European Union Reference Laboratory for **Feed** Additives (EURL-FA): Experience from 11 years organising proficiency testing

26–27 November 2024, Berlin, BFR

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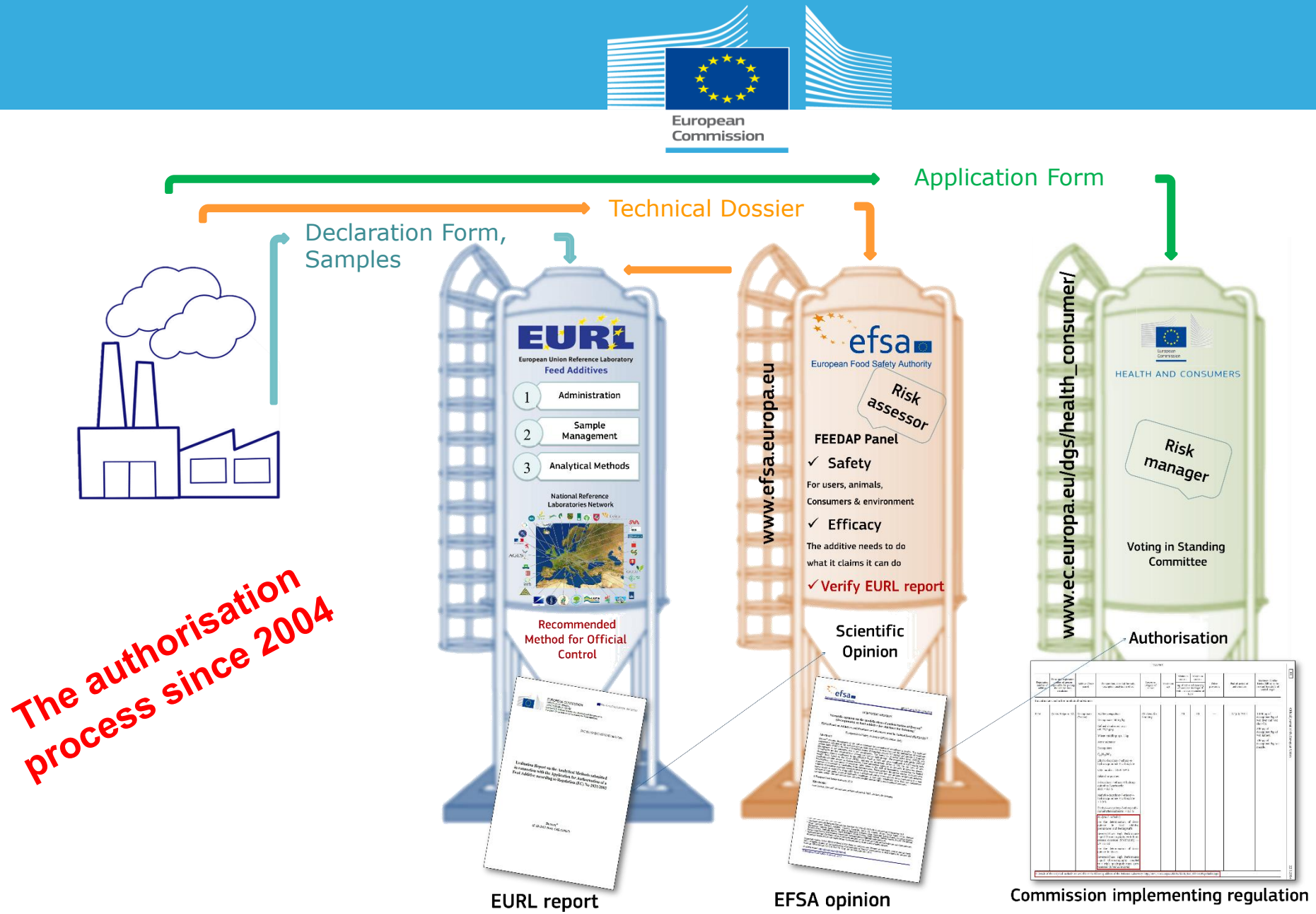
The EURL-FA: Two tasks

Support the authorisation of feed additives by evaluating analytical methods submitted by the industry

Organising proficiency testings and training courses for National Reference Laboratories


