Final results workshop of Biennial Global Assessment of POPS laboratories (2nd round)

June 24-25, 2014 , Freiburg, Germany

EU-RL Proficiency Tests for PCDD/Fs and PCBs

- Evaluation of data and scoring of results -

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Criteria in EU regulations

Current EU regulations (1)

Methods of analysis (Commission Regulations (EC) No 152/2009 (Feed), (EU) No 589/2014 (Food))

- **Requirements for laboratories:**
  - […], laboratories shall be accredited by a recognised body […] to ensure that they are applying analytical quality assurance. Laboratories shall be **accredited** following the **EN ISO/IEC 17025** standard.

EN ISO/IEC 17025:

- 5.9 Assuring the quality of test and calibration results:
  - Quality control procedures for monitoring of the validity of tests and calibrations
  - Recording of data for detection of trends and reviewing of results
  - Planning and review of monitoring may include
    - **Participation in interlaboratory comparison or proficiency testing programmes**

- Laboratory proficiency shall be proven by the **continuous successful participation in interlaboratory studies** for the determination of PCDD/Fs and dioxin-like PCBs in relevant food/feed matrices and concentration ranges.

Interlaboratory studies

Definitions:

- **Interlaboratory study:**
  A study in which **several laboratories** measure a quantity in one or more **identical portions** of homogeneous, stable **materials** under **documented conditions**, the results of which are compiled into a single report.
  [IUPAC, NOMENCLATURE OF INTERLABORATORY ANALYTICAL STUDIES, Pure & Appl. Chern., Vol. 66, No. 9, pp. 1903-191 1, 1994.]

- **Laboratory performance study:**
  An interlaboratory study that consists of one or more analyses or measurements by a **group of laboratories** on one or more **homogeneous**, stable **test samples** by the **method selected or used by each laboratory**. The reported **results** are **compared** with those from **other laboratories** or with the known or assigned **reference value**, usually with the objective of evaluating or improving laboratory performance.
  [IUPAC, NOMENCLATURE OF INTERLABORATORY ANALYTICAL STUDIES, Pure & Appl. Chern., Vol. 66, No. 9, pp. 1903-191 1, 1994.]

- **Proficiency Testing (PT):**
  Evaluation of participant performance against pre-established **criteria** by means of interlaboratory comparisons
  [EA-4/18 TA :2010– Guidance on the level and frequency of proficiency testing participation, European co-operation for Accreditation]

- **Interlaboratory Comparison:**
  Organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions
  [EA-4/18 TA :2010– Guidance on the level and frequency of proficiency testing participation, European co-operation for Accreditation]

Current EU regulations (2)

Methods of analysis (Commission Regulations (EC) No 152/2009 (Feed), (EU) No 589/2014 (Food))

- **Basic requirements for analytical procedures**

  High accuracy (trueness and precision) – valid estimate of true concentration

  - **Accuracy of the measurement**: the closeness of the agreement between the result of a measurement with the true or assigned value of the measurand.
  - **Trueness**: Difference between the mean value measured for an analyte in a certified material and its certified value, expressed as percentage of this value
  - **Precision**: Relative standard deviation calculated from results generated under reproducibility conditions

Definition in **Commission Decision 2002/657/EC:**

- "**Trueness** means the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value."
- "**Within-laboratory reproducibility** means precision obtained in the same laboratory under stipulated (predetermined) conditions over justified long time intervals."

Current EU regulations (3)

Methods of analysis (Commission Regulations (EC) No 152/2009 (Feed), (EU) No 589/2014 (Food))

- **Basic requirements for analytical procedures**

  Validation in the range of level of interest and general quality control measures:

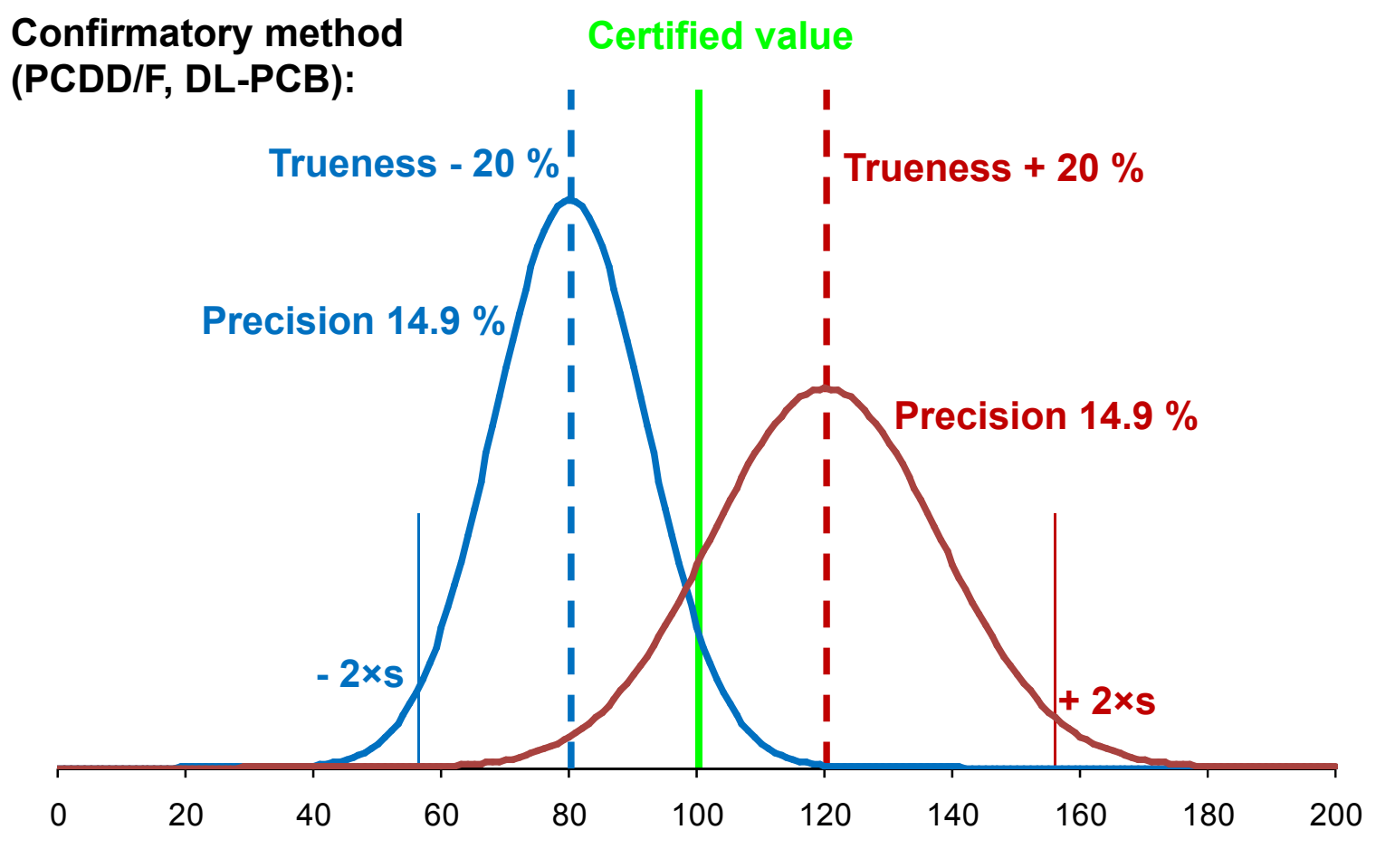
  - Demonstration of performance of method in range of level of interest with acceptable CV

