## CODEX COMMITTEE ON PESTICIDE RESIDUES

**Fortieth Session**

**Hangzhou, China, 14 - 19 April 2008**

# European Model for Pesticide Multiresidues Analysis: Experience Gained through European Proficiency Testing

**AMADEO R. FERNÁDEZ-ALBA**

Community Reference Laboratory

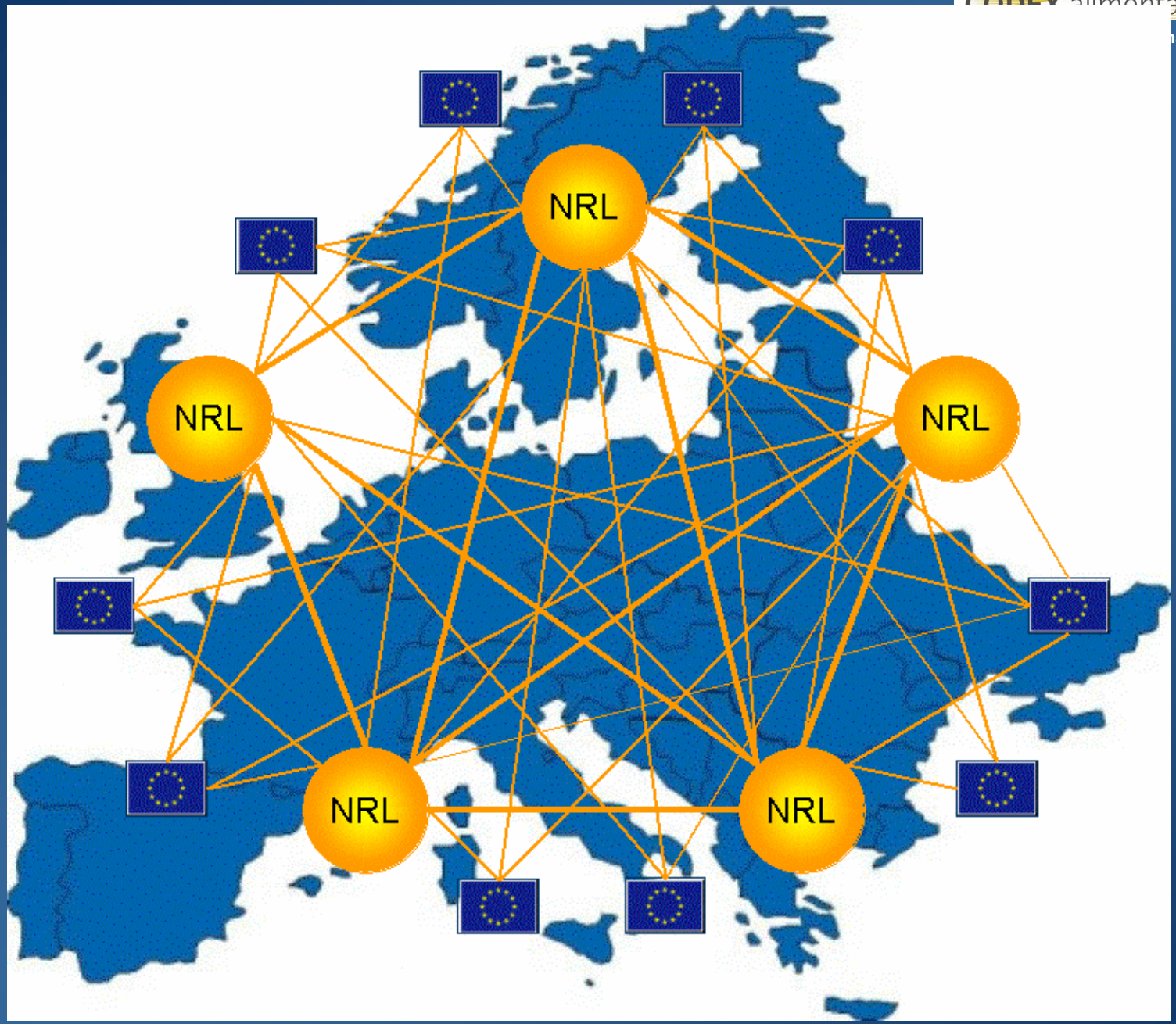
# overview

-EU model

-How EUPTs can support this model ?

-Quantifying Uncertainty

-Results


NRL
NRL
NRL
NRL
NRL

# CRL-Central Homepage: www.crl-pesticides.eu

2007
COMMUNITY REFERENCE LABORATORY
(CRL)

WORKSHOP
AND
TRAINING COURSE
FOR PESTICIDE RESIDUES
IN FOOD

Organized by:

CRL
Pesticides in Fruits And Vegetables
Community Reference Laboratory
DG SANCO
EUROPEAN COMMISSION

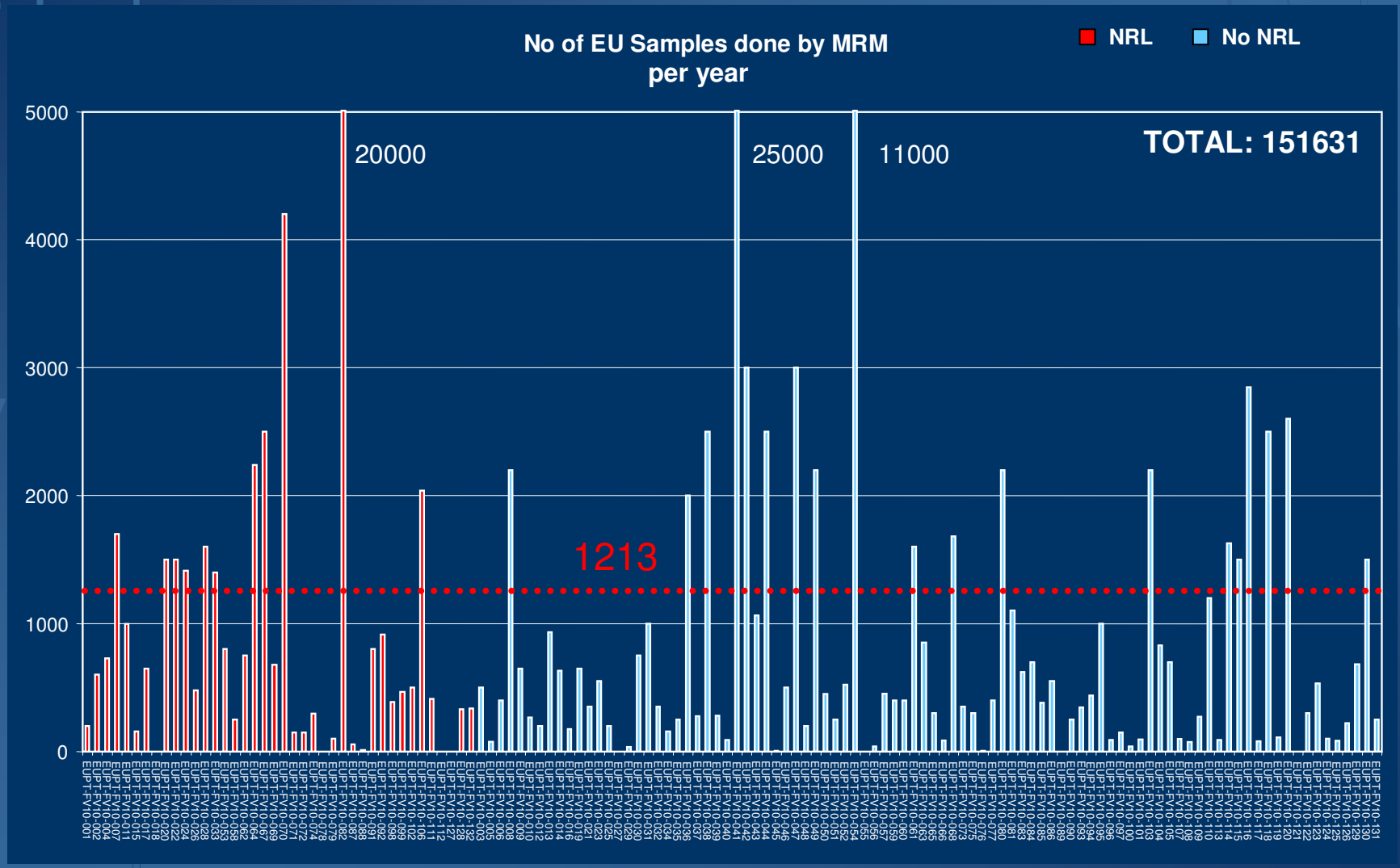
DG SANCO
EUROPEAN COMMISSION

26-29 September 2007
Valencia – SPAIN

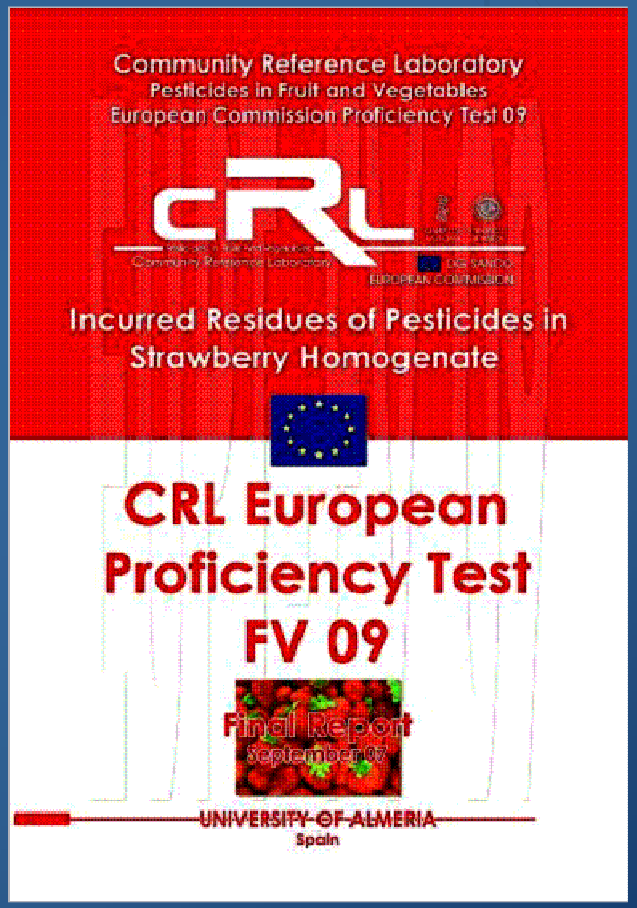
www.crl-pesticides.eu


# FIRST COMMUNITY REFERENCE LABORATORY (CRL) WORKSHOP AND TRAINING COURSE FOR PESTICIDE RESIDUES

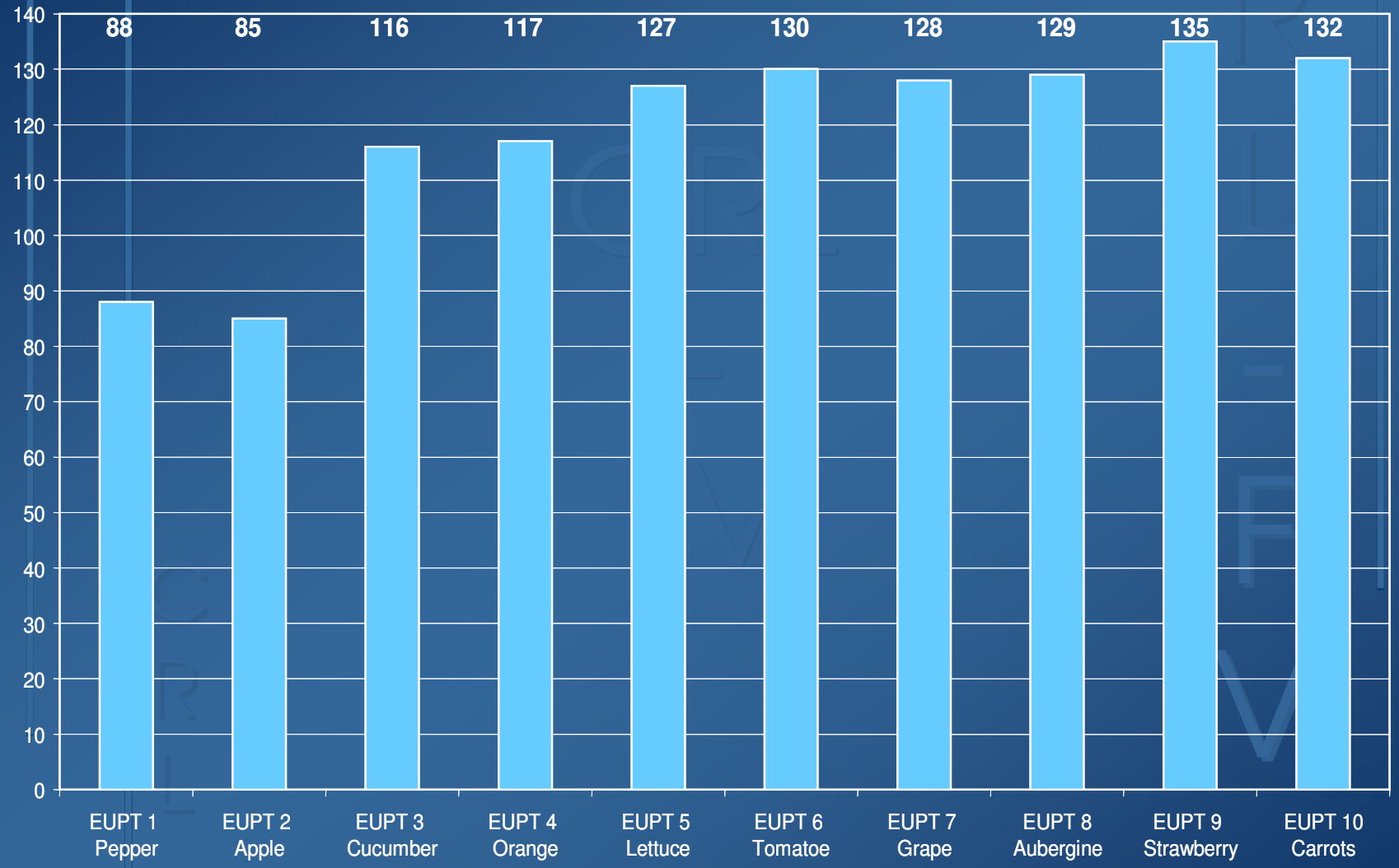
26-29 September 2007 Valencia – SPAIN


No of EU Samples done by MRM per year
NRL
No NRL
TOTAL: 151631
20000
25000
11000
1213
5000
4000
3000
2000
1000
0