European Commission
Application Form
Technical Dossier
Declaration Form, Samples
EURL
European Union Reference Laboratory
Feed Additives
1 Administration
2 Sample Management
3 Analytical Methods
National Reference Laboratories Network
Recommended Method for Official Control
www.efsa.europa.eu
efsa
European Food Safety Authority
Risk assessor
FEEDAP Panel
✓ Safety
For users, animals, Consumers & environment
✓ Efficacy
The additive needs to do what it claims it can do
✓ Verify EURL report
Scientific Opinion
www.ec.europa.eu/dgs/health_consumer/
HEALTH AND CONSUMERS
Risk manager
Voting in Standing Committee
Authorisation
The authorisation process since 2004
EURL report
EFSA opinion
Commission implementing regulation
Joint Research Centre

Analytical methods: Requirements for the applicant

Cascade approach:

1. Union methods
2. CEN/ISO method etc
3. *Single lab validated method*

Second laboratory verification

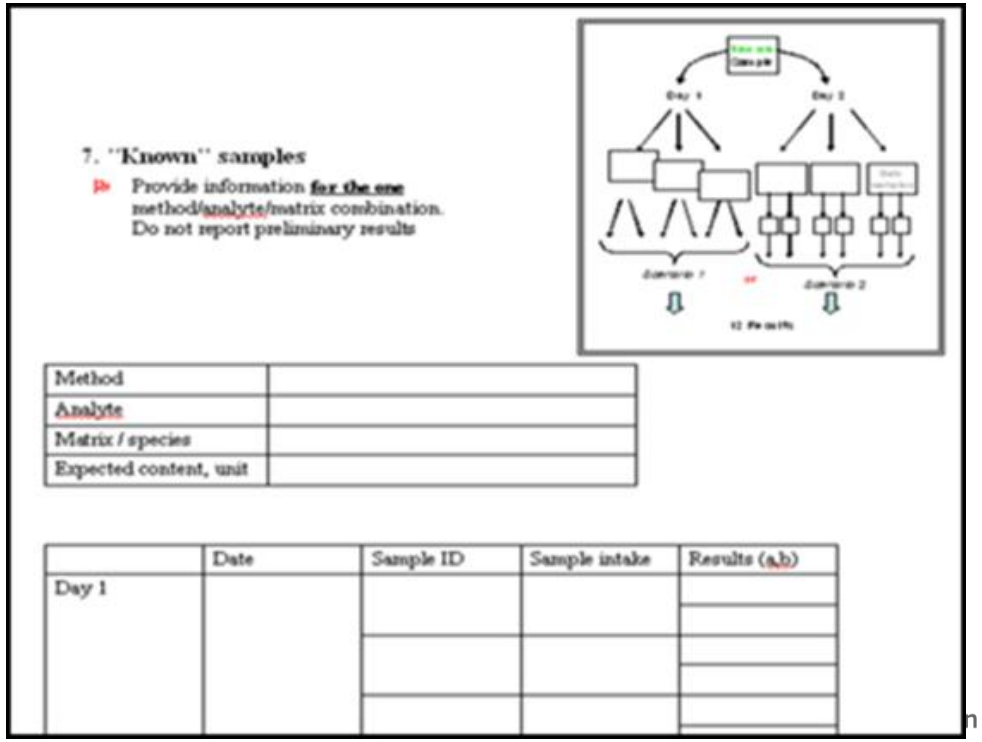
- SOP according to ISO 78-2
- In-house validation: Method performance
- Second laboratory verification

EUROPEAN COMMISSION
DIRECTORATE GENERAL
JOINT RESEARCH CENTRE
Directorate D: Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

EURL

Working document

EURL-FA
Verification form



7. "Known" samples

- Provide information for the one method/analyte/matrix combination. Do not report preliminary results