  Analytical criteria:

  - Criteria for TEQ and BEQ values for screening and confirmatory methods

	Screening with bioanalytical or physico-chemical methods	Confirmatory methods
False-compliant rate	**< 5 %**	
Trueness		**- 20 to + 20 %**
Repeatability (RSD_r)	**< 20 %**	
Within-laboratory reproducibility (RSD_R)	**< 25 %**	**< 15 %**

Current EU regulations (4)



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Proficiency tests

EU-RL proficiency tests

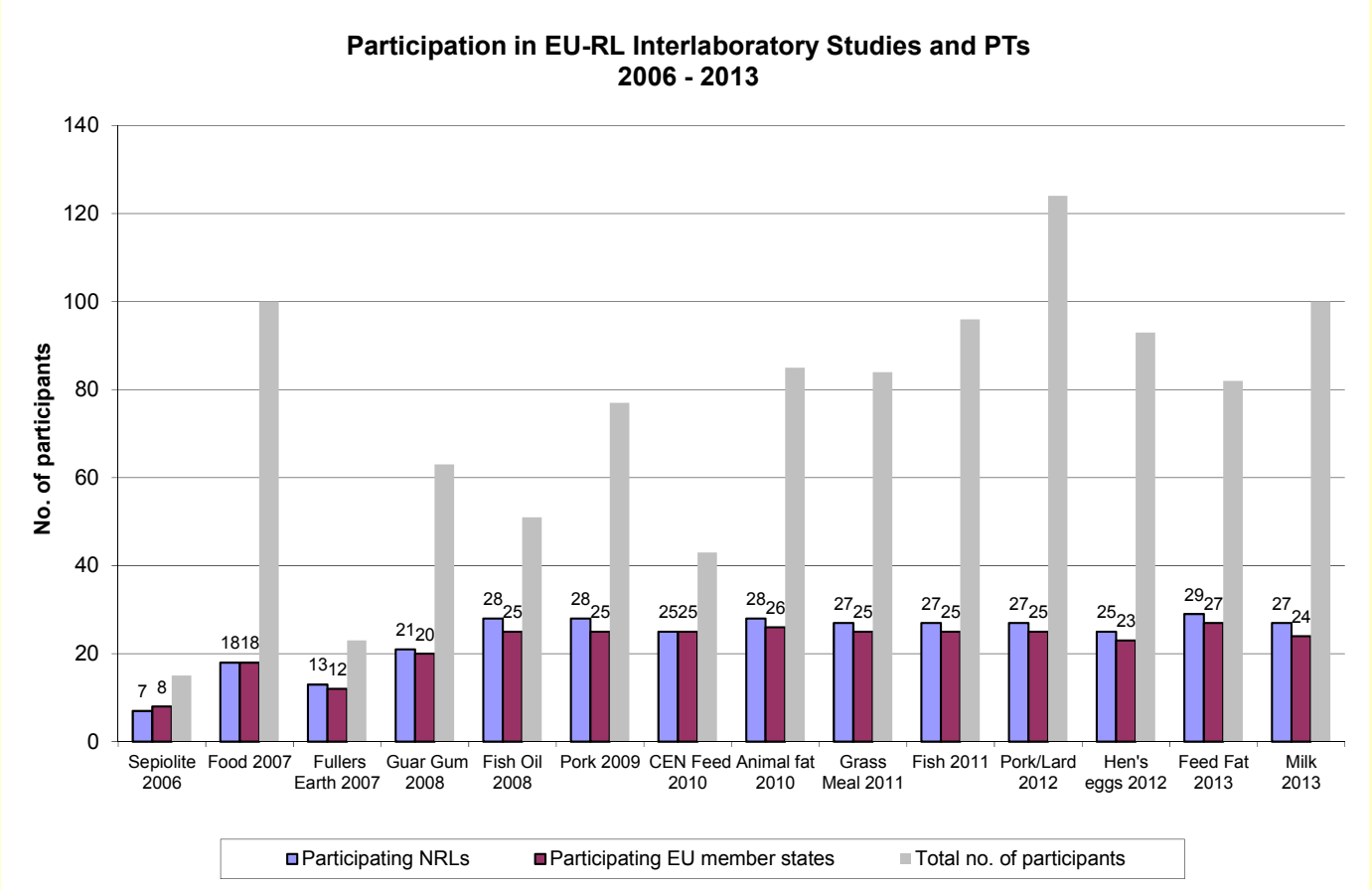
- One of the tasks of European Union Reference Laboratory (EU-RL) for Dioxins and PCBs in Feed and Food according to Regulation (EC) 882/2004:
  - **Organization of comparative tests** for National Reference Laboratories (NRLs) including appropriate follow-up
- PTs also open for official laboratories of EU member states and in certain cases also for commercial laboratories
- Organization and performance of PTs based on requirements of ISO/IEC 17043, ISO 13528 and IUPAC technical report on proficiency testing*
- Accreditation according to ISO/IEC 17043

*The international harmonized protocol for the proficiency testing of analytical chemistry laboratories" (IUPAC) Technical Report), Pure Appl. Chem, Vol. 78, No. 1, pp-145-196, 2006

Overview of EU-RL proficiency tests

- **15 Interlaboratory studies and proficiency tests performed between 2006 and 2014**
  - Sepiolite 2014 (preliminary results available)
  - Milk 2013
  - Feed Fat 2013
  - Hen‘s Eggs 2012
  - Pork sausage / lard 2012
  - Fish / fish oil 2011
  - Grass meal 2011
  - Animal fat 2010
  - *CEN PT 2010* (organized by RIKILT – Institute of Food Safety, participation of NRLs)
  - Canned Pork sausage 2009
  - Fish oil 2008
  - Guar Gum 2008
  - Fullers Earth 2007
  - *Dioxins in Food 2007* (organized by Norwegian Institute of Public Health, participation of NRLs)
  - Sepiolite 2006

Participation



Analytes of interest

- WHO-PCDD/F-PCB-TEQ (upper, middle and lower bound)
- WHO-PCDD/F-TEQ (upper, middle and lower bound)
- WHO-PCB-TEQ (upper, middle and lower bound)
- Sum of six indicator PCBs (upper, middle and lower bound)