Community Reference Laboratory
Pesticides in Fruit and Vegetables
European Commission Proficiency Test LC1
Incurred Residues of Pesticides in
Pear Homogenate
CRL European
Proficiency Test
FV LC1
Final Report
UNIVERSITY OF ALMERIA
Spain


Community Reference Laboratory
Pesticides in Fruit and Vegetables
European Commission Proficiency Test 09
Incurred Residues of Pesticides in
Strawberry Homogenate
CRL European
Proficiency Test
FV 09
Final Report
UNIVERSITY OF ALMERIA
Spain

## No of Participants throughout the EUPTs



## overview

- EU model
- How EUPTs can support this model ?
- Quantifying Uncertainty
- Results

# Harmonization

-Results ( quality + quantity)
- Acceptable Z-score
-MRRL ( 0.01 mg/k)
-Scope
-Anal. Response capability

# Advisory Group Members

## Organising Team at CRL-University of Almeria:

| | |
|---|---|
| Dr. Amadeo R. Fernández-Alba. senior Chemist. | University of Almeria |
| Ms. Paula Medina. Chemist. | University of Almeria |
| Mr. Octavio Malato. Chemist. | University of Almeria |
| Mr. Antonio V. Monterreal. Chemist. | University of Almeria |

## Advisory Group:

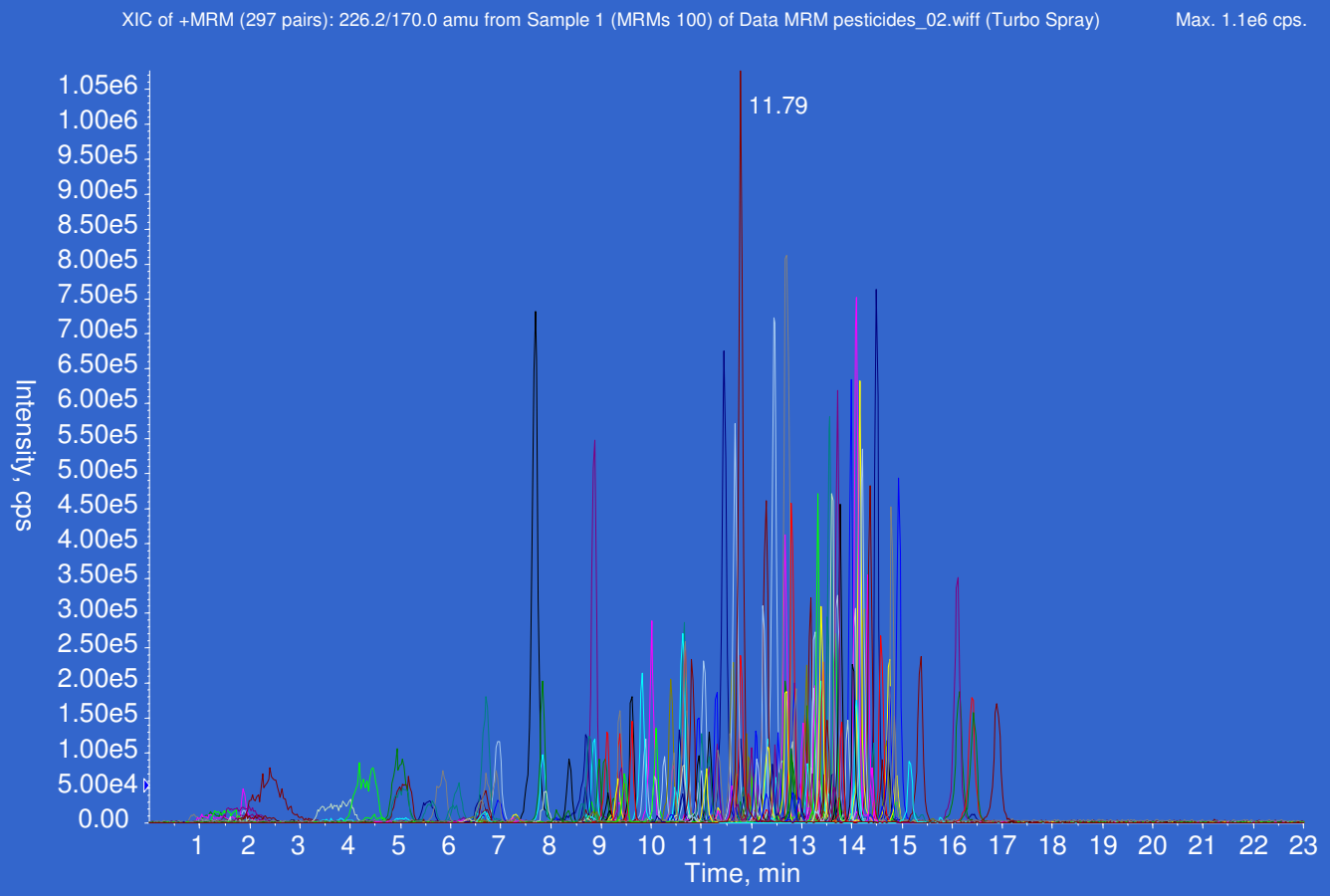
| | |
|---|---|
| Mr. Arne Andersson. Head of Division. | NFA. Uppsala. Sweden |
| Dr. Antonio Valverde García. Senior Chemist. | University of Almeria. Sain |
| Dr. Carmelo Rodríguez. Senior Chemist. | University of Almeria. Spain |
| Dr. Miguel Gamón. Senior Chemist. | Co-Head CRL-FV Generalitat Valenciana. Spain |
| Dr. Michelangelo Anastassiades. Senior Chemist. | CVUA Stuttgart. Germany |
| Dr. Mette Erecius Poulsen. Senior Chemist | Danish Institute for Food and Veterinary Research |
| Dr. André de Kok. Senior Chemist. | VWA. Amsterdam. The Netherlands |
| Dr. Tuija Pihlström. Senior Chemist. | NFA. Uppsala. Sweden |
| Mr. Stewart Reynolds. Senior Chemist. | CSL. York. United Kingdom |
| Dr. Ralf Lippold. Senior Chemist. | CVUA Freiburg. Germany |

# Harmonization

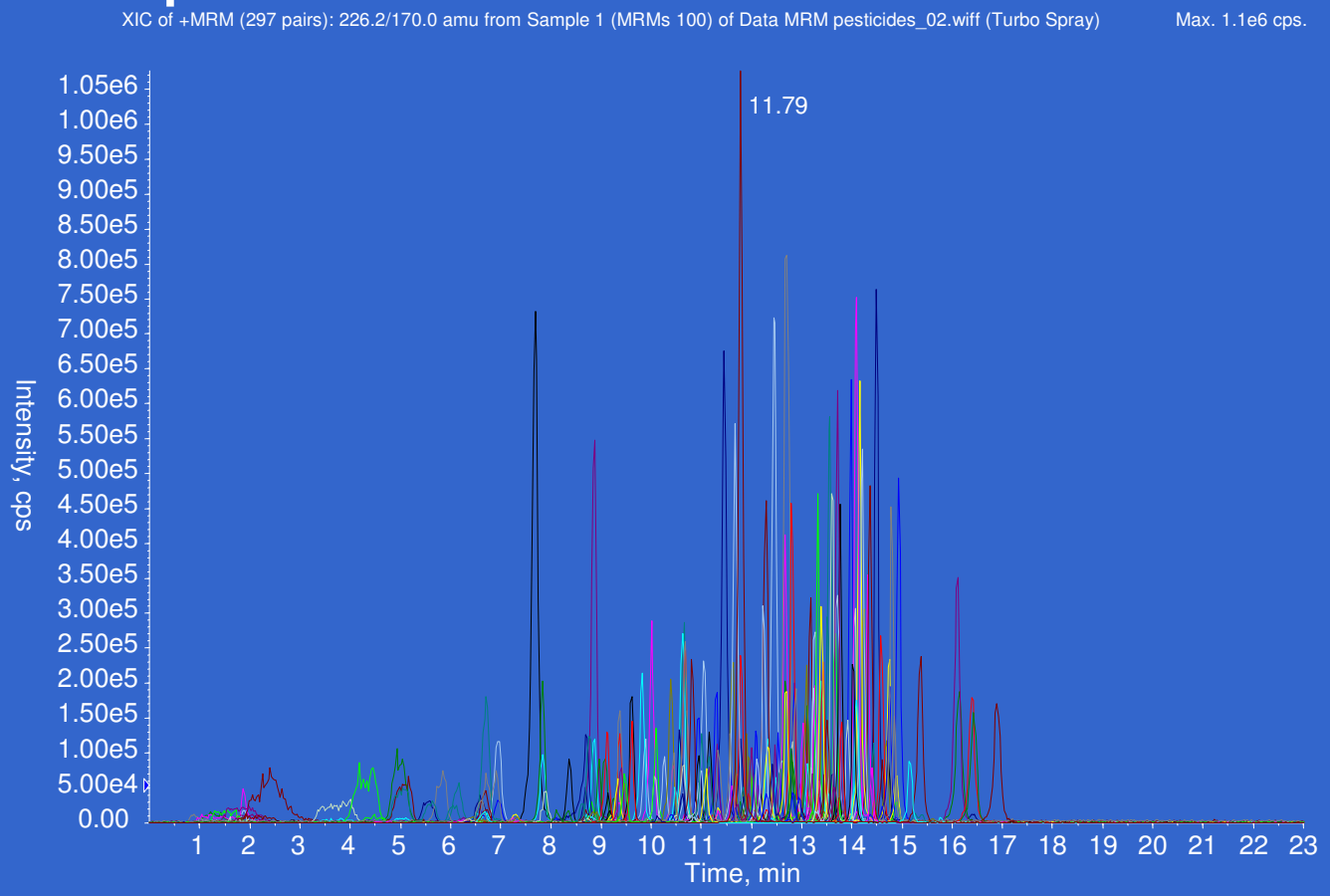
-Results ( quantity + quality)
- Acceptable Z-score
-MRRL ( 0.01 mg/k)
-Scope
-Anal. Response capability