Method	
Analyte	
Matrix / species	
Expected content, unit	

	Date	Sample ID	Sample intake	Results (a,b)
Day 1				

Authorisation regulation: Example

Identification number of the additive	Name of the holder of authorisation	Additive (Trade name)	Composition, chemical formula, description, analytical method	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	End of period of authorisation	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin
						mg of active substance/kg of complete feedingstuff with a moisture content of 12 %				
Coccidiostats and histomonostats										
5 1 771	Janssen Pharmaceutica N.V.	Diclazuril 0,5 g/100 g (Clinacox 0,5 %)	*Additive composition* Diclazuril: 0,50 g/100 g. Protein-poor soybean meal: 99,25 g/100 g Polyvidone K 30: 0,20 g/100 g Sodium hydroxide: 0,05 g/100 g *Characterisation of the active substance* Diclazuril, $C_{17}H_9Cl_3N_4O_2$, (±)-4-chlorophenyl[2,6-dichloro4-(2,3,4,5-tetrahydro-3,5-dioxo-1,2,4-triazin-2-yl)phenyl]acetonitrile, CAS number: 101831-37-2 Related impurities: Degradation compound (R064318): ≤ 0,1 % Other related impurities (T001434, R066891, R068610, R070156, R070016): ≤ 0,5 % individually Total impurities: ≤ 1,5 % *Analytical method* (1) For determination of diclazuril in feed: reversed-phase high performance liquid chromatography (HPLC) using Ultraviolet detection at 280 nm (Regulation (EC) No 152/2009) For determination of diclazuril in poultry tissues: HPLC coupled to triple quadrupole mass spectrometer (MS/MS) using one precursor ion and two product ions	Guinea fowls	—	1	1	1. The additive shall be incorporated in compound feed in form of a premixture. 