4 sum parameters

- 17 2,3,7,8-substituted PCDD/Fs
- 12 dioxin-like PCBs
- 6 Indicator PCBs (# 28, 52, 101, 138, 153, 180)

35 individual congeners

- Total-BEQ, PCDD/F-BEQ, PCB-BEQ
  (bioanalytical screening methods)

3 BEQ sum parameters

- Lipid content, moisture content

Units: Depending on requirements in EU regulations

Methods of analysis

The following detection methods can be applied:

- **GC-HRMS** methods for PCDD/Fs and dioxin-like PCBs
- **GC-MS/MS** (or other alternative methods for GC-HRMS) for PCDD/Fs and dioxin-like PCBs
- **Bioanalytical screening methods** for PCDD/Fs and dioxin-like PCBs
- **Any kind of method** for indicator PCBs

Reporting of results

Laboratories applying **physico-chemical methods**:

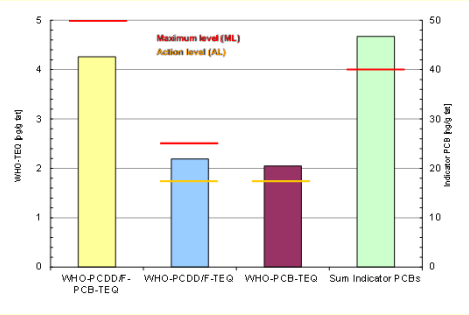
- Analytes of interest
- Indication, if test sample exceeds respective EU legal limits
- Measurement uncertainty

Laboratories applying **bioanalytical screening methods**:

- PCDD/F and DL-PCB results in bioanalytical equivalents (if applicable)
- Indication, if test sample is compliant or suspected to be noncompliant with EU legal limits and confirmation is required

Test material

- Preparation of sufficient amount of test material for proficiency test
  - Regular market food / feed:
    - **Naturally contaminated** material (fish, meat)
    - Material from **contamination incidents** (guar gum)
    - **Mixture** of contaminated and not contaminated material (hen‘s eggs)
    - **Spiking** of test material with standards, technical PCB mixtures (fat, milk powder)
- Test samples with **concentrations in the range of EU legal limits**, if possible
- Test for sufficient homogeneity performed for sum parameters and congeners



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Statistical evaluation
Assigned value

Evaluation according to **ISO 13528** and **IUPAC technical report**

Assigned values for congeners and sum parameters:

Consensus value, derived from participants' GC-MS,GC-ECD results:

- Huber robust mean after exclusion of extreme outliers (± 50 %)
- Examination of results using Histogram and Kernel density plot
- Calculation only if more than 2/3 of all reported results contributing

- **Sum parameters:**
  - Calculation of TEQ values on basis of concentrations of individual congeners (comparison with reported TEQ-values for plausibility check)

- **Individual congeners:**
  - Only for congeners with less than ⅓ of reported results below LOQ
  - Use of LOQ for evaluation, if concentrations for congeners not reported or below LOQ

Scoring of results

EU-RL for Dioxins and PCBs in Feed and Food

- **Z-scores**:

  $z = (x - x_a) / \sigma_p$

  x_a: assigned value

  x: participant's result

  σ_p: standard deviation for proficiency assessment

  WHO-TEQ: **10 %**

  Sum of indicator PCBs: **15 %**

  Evaluated individual congeners: **20 %**

- Defined criteria for standard deviation considerable stricter compared to the analytical criteria for trueness and precision as laid down in respective Commission Regulations for food and feed
  - **WHO-TEQ**: Trueness -20 to +20 %, Precision < 15 %
  - **Sum indicator PCBs**: Trueness -30 to +30 %, Precision ≤ 20 %

Standard deviation for proficiency assessment

- Definition of standard deviation by different providers:

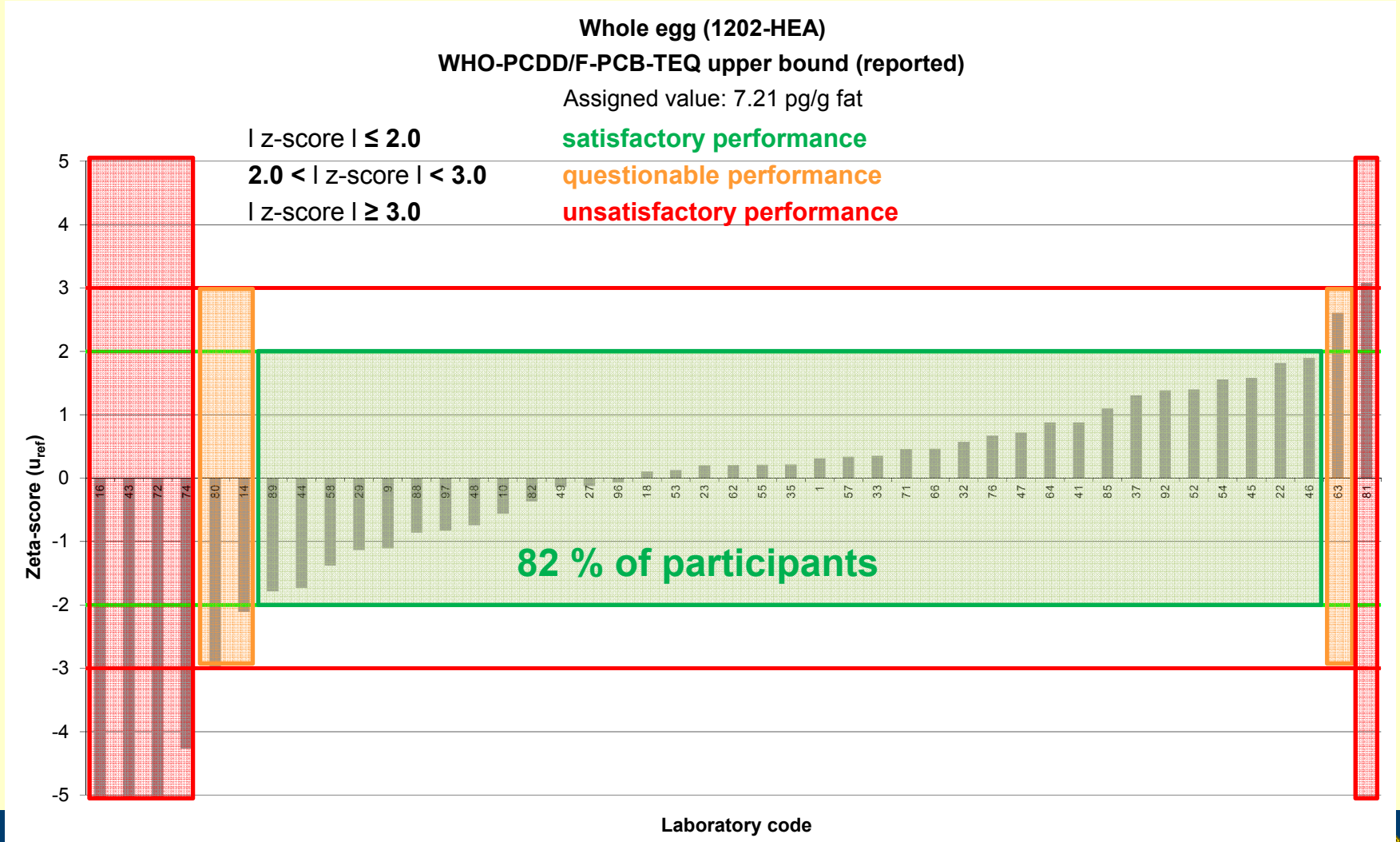
Provider	Interlaboratory study	Standard deviation
Bipea	PCB and dioxins in agri-food domain	**30 %**
Norwegian Institute of Public Health	Interlaboratory Comparisons on POPs in Food	**20 %**
FAPAS	Proficiency Tests Environmental Contaminants (PCBs and Dioxins)	**22 %**
Quasimeme	Laboratory Performance Studies	**12.5 %** + constant error
EU-RL for Dioxins and PCBs in feed and food	Proficiency Tests for food and feed	**10 %, 15 %** (sum parameters), **20 %** (congeners)