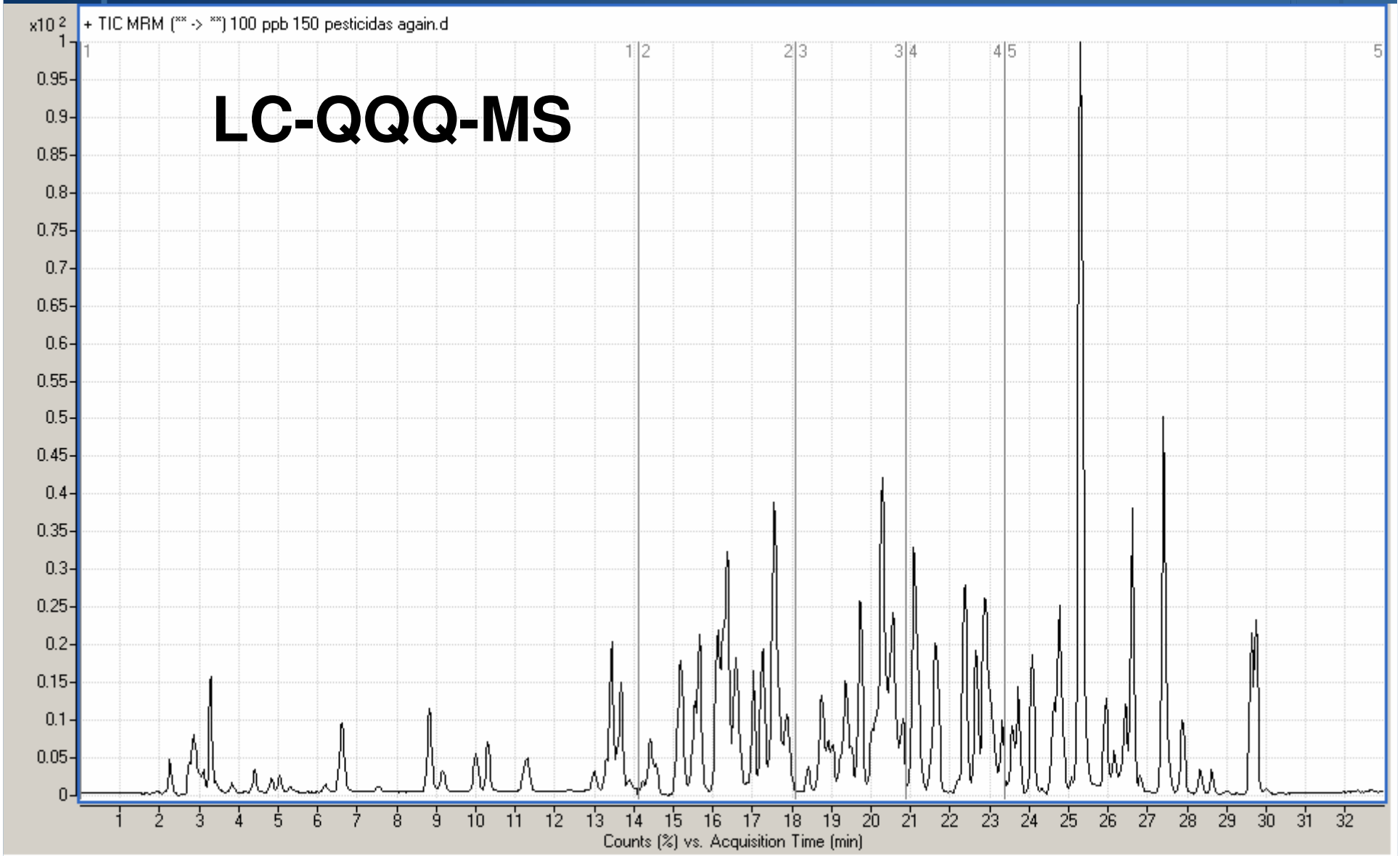
# MRM SCOPE

# ? pesticides in MRM



# ? pesticides in MRM and ?pesticides present in the PT



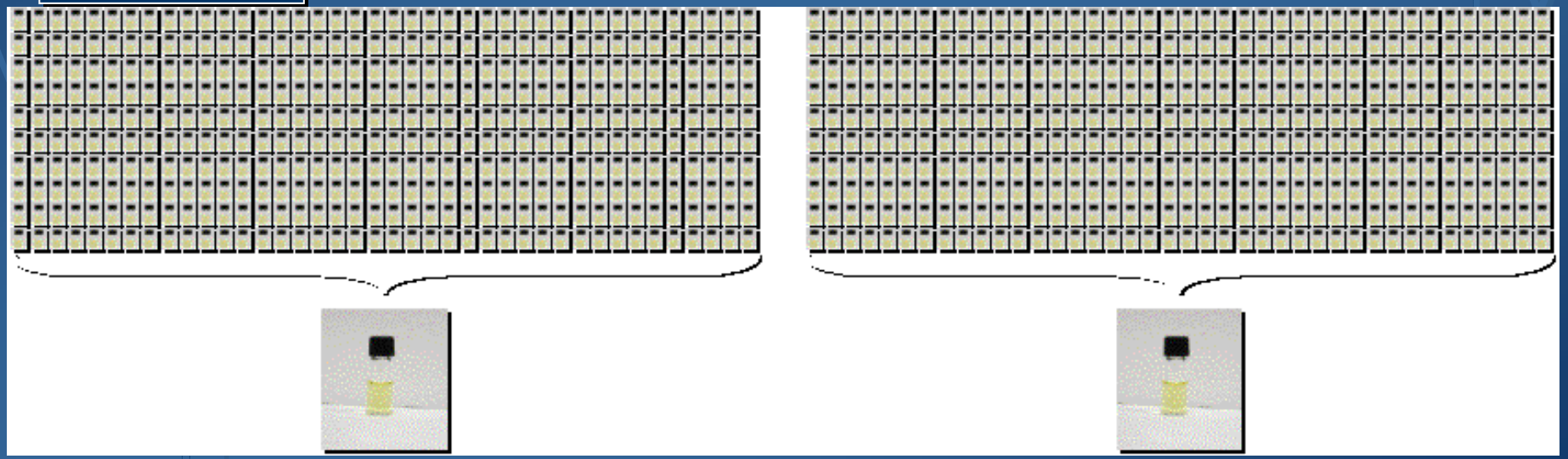

+ TIC MRM (** -> **) 100 ppb 150 pesticidas again.d
x10 2
LC-QQQ-MS
Counts (%) vs. Acquisition Time (min)

## STANDARDS

A) 100 Standards x costs €/Standard
B) 200 Standards x costs €/Standard
C) 600 Standards x costs €/Standard

## SOLUTIONS

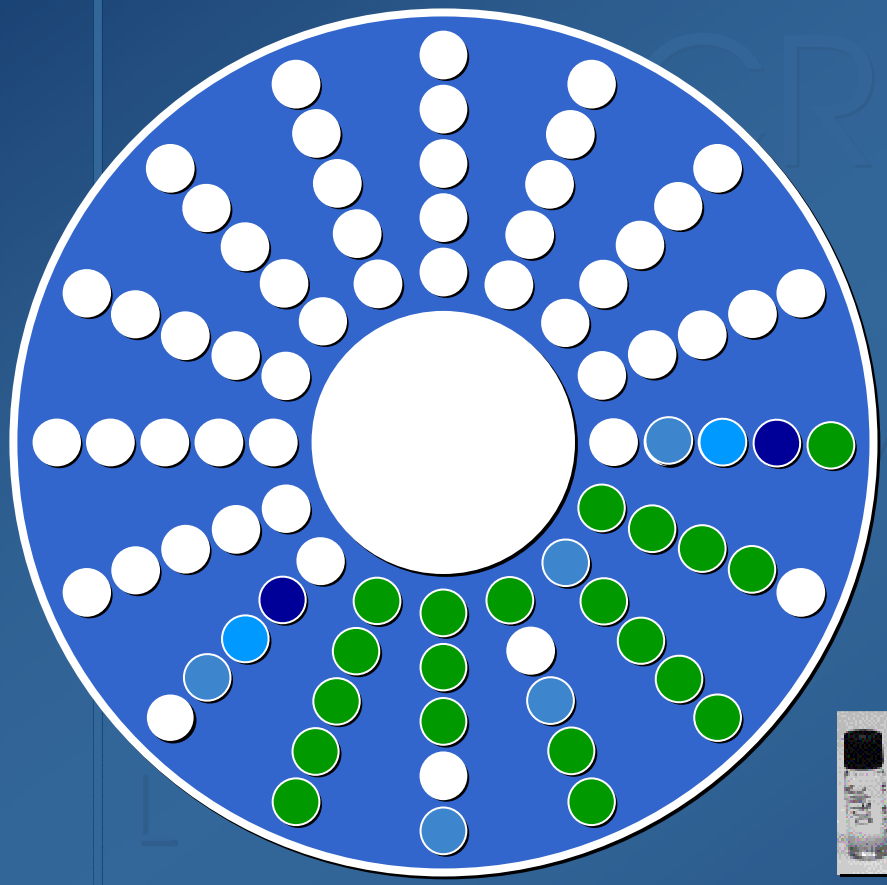
3-6 months



QC Double Check
(every day)

Stability?

# Practical design of a batch of samples



**QC PROCEDURES FOR PEST. RES. ANAL. Guidelines for Residues Monitoring Control in the European Union**

**Document 7826/VI/97**

| PESTICIDES | Median (mg/Kg) | Nº of reported results | Nº of NA (not sought) | False negatives | % of results from the total 132 |
|---|---|---|---|---|---|
| Bupirimate | 0.790 | 110 | 20 | 2 | **83** |
| Cyprodinil | 0.954 | 115 | 15 | 2 | **87** |
| Diazinon | 0.272 | 130 | 2 | 0 | **98** |
| Endosulfan α | 0.411 | 124 | 4 | 0 | **95** |
| Endosulfan β | 0.326 | 124 | 4 | 0 | **95** |
| Fenhexamid | 0.338 | 96 | 29 | 7 | **73** |
| Fenitrothion | 0.791 | 123 | 7 | 2 | **93** |
| Fludioxonil | 0.516 | 103 | 28 | 1 | **78** |
| Hexythiazox | 1.05 | 71 | 60 | 1 | **54** |
| Iprodione | 0.725 | 117 | 8 | 7 | **89** |
| Myclobutanil | 0.759 | 118 | 13 | 1 | **89** |
| Oxamyl | 0.756 | 111 | 15 | 6 | **84** |
| Penconazole | 0.486 | 116 | 14 | 2 | **88** |
| Procymidone | 0.606 | 129 | 3 | 0 | **98** |
| Pyrimethanil | 0.880 | 116 | 15 | 1 | **88** |
| Quinoxifen | 1.03 | 90 | 41 | 1 | **68** |
| Tebuconazole | 0.800 | 107 | 22 | 3 | **81** |
| Tolylfluanid | 1.60 | 118 | 14 | 0 | **89** |
| Triadimenol | 1.32 | 100 | 27 | 5 | **76** |
| TOTAL | | 2018 | 341 (14%) | 41 (2%) | |

# CATEGORY A ( 90% + COMPOUNDS*)

# CATEGORY B ( <90% OR FALSE POSITIVE)

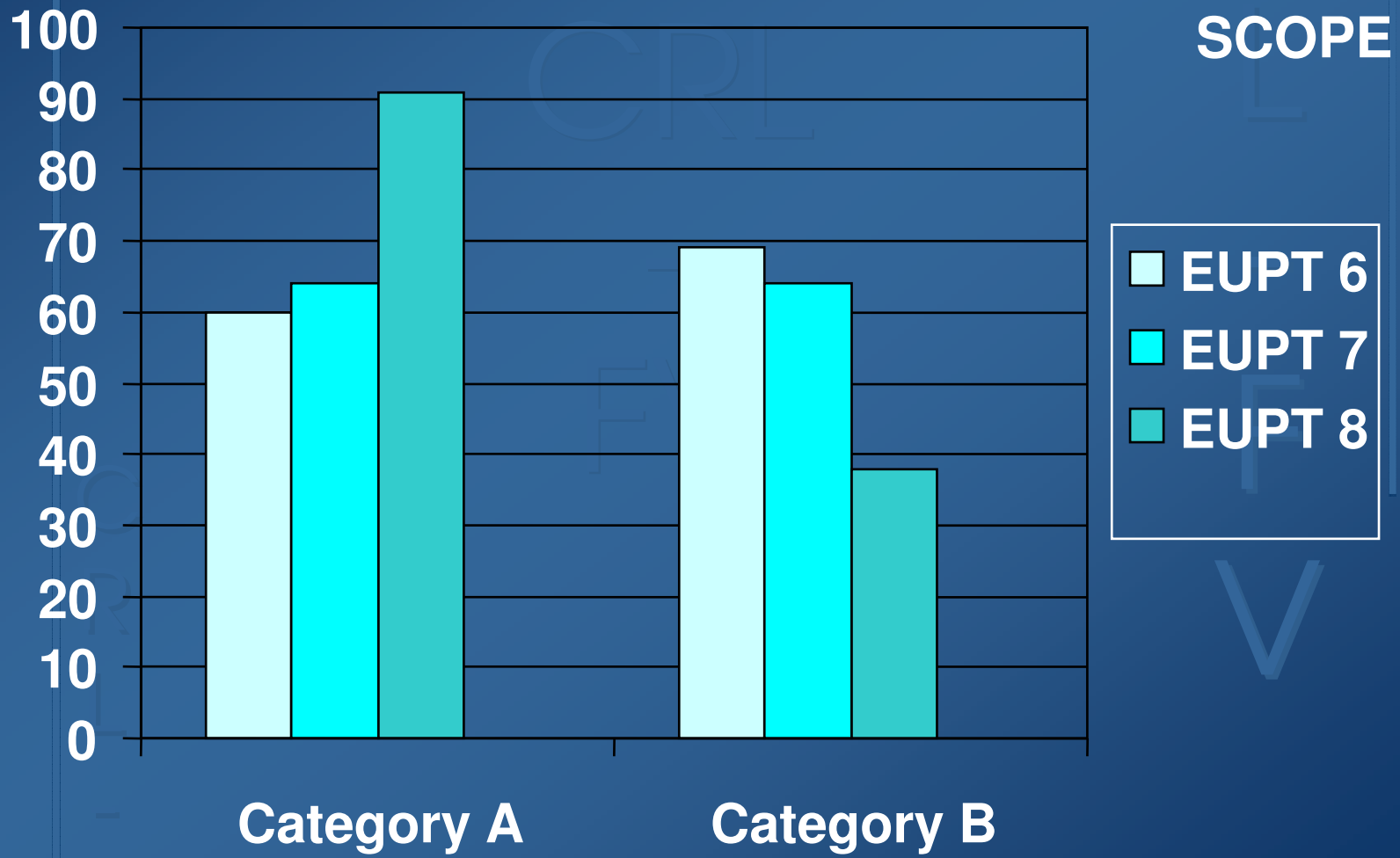
# EUPT-FV9 Possible Pesticide List

* Acephate
Acetamiprid
Acrinathrin
* Aldicarb
Azinphos-methyl
* Azoxystrobin
* Bifenthrin
Bromopropylate
**Bupirimate**
Buprofezin
* Captan
* Carbaryl
* Carbendazim
Chlofentezin
* Chlorothalonil
Chlorpropham
* Chlorpyrifos
Chlorpyrifos-methyl
Cypermethrin
**Cyprodinil**
* Deltamethrin
*** Diazinon**
Dichlofluanid
* Dichlorvos
* Dicofol
* Dimethoate
Dimethomorph
Diphenylamine
*** Endosulfan**
Fenarimol
**Fenhexamid**
*** Fenitrothion**
Flusilazole
**Fludioxonil**
Folpet
* Imazalil
Hexaconazole
**Hexythiazox**
* Imidacloprid
Indoxacarb
*** Iprodione**
Iprovalicarb
Kresoxim-methyl
* Lambda-cyhalothrin
* Malathion
Mepanipyrim
* Metalaxyl
* Methamidophos
Methidathion
* Methiocarb
* Methomyl
* Monocrotophos
**Myclobutanil**
*** Oxamyl**
* Oxydemeton-methyl
Parathion
* Parathion-methyl
**Penconazole**
Phosalone
* Pirimicarb
* Pirimiphos-methyl
* Prochloraz
*** Procymidone**
Profenofos
Propargite
* Propiconazole
*** Pyrimethanil**
Pyriproxyfen
**Quinoxifen**
Spiroxamine
*** Tebuconazole**
Tebufenozide
Tetraconazole
* Thiabendazole
* Thiophanate-methyl
Tolclofos-methyl
*** Tolylfluanid**
*** Triadimefon**
Triazophos
Trifloxystrobin
* Vinclozolin