2. Diclazuril shall not be mixed with other coccidiostats. 3. For safety: breathing protection, glasses and gloves shall be used during handling. 4. The holder of the authorisation shall carry out a post-market monitoring programme on the resistance to bacteria and *Eimeria* spp.	16 March 2021	1 500 µg diclazuril/kg of wet liver 1 000 µg diclazuril/kg of wet kidney 500 µg diclazuril/kg of wet muscle 500 µg diclazuril/kg of wet skin/fat

(1) Details of the analytical methods are available at the following address of the Community Reference Laboratory: www.irmm.jrc.be/crl-feed-additives

Evaluation of results from proficiency testing (PTs)

The actual pupose of PTs is to evaluate the proficiency of laboratories

Here we use the results to gain even more information from PTs

4 Examples are given

The z-score: Some key aspects

Reference: ISO 13528

$$z = \frac{(x_i - x_{pt})}{\sigma_{pt}}$$

x_i	Result reported by laboratory i
x_{PT}	Assigned value
σ_{PT}	Standard deviation for proficiency assessment reflecting the acceptable uncertainty of the results of the laboratories

Performance of *individual* laboratories:

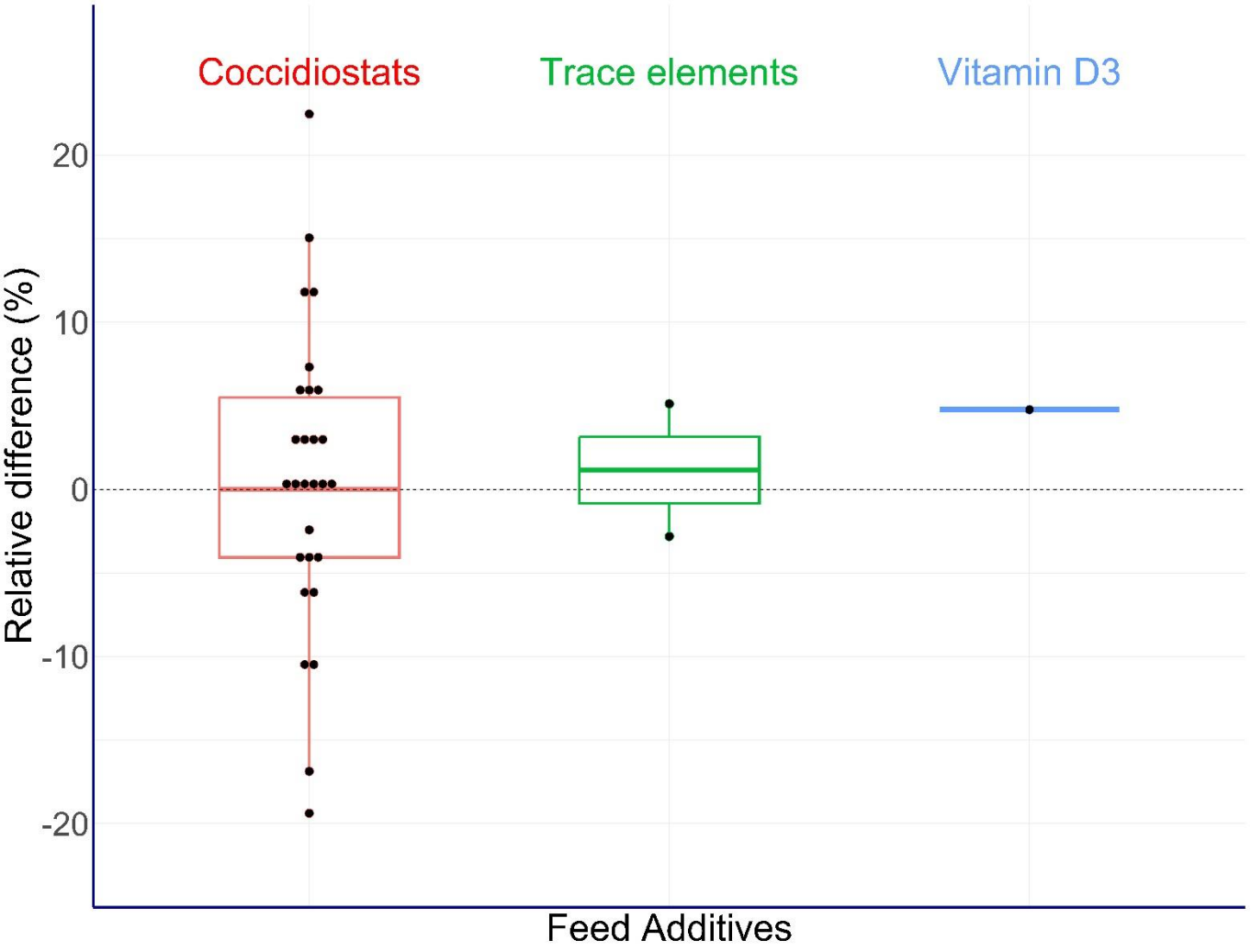
- A result that gives $|z| \leq 2{,}0$ is considered to be acceptable.
- A result that gives $2{,}0 < |z| < 3{,}0$ is considered to give a warning signal.
- A result that gives $|z| \geq 3{,}0$ is considered to be unacceptable (or action signal)