Evaluation of performance (1)

- Interpretation of z-scores (ISO/IEC 17043)

l z-score l **≤ 2.0**	**satisfactory performance**
2.0 < l z-score l **< 3.0**	**questionable performance** → "warning signal"
l z-score l **≥ 3.0**	**unsatisfactory performance** → "action signal"

Evaluation of performance (2)



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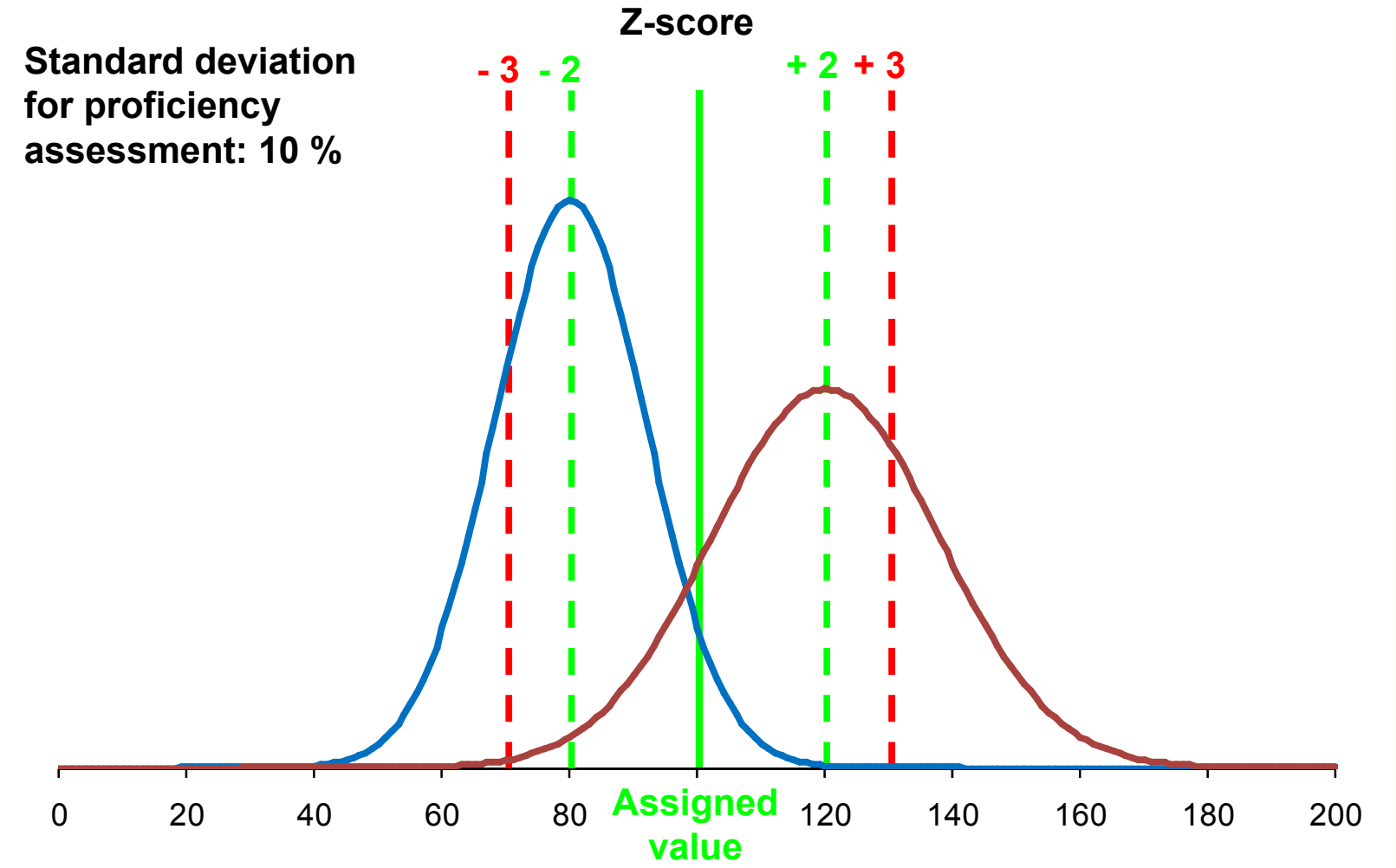
Scoring of results