**Possible:**
**82 pesticides (FV10: 108)**

**Total Applied:**
**18 pesticides (A16)**
*** mandatory**

## Number of laboratories in each Category for the last four EUPTs



# Harmonization

-Results ( quantity + quality)
- Acceptable Z-score
-MRRL ( 0.01 mg/k)
-Scope
-Anal. Response capability

# False negatives: x = MRRL

$$z\text{-Score} = (x - \mu) / \sigma$$

μ : Assigned value (median)
σ : Standard deviation (FFP RSD 25%)

| | |
|---|---|
| $\lvert z \rvert \leq 2$ | Acceptable |
| $2 < \lvert z \rvert \leq 3$ | Questionable |
| $3 < \lvert z \rvert$ | Unacceptable |

## overview

- EU model
- How EUPTs can support this model ?
- Quantifying Uncertainty in MRMs
- Results

# Quantifying uncertainty in MRMs

Amadeo R. Fernández-Alba – COMMUNITY REFERENCE LABORATORY

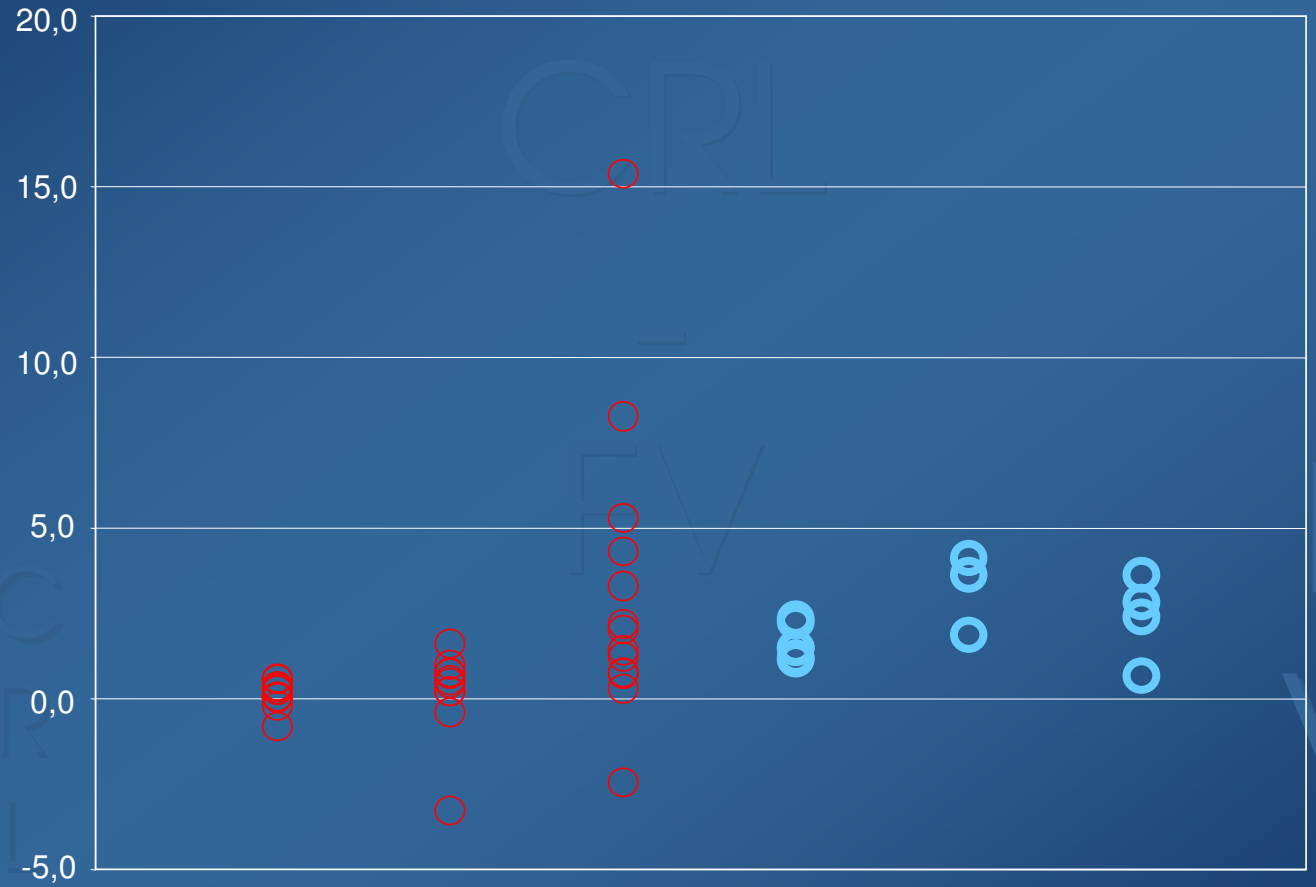
# z-Score $= (x - \mu) / \sigma$

$\mu$ : Assigned value (median)
$\sigma$ : Standard deviation (FFP RSD 25%)

| | |
|---|---|
| $\lvert z \rvert \leq 2$ | Acceptable |
| $2 < \lvert z \rvert \leq 3$ | Questionable |
| $3 < \lvert z \rvert$ | Unacceptable |

σ based on:

- Data
- Requeriments



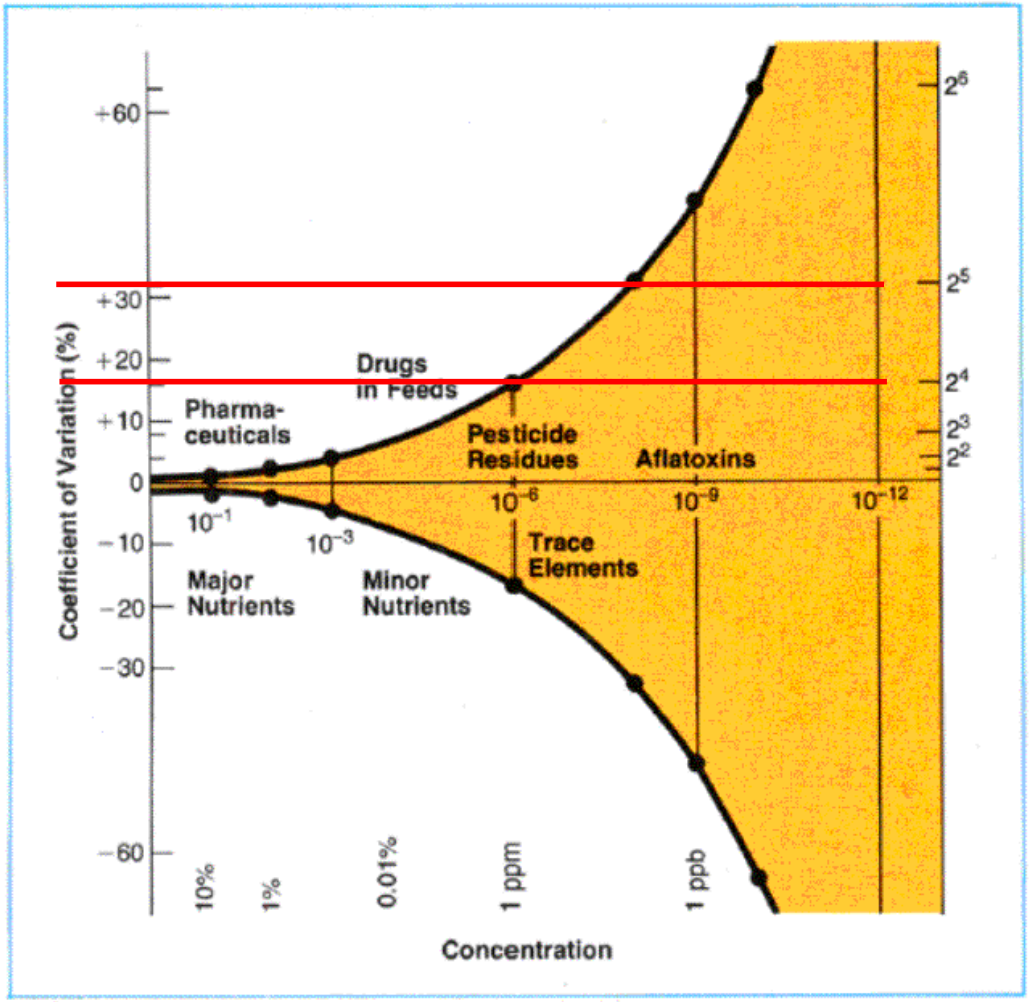
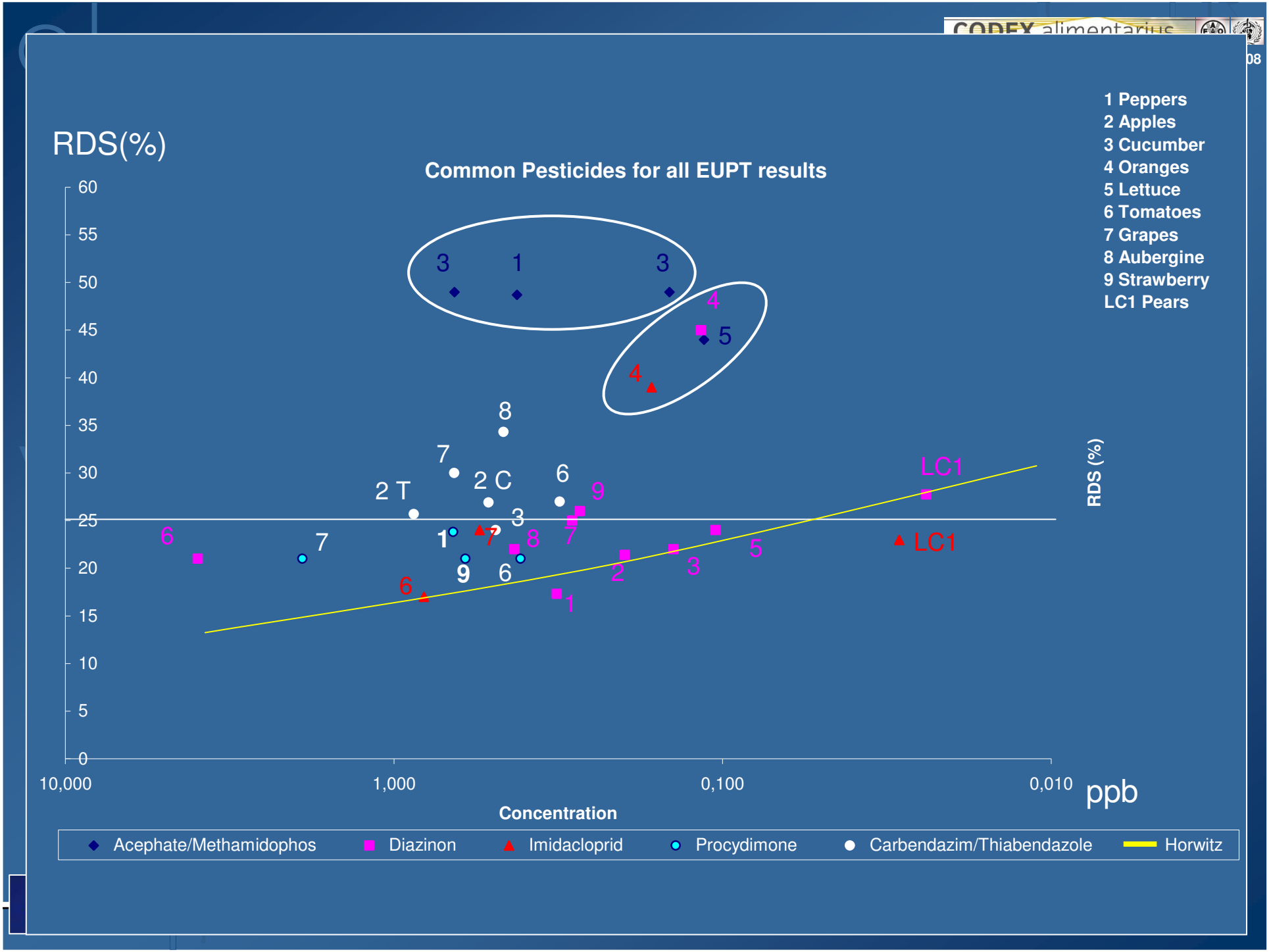
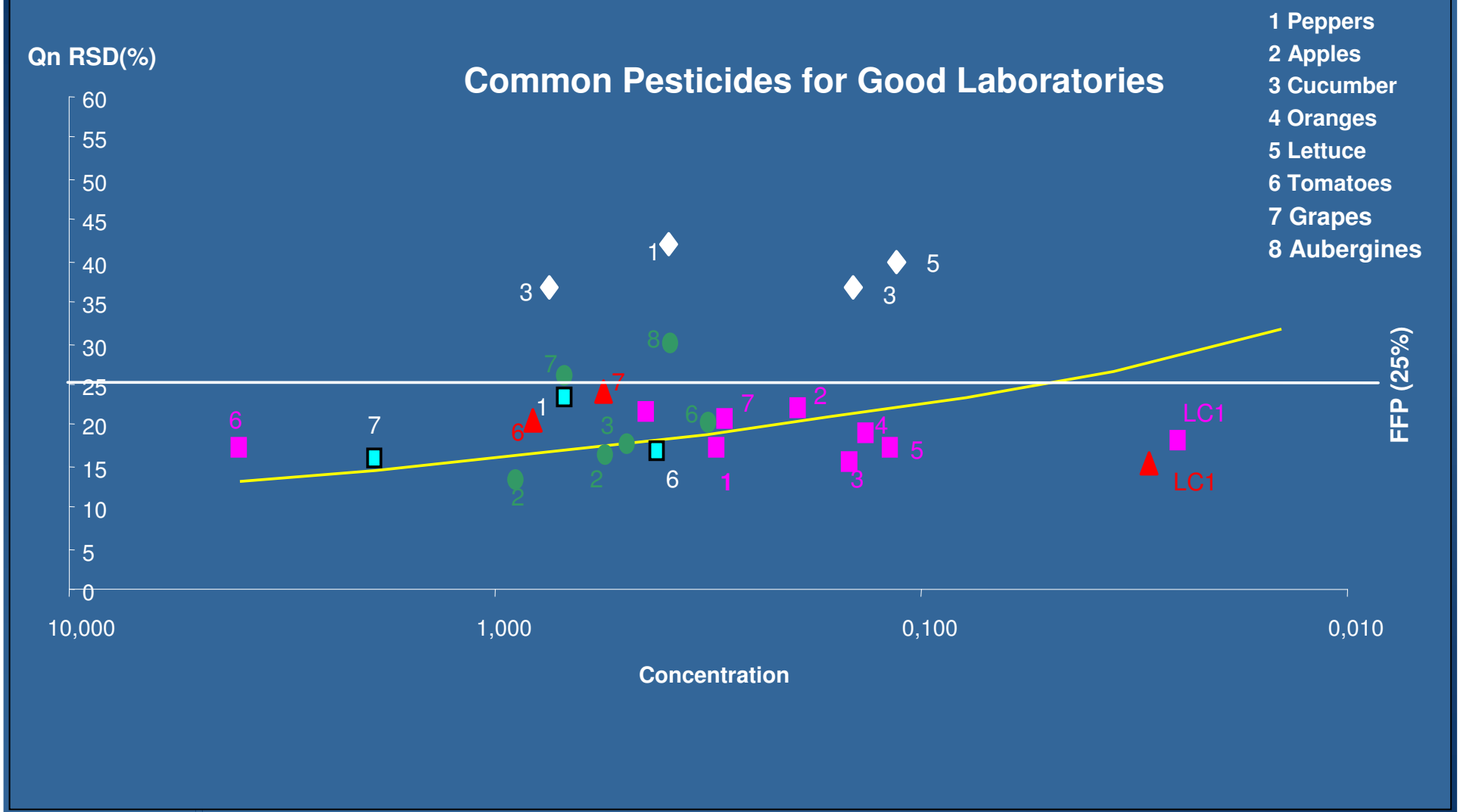
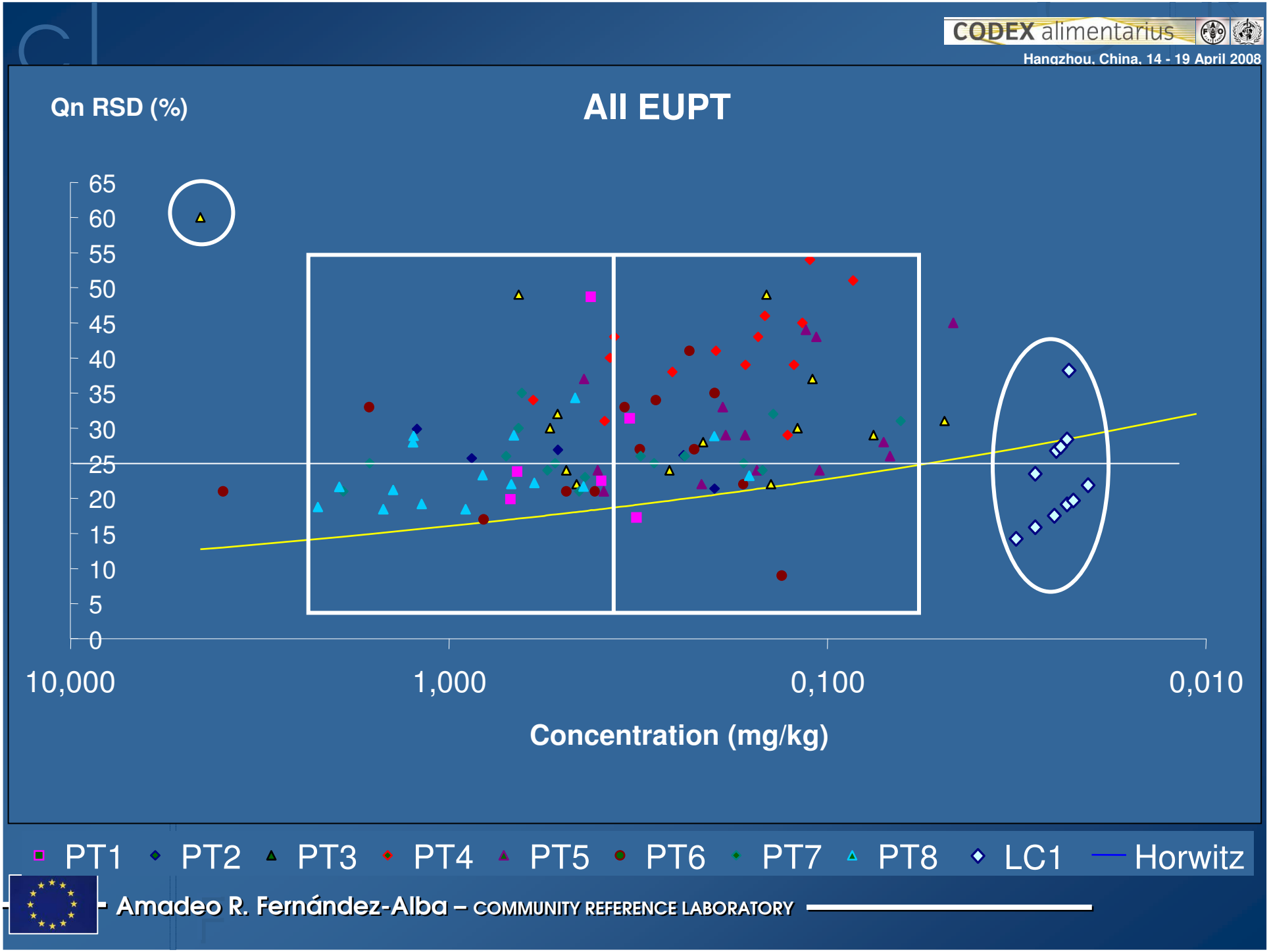
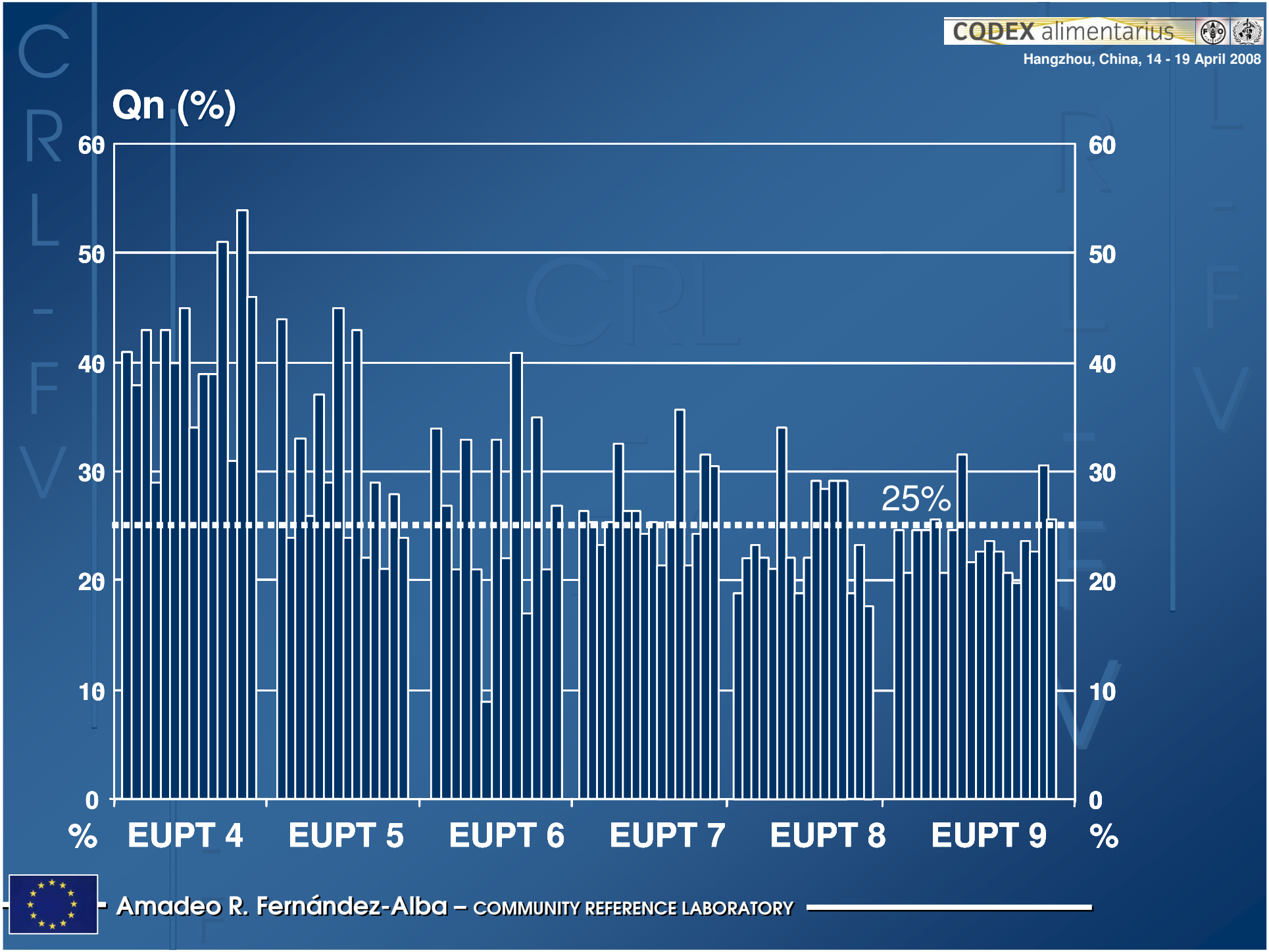



**Figure 1.** The general curve relating interlaboratory coefficients of variation (expressed as powers of two on the right) with concentration (expressed as powers of 10) along the horizontal center axis

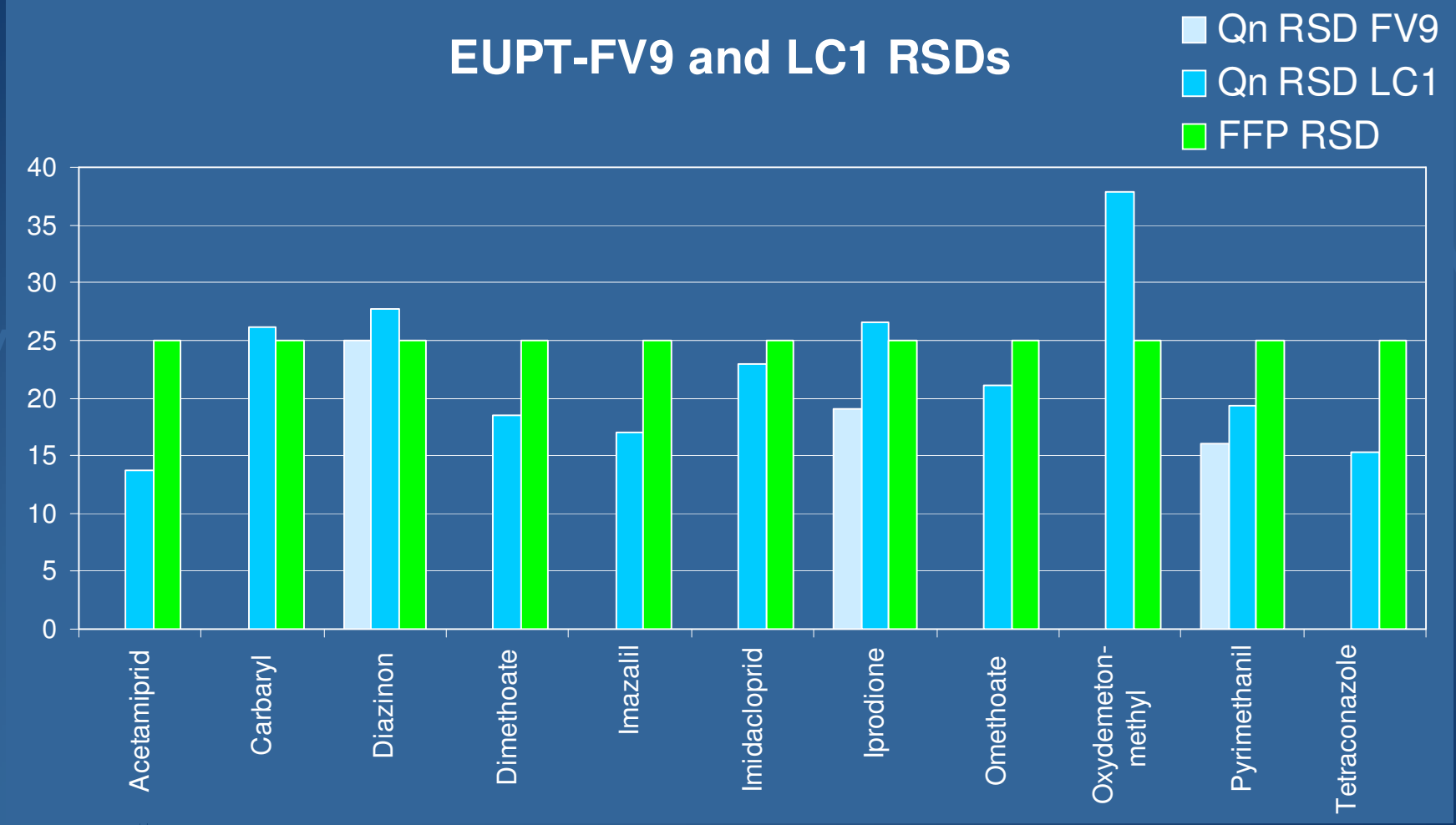

RDS(%)
Common Pesticides for all EUPT results
1 Peppers
2 Apples
3 Cucumber
4 Oranges
5 Lettuce
6 Tomatoes
7 Grapes
8 Aubergine
9 Strawberry
LC1 Pears
RDS (%)
Concentration
ppb
10,000
1,000
0,100
0,010
Acephate/Methamidophos
Diazinon
Imidacloprid
Procydimone
Carbendazim/Thiabendazole
Horwitz