Assigned value and average bias of laboratories

- Assigned value from *external* sources
- Bias assement: Comparison of robust mean of laboratories with assigned value

Feed additve	x_{pt}
Coccidiostats	Formulation (spiking with solution of pure substances)
Vitamin D_3	Formulation (mixing with preparation)
Cobalt, Selenium	External laboratories using reference methods

Assigned value and bias of laboratories



$$Rel\_Diff\,(\%) = \frac{100 \times (x_{Rob} - x_{pt})}{x_{pt}}$$

Rel_Diff (%)= Relative difference

X_{Rob} = Robust mean of laboratories´ results

X_{pt} = Assigned value

Data basis

- Each dot reflects a PT for a single analyte
- 31 coccidiostats
- 2 trace elements
- 1 vitamin D_3

Results

- Overall average bias below 6%

European Commission

Test material with carotenoids: Via encapsulated products

Production of homegenous material: Do I have a sampling problem?

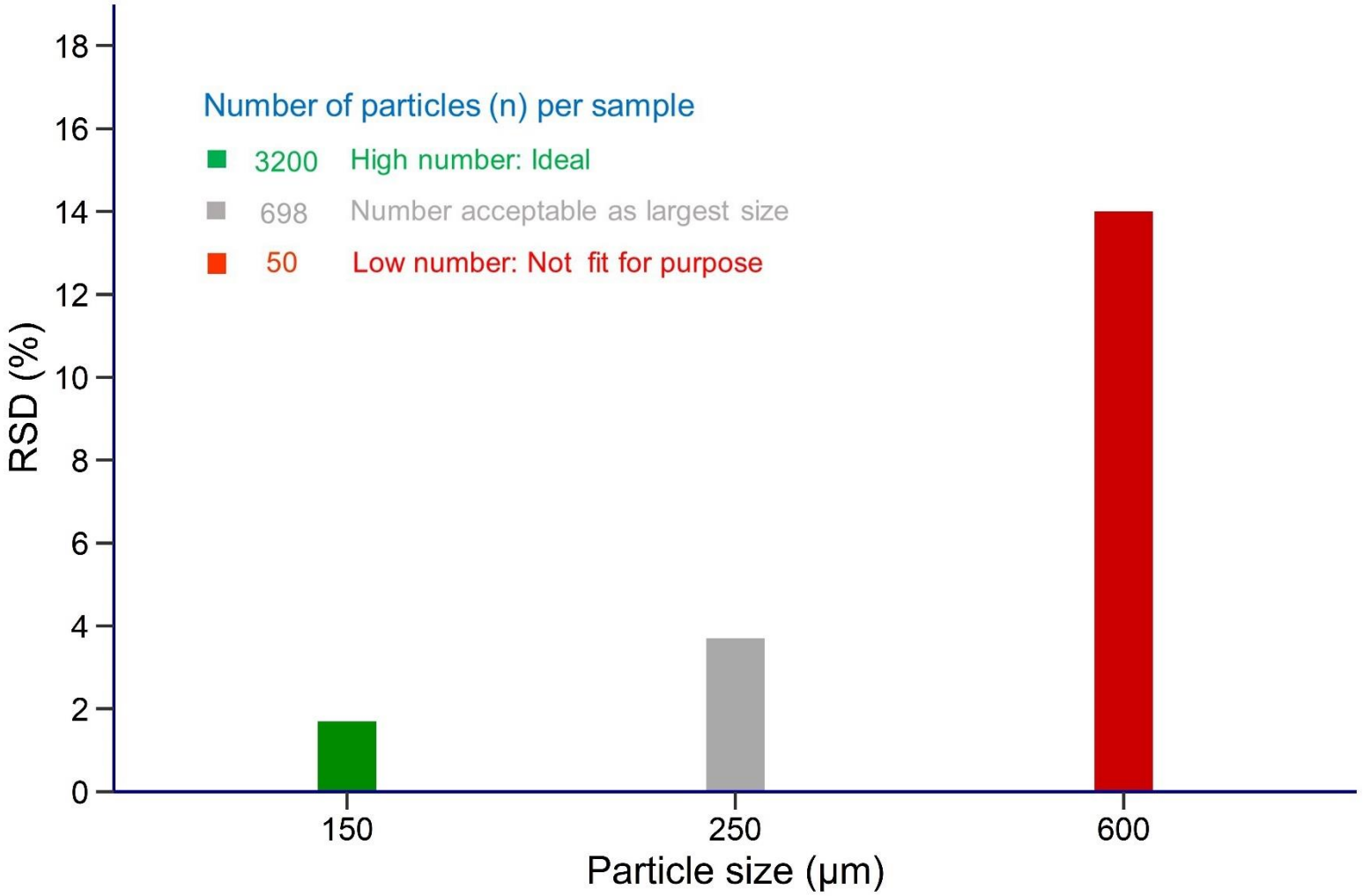
Mass fraction carotenoid in the product	10 %
Mass fraction carotenoid in feed	20 mg/kg
Required amount of the product in 20 g feed	4 mg

Estimation of sampling error (SE)

$$SE\ (\%) = 100 \times \frac{\sqrt{n}}{n}$$

Particle size (µm)	Absolute number in 20 g sample
600	16
250	129
150	3200

Product characteristics and target homogeneity



- Target relative standard deviation σ_{PT} : 10%
- Homogeneity criterion:

$$s_s \leq 0{,}3\ \sigma_{pt}$$

- Sieve fraction used: 150 to 250 µm
- Production by stepwise dilution
- Experimental study confirmed sufficient homogeneity