EU-RL for Dioxins and PCBs in Feed and Food



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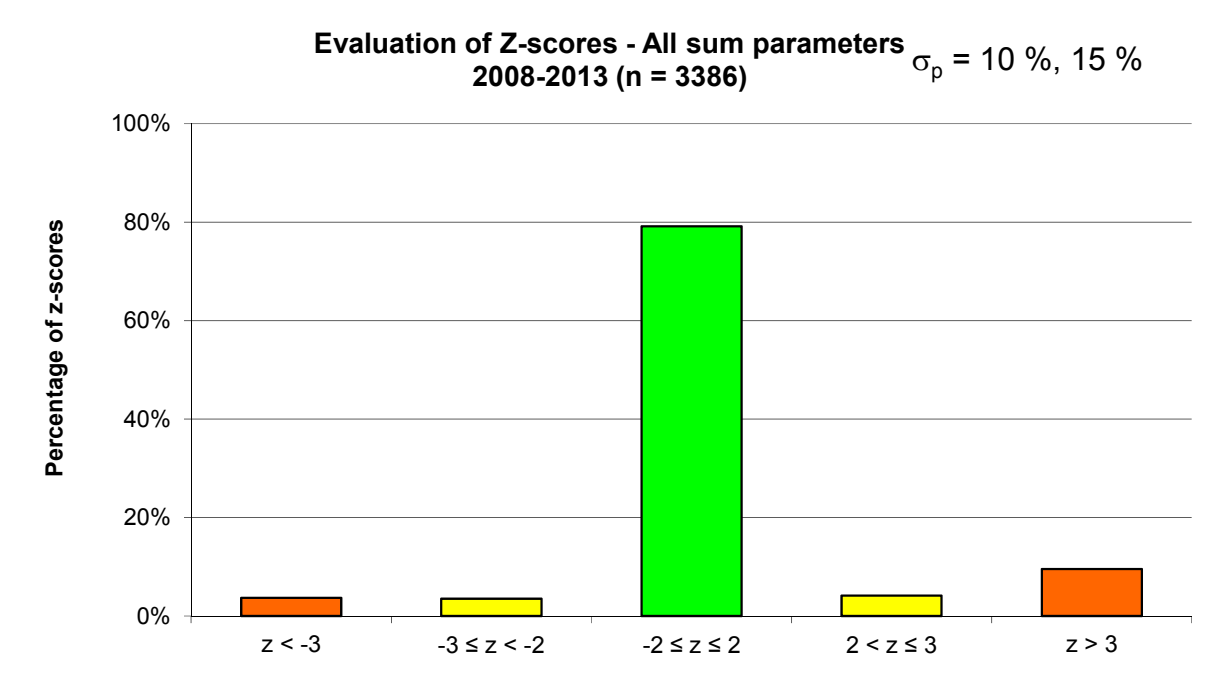
Evaluation of z-scores
Physico-chemical methods



- Percentage rate of z-scores for **10** PTs including **18** matrices
- In total **3386** matrix/analyte combinations

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Scoring system

- **„Positive scoring system“**
  - Developed within EURL/NRL network
  - **One assessment for each PT** sample covering all relevant sum parameters and congeners
  - Scoring system applicable for sum parameter concentrations in the range (about 0.5 to 4 times) of the level of interest (maximum or action level)

Positive scoring system (1)

- Principles:
  - Calculation of z-scores for sum parameters and evaluated individual congeners
  - Calculation of the **positive scores** according to:

Positive scoring system	I z-score I ≤ 2	2 < I z-score I ≤ 3	I z-score I > 3
Individual congeners	Positive score	Positive score	Positive score
Contribution to sum parameter* > 10 %	12	6	0
Contribution to sum parameter* 3 – 10 %	8	4	0
Contribution to sum parameter* < 3 %	6	3	0
Not evaluated congeners	0	0	0

*separately for the respective sum parameters WHO-PCDD/F-TEQ, WHO-PCB-TEQ and the sum of six indicator PCBs

Positive scoring system (2)

- **Calculations**:
  - Calculation of **maximum achievable scores** (I z-score I ≤ 2) for PCDD/F and DL-PCB and indicator PCB congeners separately:

    $$\textit{Maximum score} = \Sigma\textit{max. score}_{(> 10\ \%)} + \Sigma\textit{max. score}_{(3\text{-}10\ \%)} + \Sigma\textit{max. score}_{(< 3\ \%)}$$

  - Calculation of the **participant's scores** for PCDD/F and DL-PCB and indicator PCB congeners separately:

    $$\textit{Participant's score} = \Sigma\textit{score}_{(> 10\ \%)} + \Sigma\textit{score}_{(3\text{-}10\ \%)} + \Sigma\textit{score}_{(< 3\ \%)}$$

  - Calculation of achieved **scoring percentage** for each participant:

    $$\textit{Participant's scoring percentage} = \textit{Participant's score} / \textit{Maximum score} \bullet 100$$

Positive scoring system (3)

- Criteria for successful participation:

Sum parameters:	**≤ 1 parameter with l z-score l > 2, no parameter with l z-score l > 3**
PCDD/F congeners:	≥ 75 % of maximum score
DL-PCB congeners:	≥ 75 % of maximum score
Indicator PCB congeners:	≥ 75 % of maximum score

- **Assessment based on the positive scoring system performed for each PT test sample**
- **A laboratory participates successfully in a PT, if all above mentioned criteria for the reported analytes are met for each PT test sample**

Evaluation of results
Bioanalytical screening methods

*According to Commission Regulations (EU) No 278/2012 and 589/2014, "a **screening method** in principle **classifies** a sample as **compliant** or **suspected to be non-compliant**. For this, the calculated **BEQ level** is compared to the **cut-off value** […]. Samples below the cut-off value are declared compliant, samples equal or above the cut-off value as suspected to be non-compliant, requiring analysis by a confirmatory method."*

- **Main criterion** for evaluation of results from bioanalytical screening methods:
  - **Ability to reliably identify compliant samples and samples suspected to be non-compliant with established legal limits**

- Evaluation of test samples:
  - Comparison of assigned values with legal limits

Scoring of results
Bioanalytical screening methods

- **Bioassay-scores**:
  - Direct comparison of bioassay-scores and z-scores not possible (focus of bioanalytical screening methods on the identification of compliance or potential non-compliance of a sample)
  - Tool to assess method performance within the scope of external quality control measures

Bioassay-score = $(x - x_a) / \sigma_{bioassay}$

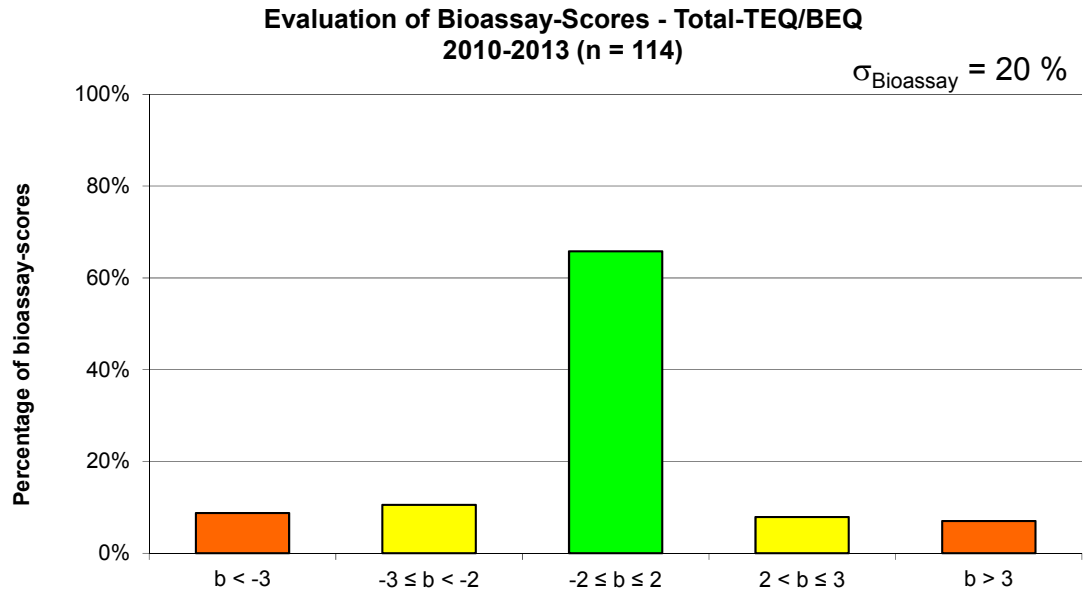
x_a: assigned value (results of physical-chemical methods)

x: participants result (BEQ from bioanalytical screening method)