Common Pesticides for Good Laboratories
Qn RSD(%)
60
55
50
45
40
35
30
25
20
15
10
5
0
10,000
1,000
0,100
0,010
Concentration
FFP (25%)
1 Peppers
2 Apples
3 Cucumber
4 Oranges
5 Lettuce
6 Tomatoes
7 Grapes
8 Aubergines
LC1
LC1
Acephate/Methamidophos
Diazinon
Imidacloprid
Procymidone
Carbendazim/Thiabendazole
Horwitz

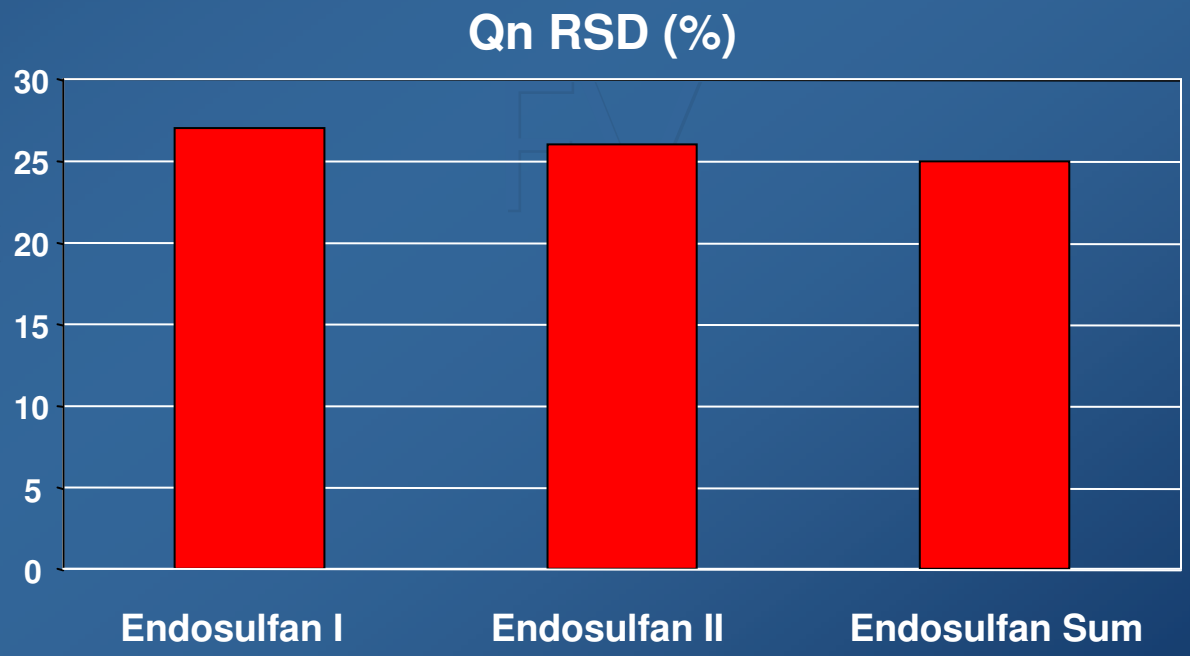

Qn RSD (%)
All EUPT
65
60
55
50
45
40
35
30
25
20
15
10
5
0
10,000
1,000
0,100
0,010
Concentration (mg/kg)
PT1
PT2
PT3
PT4
PT5
PT6
PT7
PT8
LC1
Horwitz


Qn (%)
60
50
40
30
20
10
0
25%
% EUPT 4 EUPT 5 EUPT 6 EUPT 7 EUPT 8 EUPT 9 %

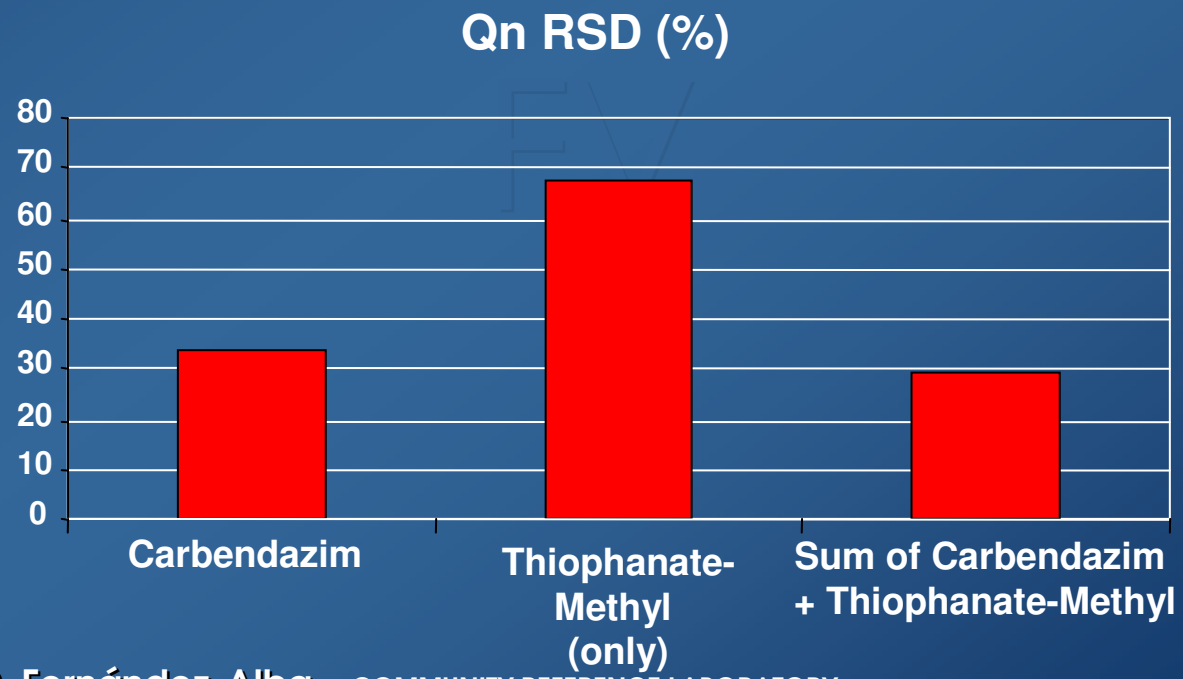
# STANDARD DEVIATION



| Code | Endosulfan I | Endosulfan II | Endosulfan Sum |
|---|---|---|---|
| Median | 0,411 | 0,326 | 0,750 |
| Qn (%) | 27 | 26 | 25 |



| Code | Carbendazim | Thiophanate-Methyl (only) | Sum of Carbendazim + Thiophanate-Methyl |
|---|---|---|---|
| Median | 0,414 | 0,273 | 0,449 |
| Qn (%) | 34 | 68 | 29 |



**Interlab. relative standard deviation ± 25%**

**Expanded uncertainty (K=2) ± 50%**

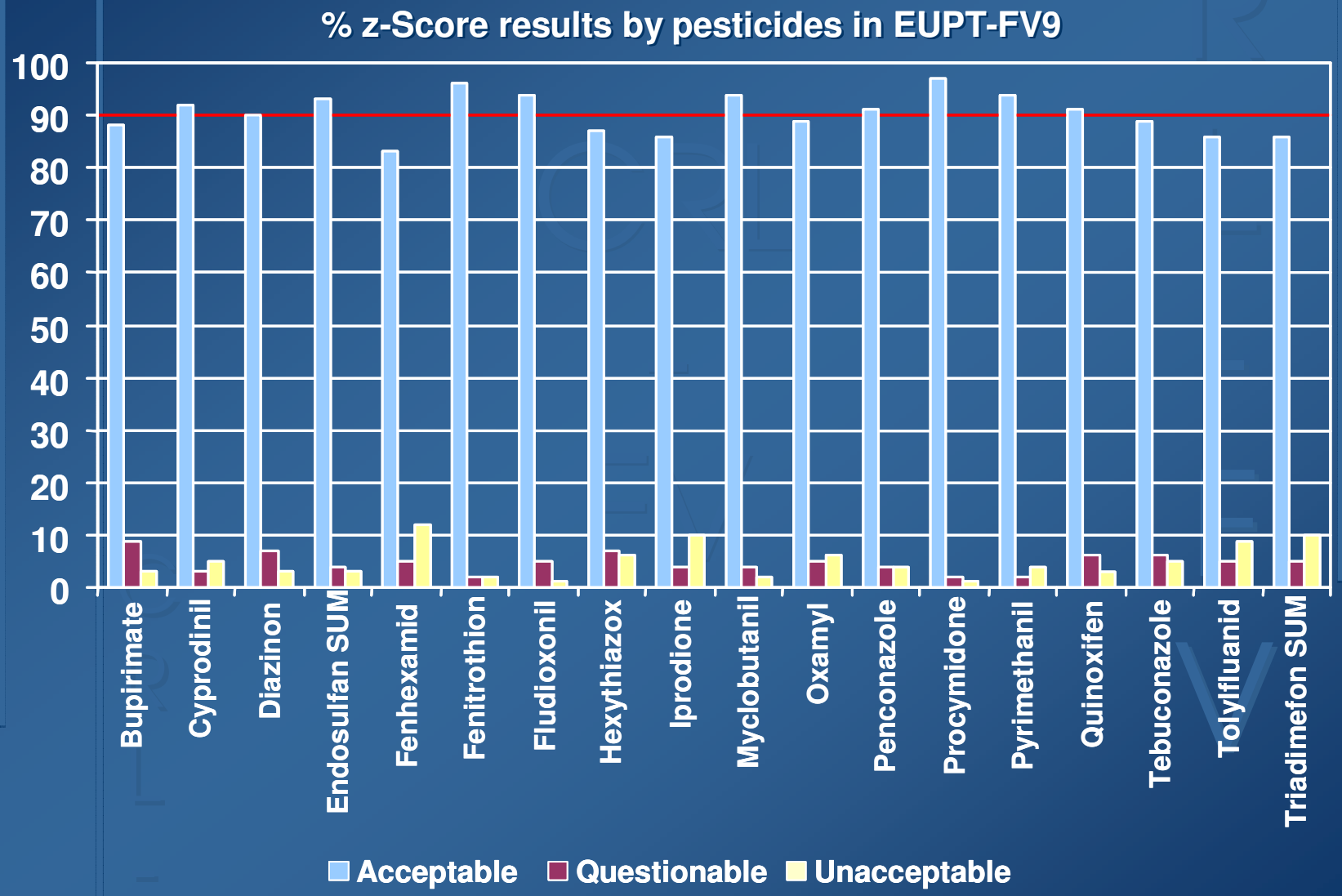
## overview

-EU model

-How EUPTs can support this model ?