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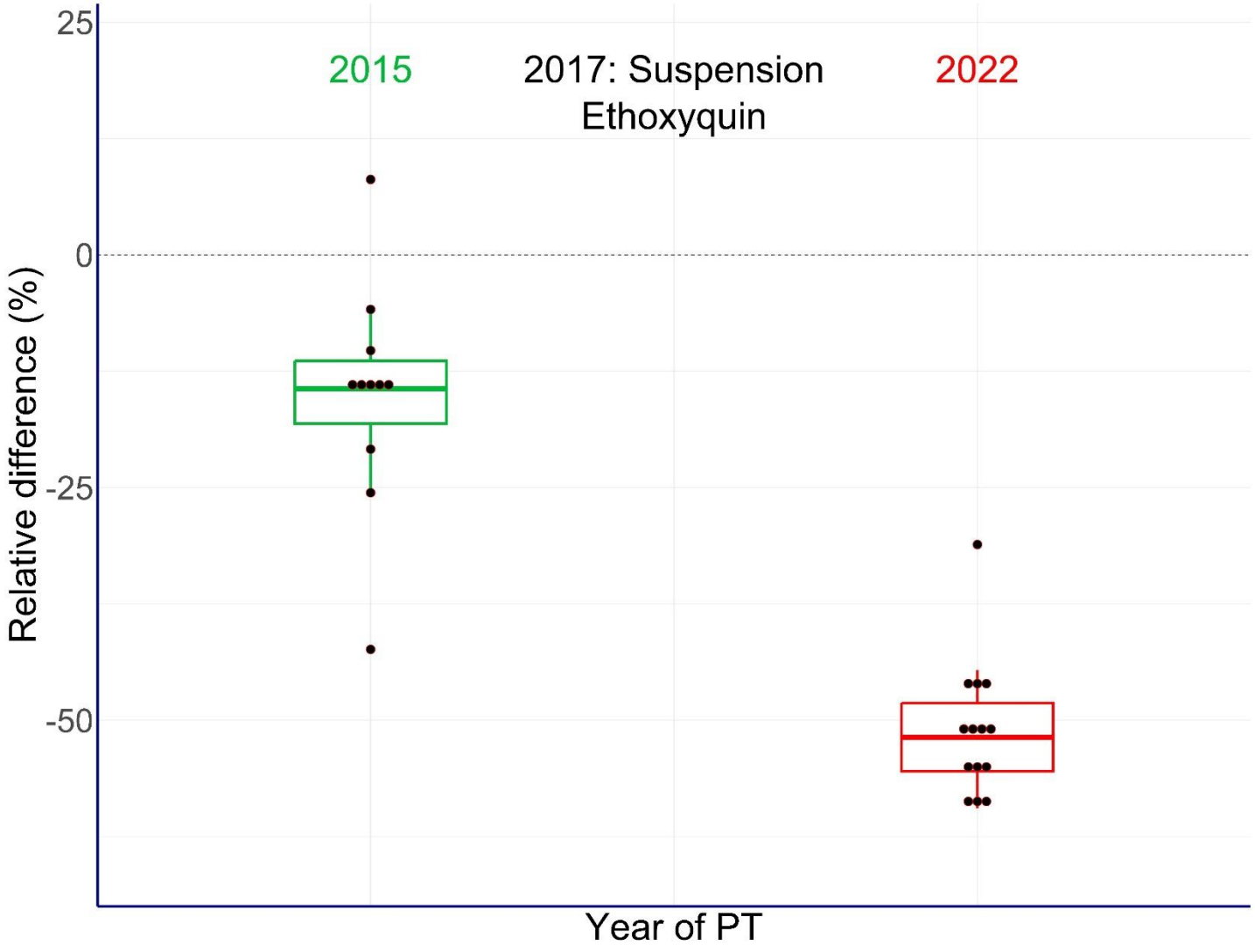
Ethoxyquin is an antioxidant

- Authorised for many years as feed additive as important antixidiant
- Helps stabilise **vitamin A** in preparation
- Contains mutagen p-phenetidine

2015/2022	EFSA cannot conclude on safe levels of ethoxyquin in feed
2017	The Commission suspended the authorisiation of ethoxyquin
2024	The Commission denies the authorisation of ethoxyquin

Is there a problem with vitamin A determination ?



$$Rel\_Diff(\%) = \frac{100 \times (x_{lab} - x_{label})}{x_{label}}$$

Rel_Diff (%)= Relative Difference (%)