$\sigma_{bioassay}$: bioassay target deviation (= 20 %)

Bioanalytical screening methods

Bioassay-scores

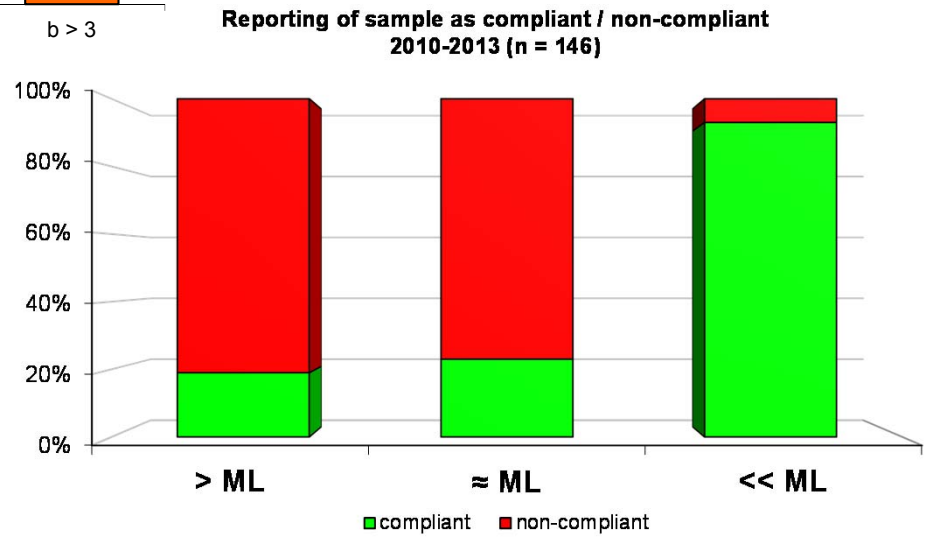


Evaluation of **6** PTs (10 (12) matrices)

- **114** matrix/analyte combinations for Total-TEQ/BEQ
- **146** assessments of analytical results

Assessment of analytical results:

- For concentrations **above** or in the range of **maximum levels** about **80 %** of participants report "**non-compliant**"
- For concentrations **below ML** more than **90 %** report **compliant**



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Further assessment of results

Assessment of analytical results
Regulation

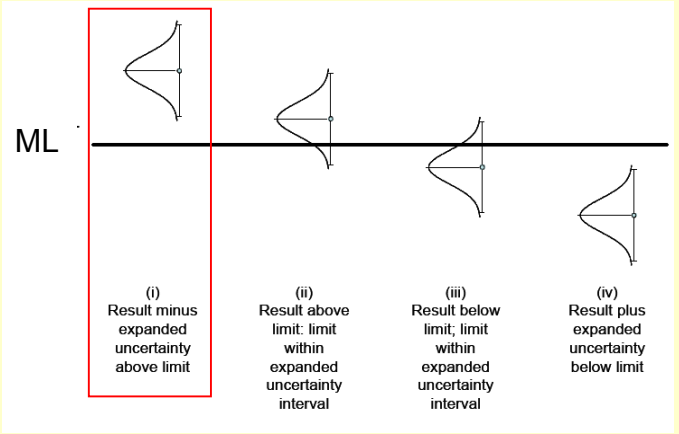
Compliance with legal limits (Commission Regulations (EU No 278/2012 and 589/2014)

- The lot is **accepted**, if the result of a single analysis performed by a confirmatory method does **not exceed the respective maximum level** […] taking into account the measurement uncertainty.
- The lot is **non-compliant** with the maximum level […], if the upperbound analytical result obtained with a confirmatory method and confirmed by duplicate analysis, **exceeds the maximum level** beyond reasonable doubt taking into account the measurement uncertainty.
  [Com.Reg. (EU) No 589/2014: The mean of two determinations is used for verification of compliance.]

- The **measurement uncertainty** may be taken into account according to one of the following approaches:
  - by calculating the **expanded uncertainty**, using a coverage factor of 2 which gives a level of confidence of approximately 95 %.
  - by establishing the **decision limit (CCα)** according to the provisions of Decision 2002/657/EC