-Quantifying Uncertainty

-Results (Z-score)


% z-Score results by pesticides in EUPT-FV9
100
90
80
70
60
50
40
30
20
10
0
Bupirimate
Cyprodinil
Diazinon
Endosulfan SUM
Fenhexamid
Fenitrothion
Fludioxonil
Hexythiazox
Iprodione
Myclobutanil
Oxamyl
Penconazole
Procymidone
Pyrimethanil
Quinoxifen
Tebuconazole
Tolylfluanid
Triadimefon SUM
Acceptable
Questionable
Unacceptable

# Pyrimethanil

CODEX alimentarius

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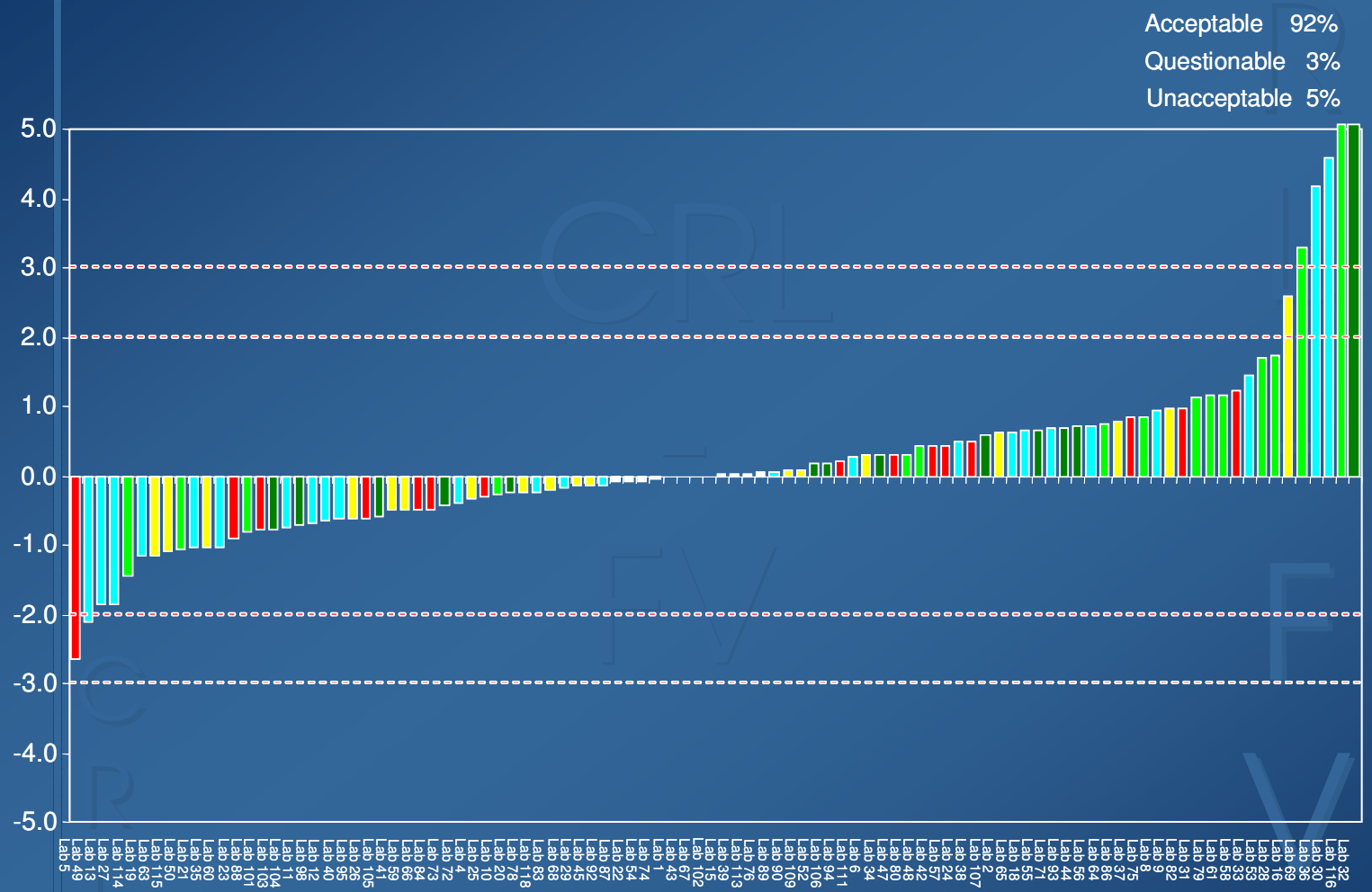
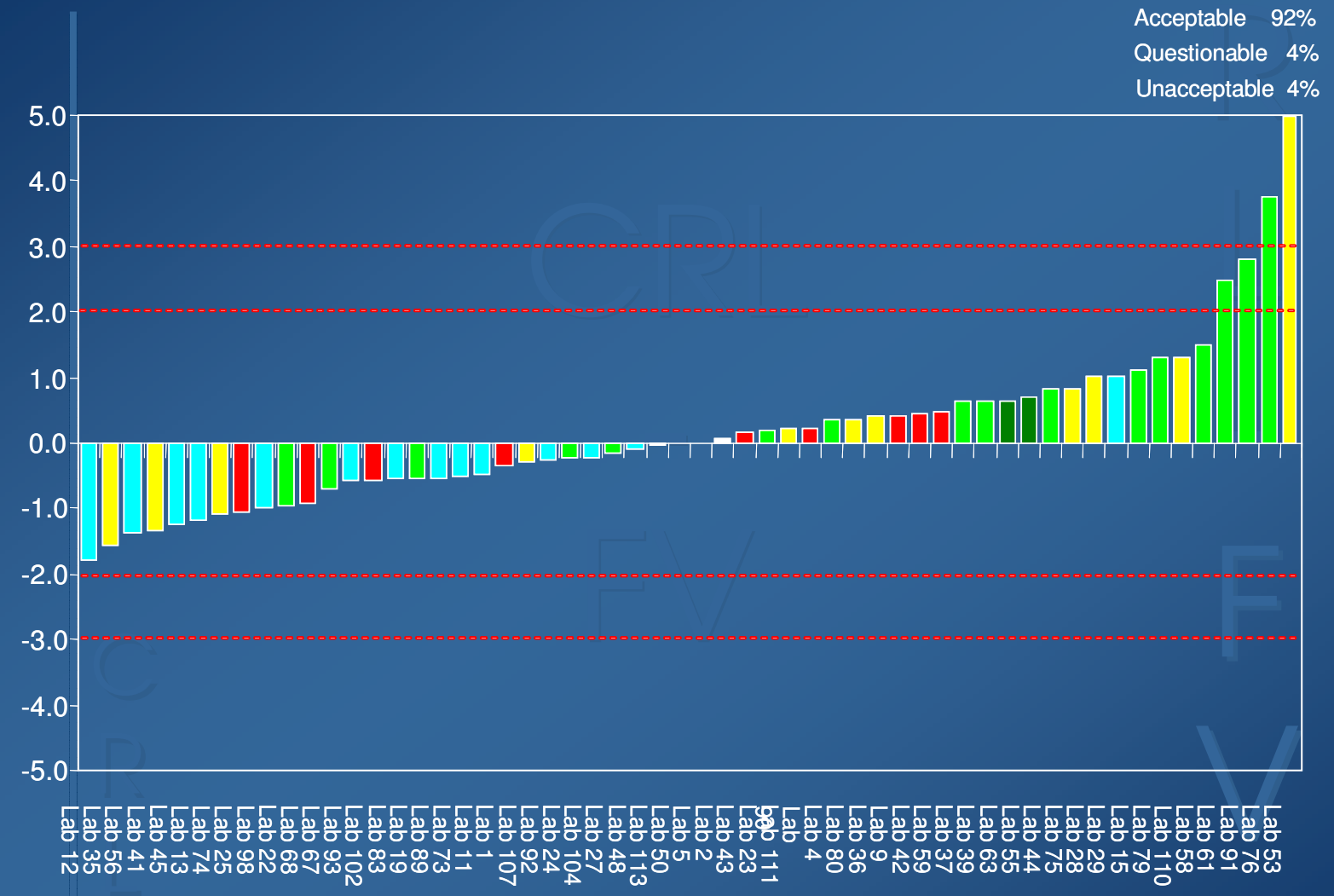



Quechers Luke Acetato de Etilo § 35 LMBG Klein & Alder

CODEX alimentarius

Hangzhou, China, 14 - 19 April 2008

# Acetamiprid

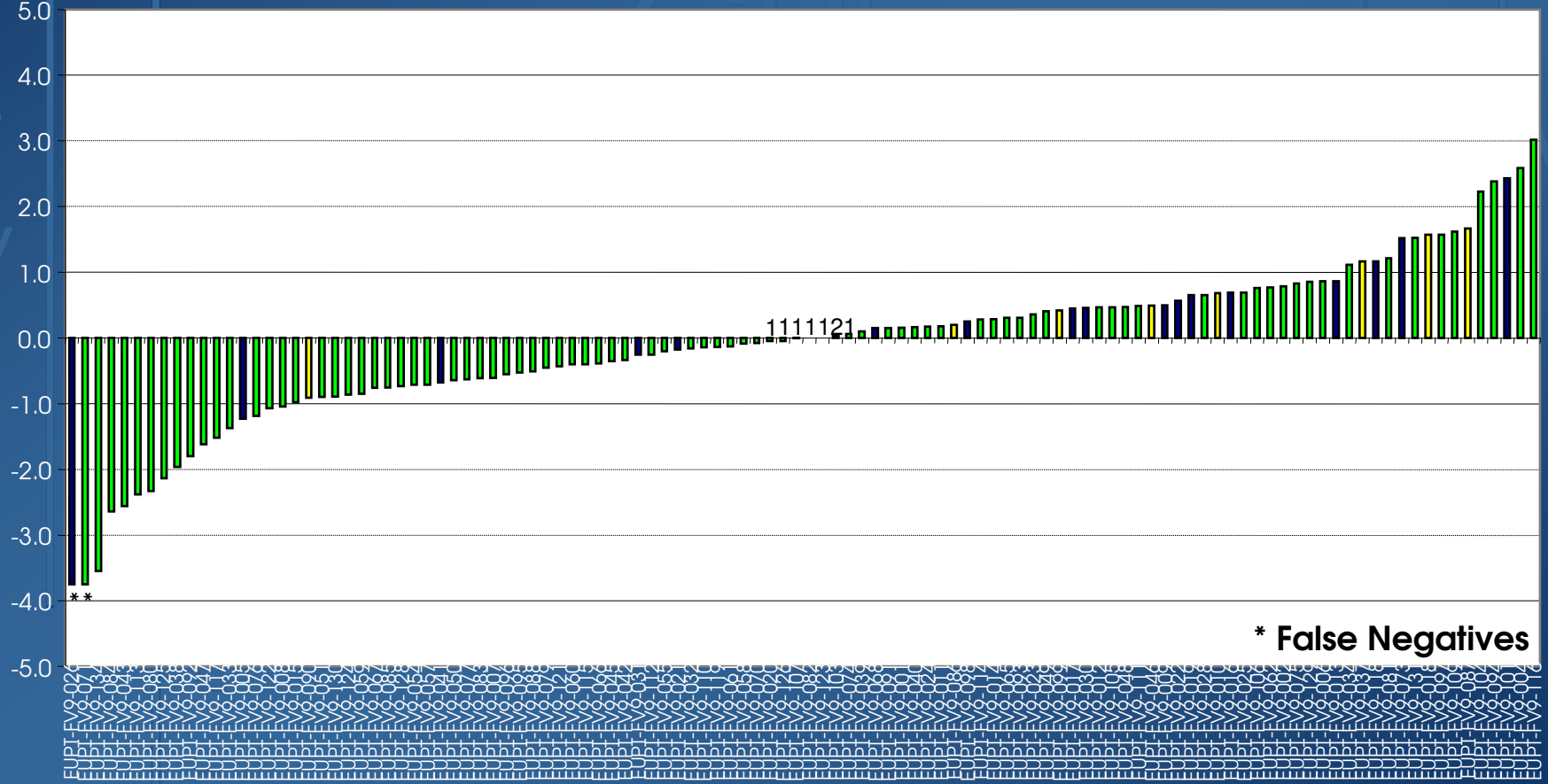



Quechers Luke Acetato de Etilo § 35 LMBG Klein & Alder

# Bupirimate

or '1' : GC
or '2' : LC
or '3' : GC and LC

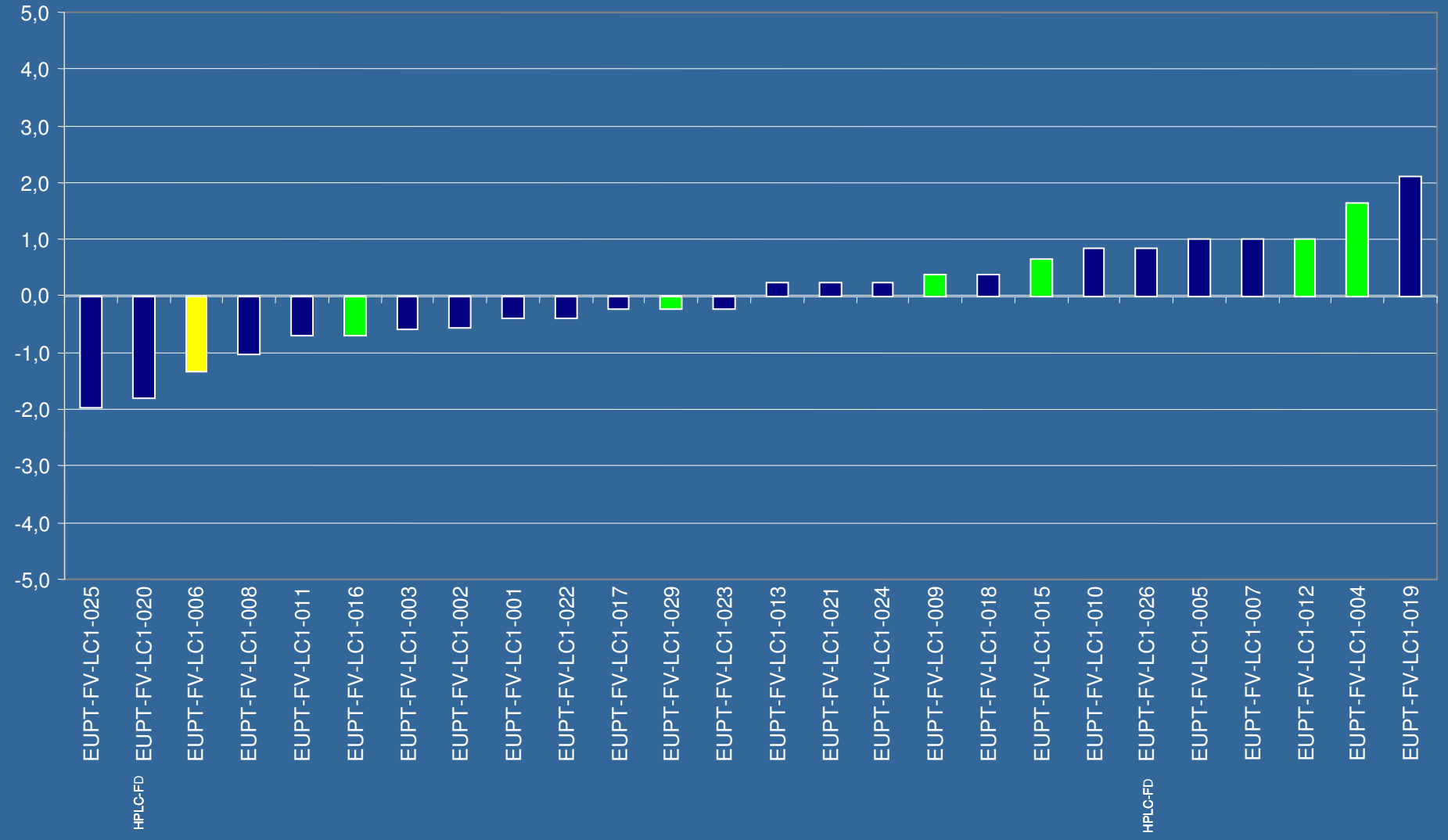
| | |
|---|---|
| Acceptable | 88% |
| Questionable | 9% |
| Unacceptable | 3% |