X_{lab} = Result of laboratory

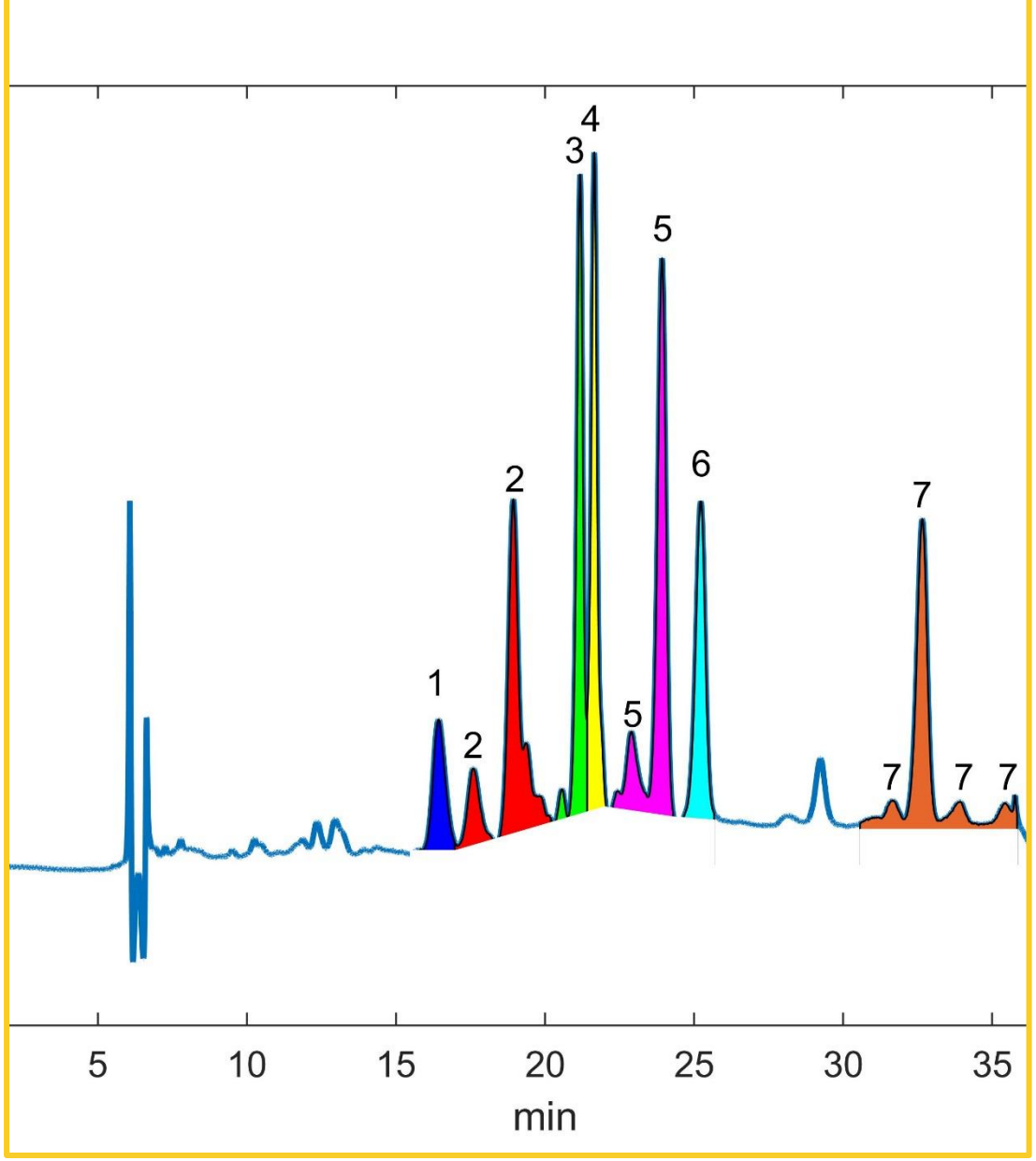
X_{label} = Vitamin A content on the label

- PT data used to simulate official control for label information
- Difference aboratories´results from labelled vitamin A level
  - 2015: low
  - 2022: Large
- Hypothesis: Stability problems linked to ban of ethoxyquin

European Commission

Determination of carotenoids

- Challenging task
- Very sensitive substances measured with LC-UV/DAD
- Multianalyte method for 10 authorised carotenoids
- Encapsulated products require enzymatic step
- Cis trans isomers
- Normal versus RP LC
- 2022 CEN method 17550
  - Isosbestic approach
  - RP LC method