Assessment of analytical results
Physico-chemical methods

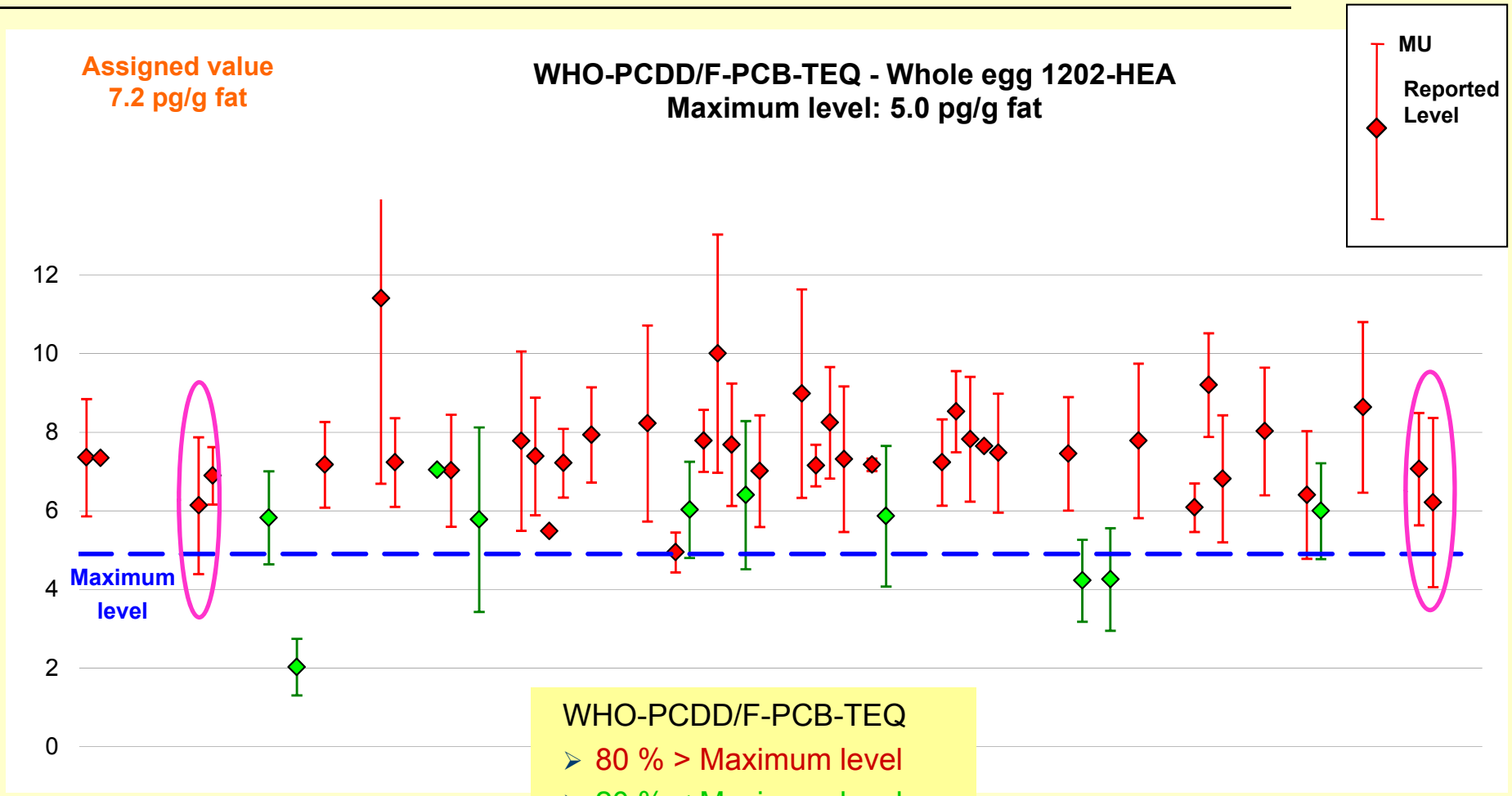
- Comparison of reported concentrations for sum parameters with respective EU legal limits
- Application of **measurement uncertainty** to analytical result
- Is the estimation of the measurement uncertainty realistic?
  - Comparison of the reported results including measurement uncertainty with assigned value
  - Comparison of uncertainty estimate with reproducibility standard deviation for collaborative trial
  - E_n-number and Zeta(ζ)-score



Assessment of Compliance with an Upper Limit (Eurachem/CITAC Guide: Use of uncertainty information in compliance assessment)

Assessment of analytical results

Comparison with legal limits



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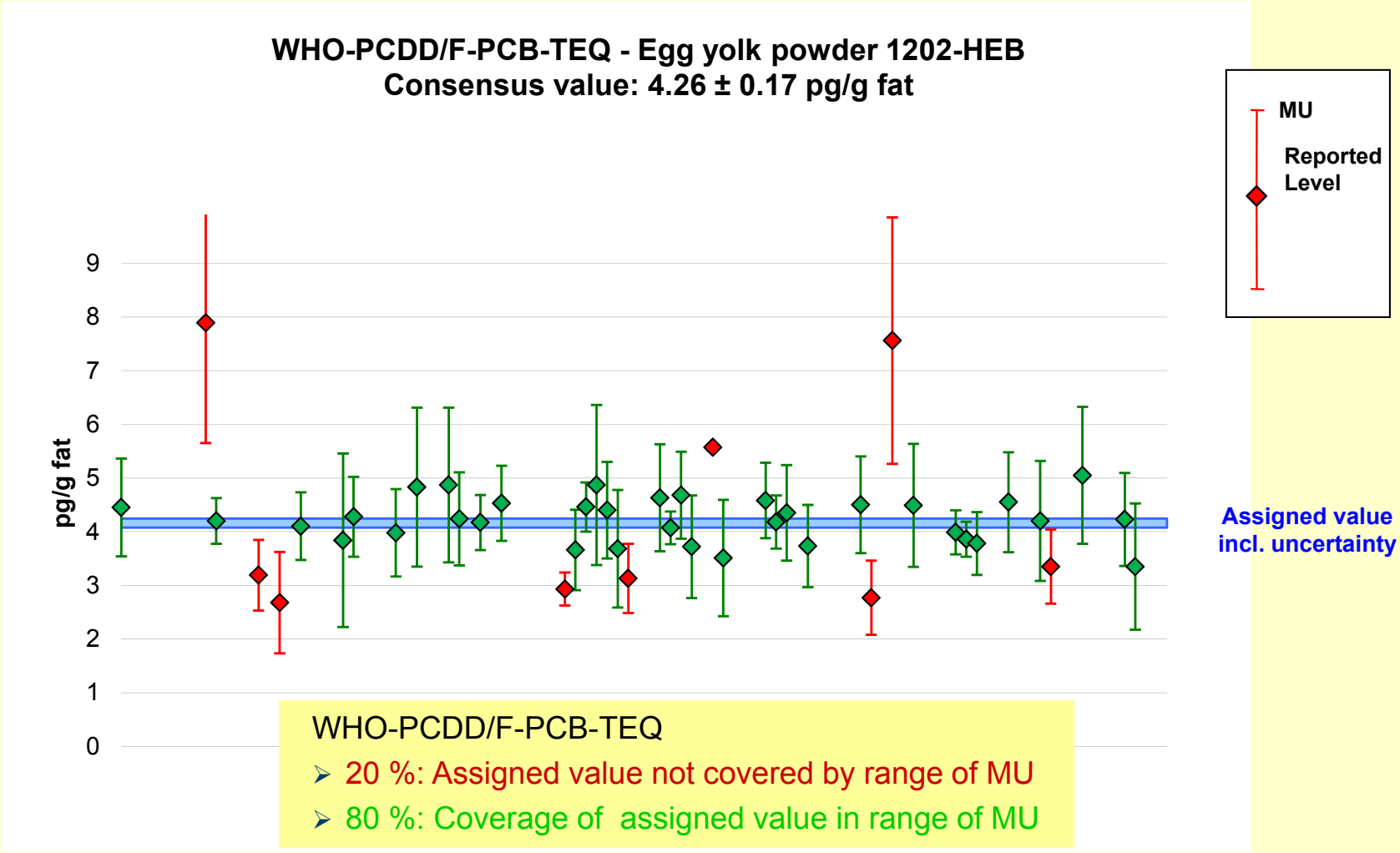
Comparison of MU and assigned value



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Scoring of results with uncertainty (1)

E_n-number and Zeta(ζ)-score

- E_n-number:

$$E_n = \frac{x_{lab} - xa}{\sqrt{U_{lab}{}^2 + U_{av}{}^2}}$$

x_a: assigned value
x_{lab}: participants result
U_{lab}: expanded uncertainty of participant's result
U_{av}: expanded uncertainty of assigned value

