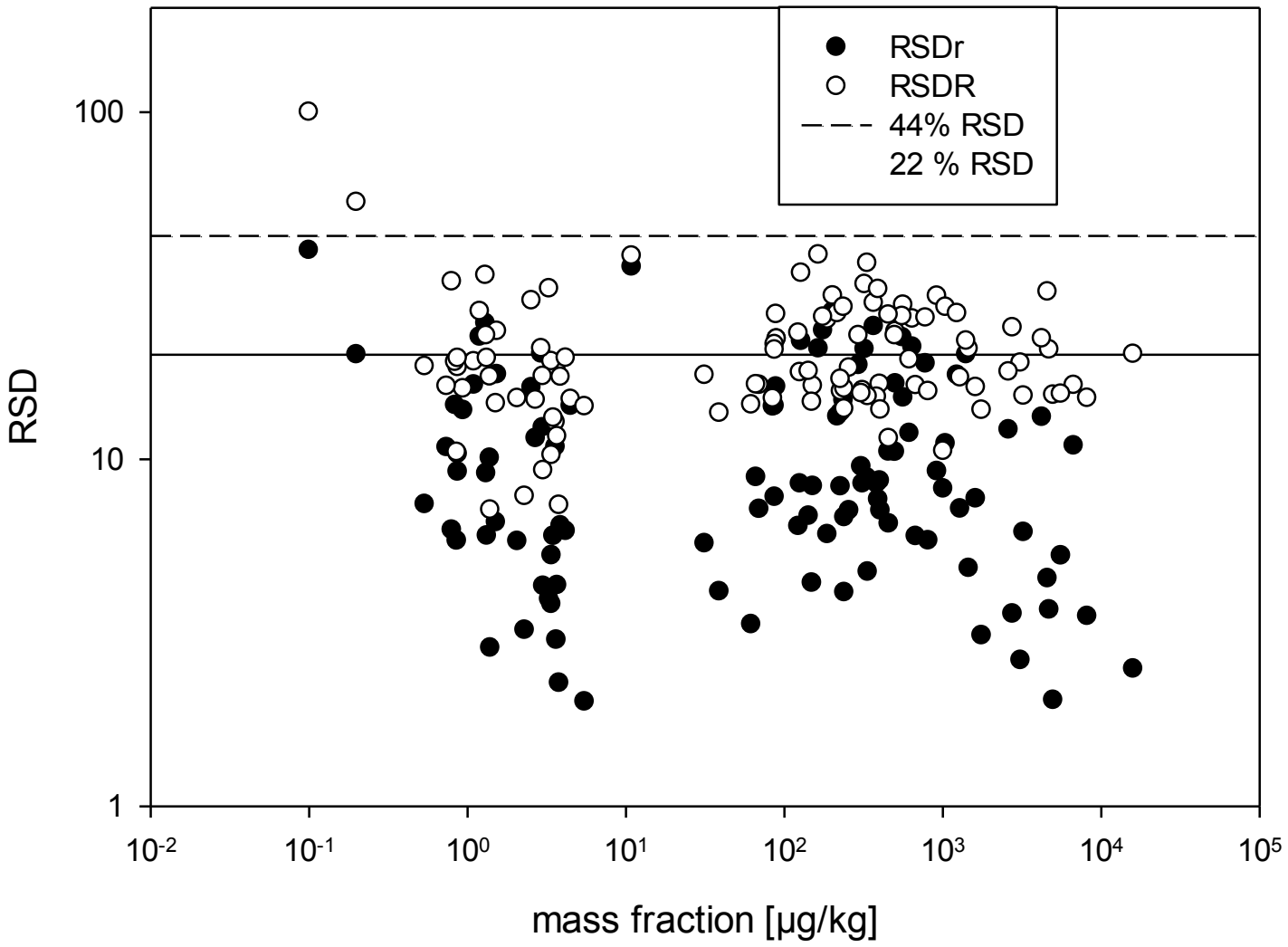
Expected RSD_R under 120 µg/kg → 22%

$$\textbf{HorRat} = \frac{\textbf{Observed RSD}}{\textbf{Expected RSD}}$$

European Commission



European Commission



European Commission

Acknowledgements

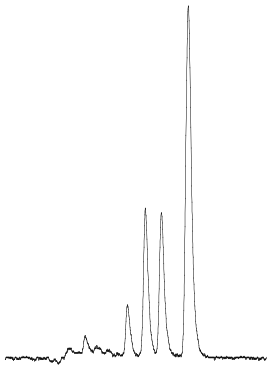
All EURL teams but in particular:

Partners in the NRL network

EURL for dioxins and PCBs

European Commission

At the end of a long(?) road…



!?!

Vision for the future:

"Measured once accepted everywhere"

European Commission

Stay in touch

JRC Science Hub: www.ec.europa.eu/jrc

Twitter: @EU_ScienceHub

LinkedIn: european-commission-joint-research-centre

YouTube: JRC Audiovisuals

Vimeo: Science@EC

European Commission

European Commission