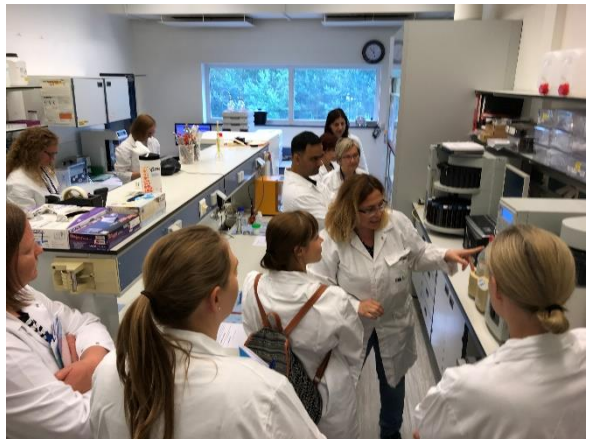


Training and NRLs: Effect shown by PTs

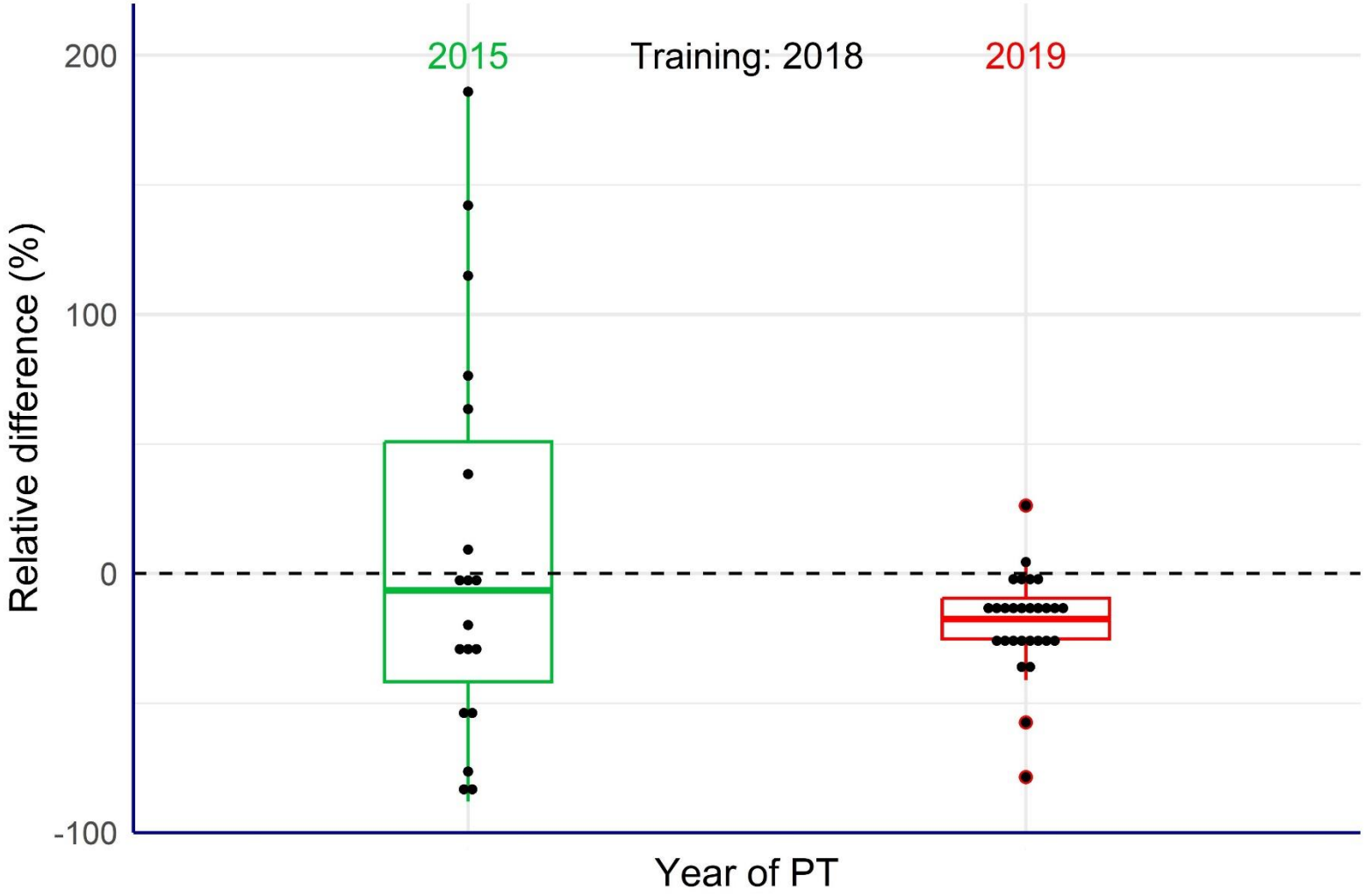
$$Rel\_Diff(\%) = \frac{100 \times (x_{lab} - x_{pt})}{x_{pt}}$$

2015 PT showed poor results

2018 EURL organised training



2019 PT showed significant improvement



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Key aspects

- Feed additives cover a large range of different products
- Likewise, analytical methods are quite different
- For important feed additives, Union methods or CEN standards are available
- Feed additive register: https://food.ec.europa.eu/food-safety/animal-feed/feed-additives_en
- EURL reports and methods: https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives_en
- If you have questions: christoph.von-holst@ext.ec.europa.eu