- Use of expanded uncertainties
- Use of 1 as critical value for E_n-numbers

- Zeta(ζ)-score:

$$\zeta = \frac{x_{lab} - xa}{\sqrt{u_{lab}{}^2 + u_{av}{}^2}}$$

x_a: assigned value
x_{lab}: participants result
u_{lab}: combined standard uncertainty of part.'s result
u_{av}: standard uncertainty of assigned value

- Use of standard uncertainties
- Critical values for ζ-scores comparable to z-scores

[ISO/IEC 17043, ISO 13528]

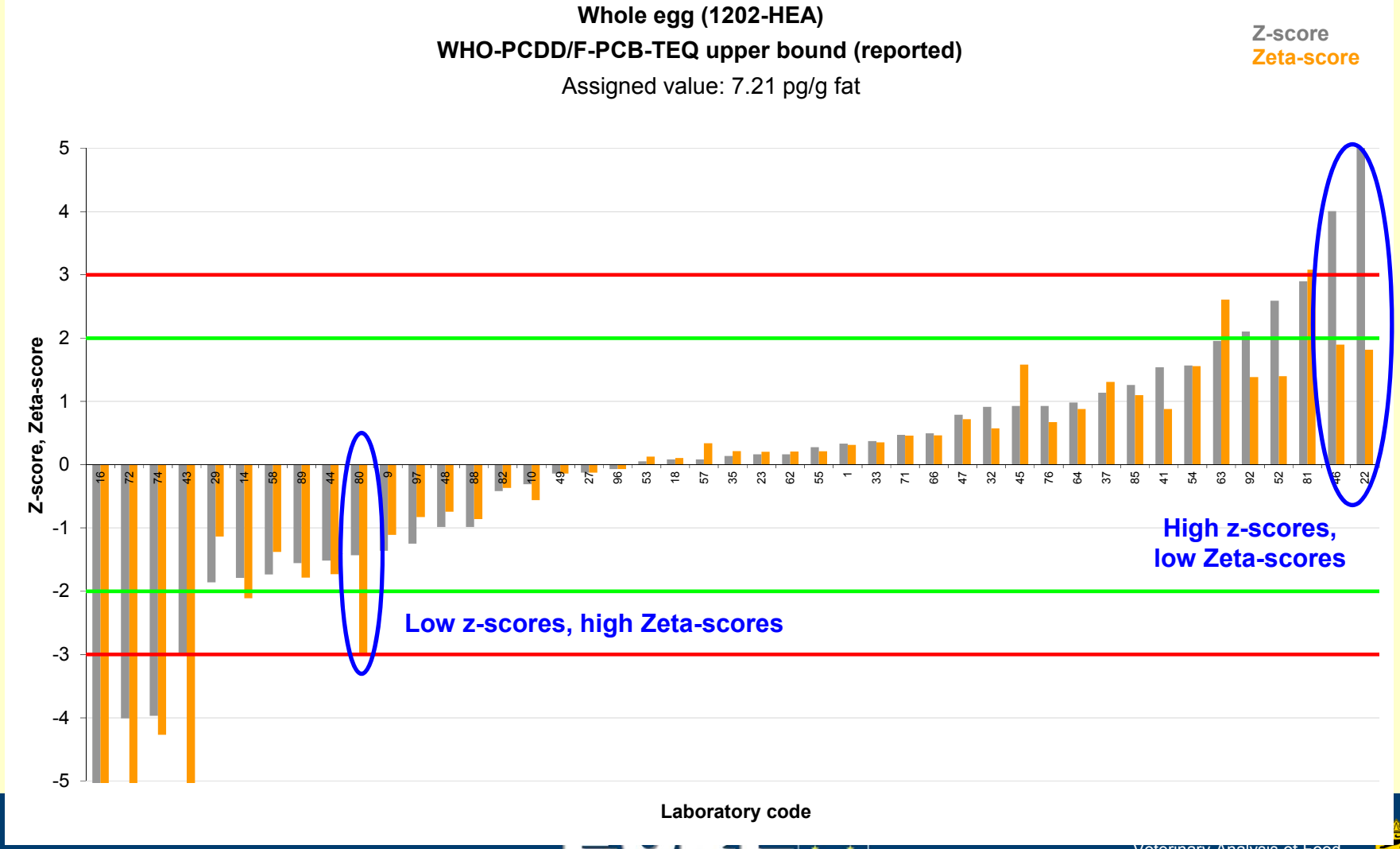
Scoring of results with uncertainty (2)

E_n-number and Zeta(ζ)-score

- E_n-number and Zeta(ζ)-score provide indication, if applied measurement uncertainty consistent with deviation from assigned value
- Useful only in conjunction with z-scores
- Tool for participants to check own estimates of uncertainty

- For evaluation in PT only meaningful, if uncertainty estimates determined in consistent manner by all participants
- Calculations correct only if x_{lab} and x_{av} independent
  - in principle not applicable for use of consensus values of all participants

Comparison
Z-score – Zeta-score



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Summary

- Evaluation of results of EU-RL PTs based on international standards and IUPAC-protocol
- Evaluation of performance of participants based on ...
  - Deviation of participants‘ results from assigned values
  - Assessment of analytical results using physico-chemical and bioanalytical screening methods
- Evaluation of application of measurement uncertainty

- Criteria for evaluation of performance of results stricter than analytical criteria for trueness and precision as laid down in Commission Regulations (EU) 589/2014 and 278/2012

➢ **Approach supports attempt to demonstrate and maintain the required high analytical quality of European NRLs**

References

- ISO/IEC 17043: Conformity assessment – General requirements for proficiency testing
- ISO 13528: Statistical methods for use in proficiency testing by interlaboratory comparisons
- The international harmonized protocol for the proficiency testing of analytical chemistry laboratories" (IUPAC) Technical Report), Pure Appl. Chem, Vol. 78, No. 1, pp-145-196, 2006
- IUPAC, NOMENCLATURE OF INTERLABORATORY ANALYTICAL STUDIES, Pure & Appl. Chern., Vol. 66, No. 9, pp. 1903-191 1, 1994
- EA-4/18 TA :2010– Guidance on the level and frequency of proficiency testing participation, European co-operation for Accreditation
- AMC Technical Brief No. 2: The z_L-score--combining your proficiency test results with your own fitness for purpose criterion
- AMC Technical Brief No. 11: Understanding and acting on scores obtained in proficiency testing schemes
- AMC Technical Brief No. 15: Is my uncertainty estimate realistic?
- AMC Technical Brief No. 16: Proficiency testing: assessing z-scores in the longer term
- AMC Technical Brief No. 18a: What is proficiency testing? A guide for end-users of chemical data

[http://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/TechnicalBriefs.asp]

Thank you very much for your attention !