GC
LC
GC and LC

*** False Negatives**

# Carbaryl



# Extraction Methods

- Quechers

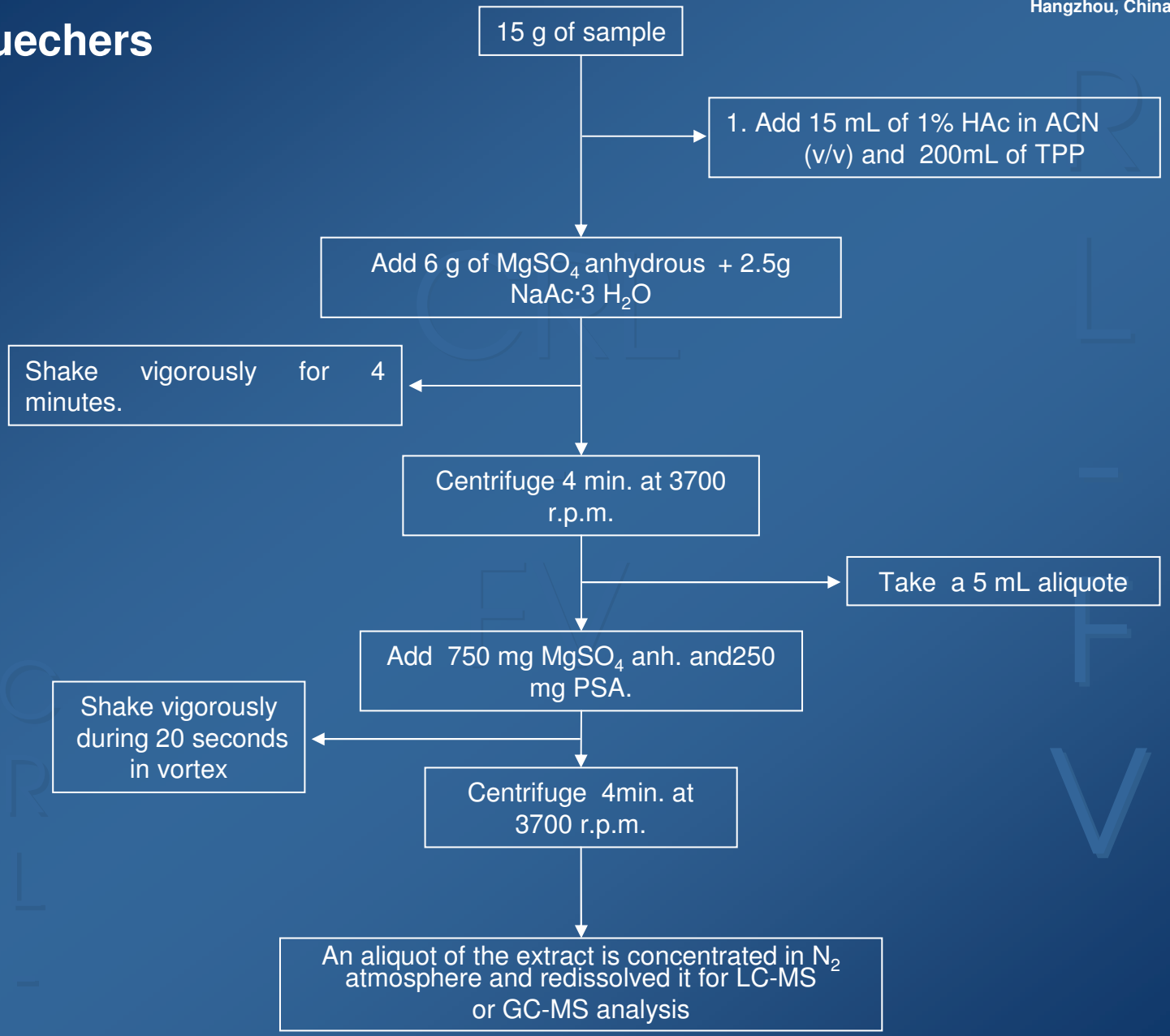
- Ethyl Acetate

- Luke

- Klein & Alder

- § 35 LMBG

# 1) Quechers


15 g of sample
1. Add 15 mL of 1% HAc in ACN (v/v) and 200mL of TPP
Add 6 g of $MgSO_4$ anhydrous + 2.5g NaAc·3 $H_2O$
Shake vigorously for 4 minutes.
Centrifuge 4 min. at 3700 r.p.m.
Take a 5 mL aliquote
Add 750 mg $MgSO_4$ anh. and250 mg PSA.
Shake vigorously during 20 seconds in vortex
Centrifuge 4min. at 3700 r.p.m.
An aliquot of the extract is concentrated in $N_2$ atmosphere and redissolved it for LC-MS or GC-MS analysis

**15 g sample**

**Add 15 mL AcN (1% Acetic Acid)**

**Add 6 g $MgSO_4$ + 2.5 g NaAc·3 $H_2O$**

**Shake hard (3 minutes)**

**Centrifuge 5 min. at 3700 r.p.m.**

**Take aliquot of 5 mL**

**Add 750 mg $MgSO_4$ + 250 mg PSA**

**Shake hard (20 seconds)**

**Centrifuge 5 min. at 3700 r.p.m.**

**Analysis by LC/MS-MS**

## 2) Ethyl Acetate

15g sample

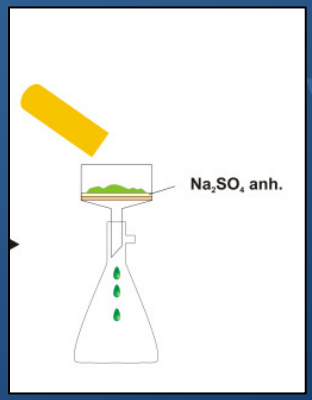
15 mL ethyl acetate


1) 30s extractions

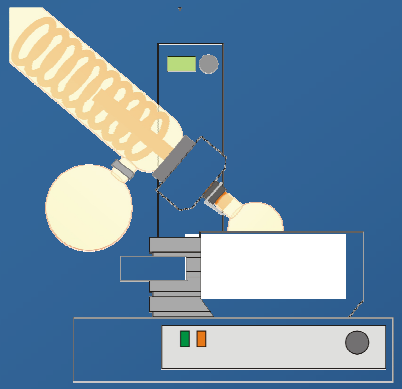
2) 15mL + 10 mL ethyl acetate


Filtrate with

20 g $Na_2SO_4$ anhydrou




Evaporate until dryness



15 mL MeOH
(1g/mL)

15 mL Ciclohexane
(1g/mL)

Recompose

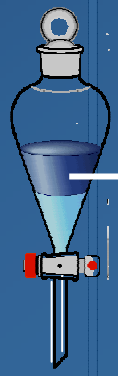
# 3) Luke's Method

## Extraction

100 g Sample
200 mL Acetone

## Filtration

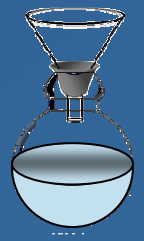
## Portioning



200mL Dichloromethane:
Petroleum Ether (1:1)

Sake 3 min
Wait 10 min

Organic Fase



Dryness: $Na_2SO_4$

Evaporation
Redissolved

Aqua Fase: 7g NaCl
100mL Dichloromethane x2

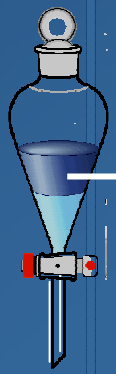
# 4) § 35 LMBG

**Extraction**

100 g Sample
200 mL Acetone

**Filtration**

**Portioning**



100mL Ethyl Acetate
:Ciclohexane (1:1)

Sake 2 min
Wait 10 min

Organic Fase

Dryness: $Na_2SO_4$

Evaporation
Redissolved with
Ethyl Acetate

## 5) Klein & Alder

Add water to 5g or 10g of dry or water-containing sample (sum 10mL)

↓

Homogenise with 20mL methanol; if necessary filtration or centrifugation

↓

Add NaCl solution to an aliquot and soak diatomeas earth (ChemElut)

↓

Elution with dichloromethane evaporate to dryness

↓

Reconstitute in MeOH:$H_2O$ (final sample concentration 1g/mL)

↓

Pass through a 0.45µm syringe filter

↓

Chromatography on 5cm x 2mm RP18-column, LC-MS/MS in MRM

# Single vs multiple Z-score values

# Combined Z-scores

$$RSZ = \sum_i Z_i / \sqrt{n}$$

$$SSZ = \sum_i Z_i^2$$

$$WSZ = \frac{\sum_i |Z_{accep.}| \times 1 + \sum_i |Z_{quest}| \times 3 + \sum_i |Z_{unaccep.}| \times 5}{n}$$

# Combined Weight Sum z-Scores

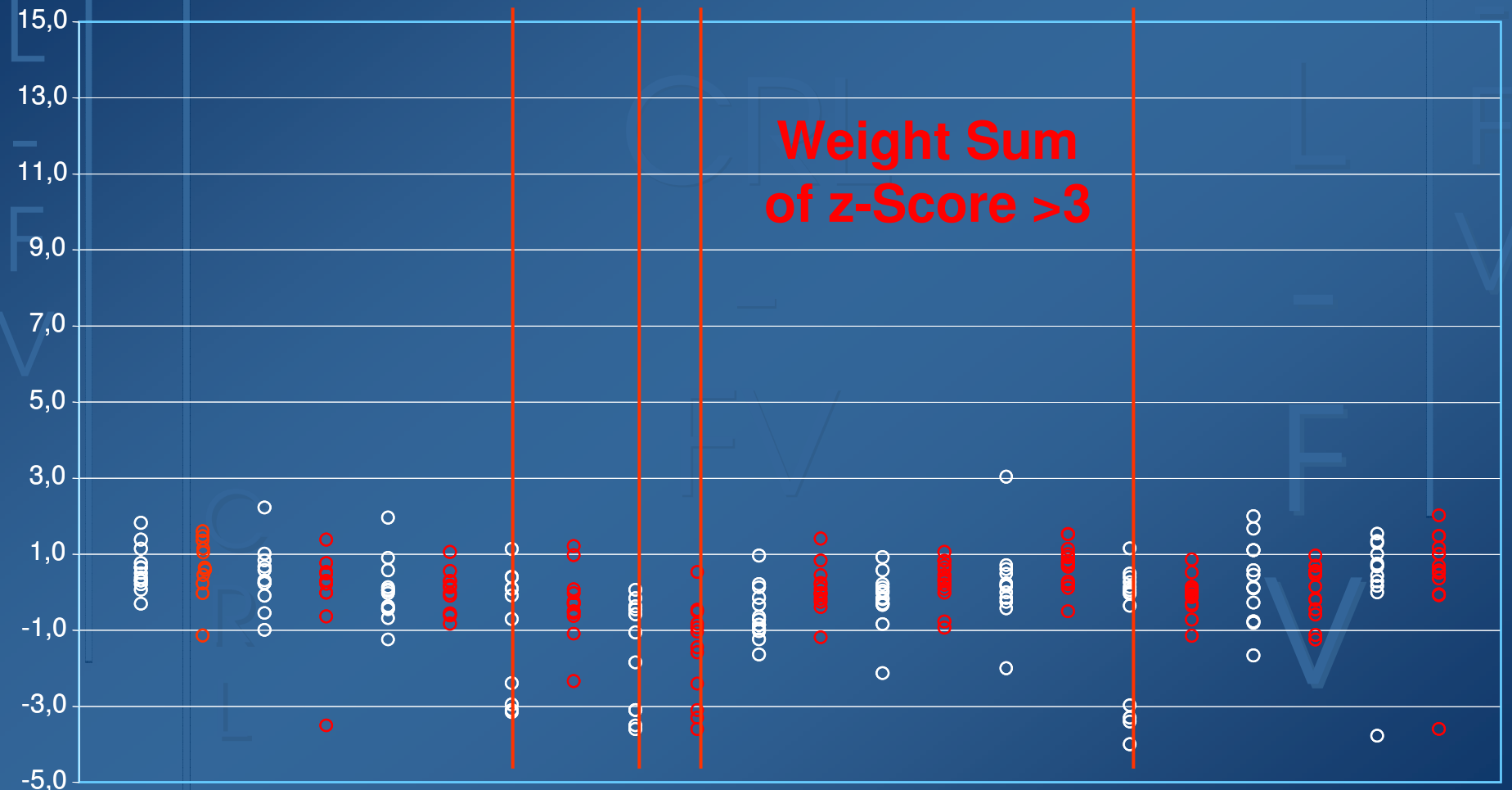
$$WSZ = \frac{\sum_i |Z_{accep.}| \times 1 + \sum_i |Z_{quest}| \times 3 + \sum_i |Z_{unaccep.}| \times 5}{n}$$

# Combined Weight Sum z-Scores

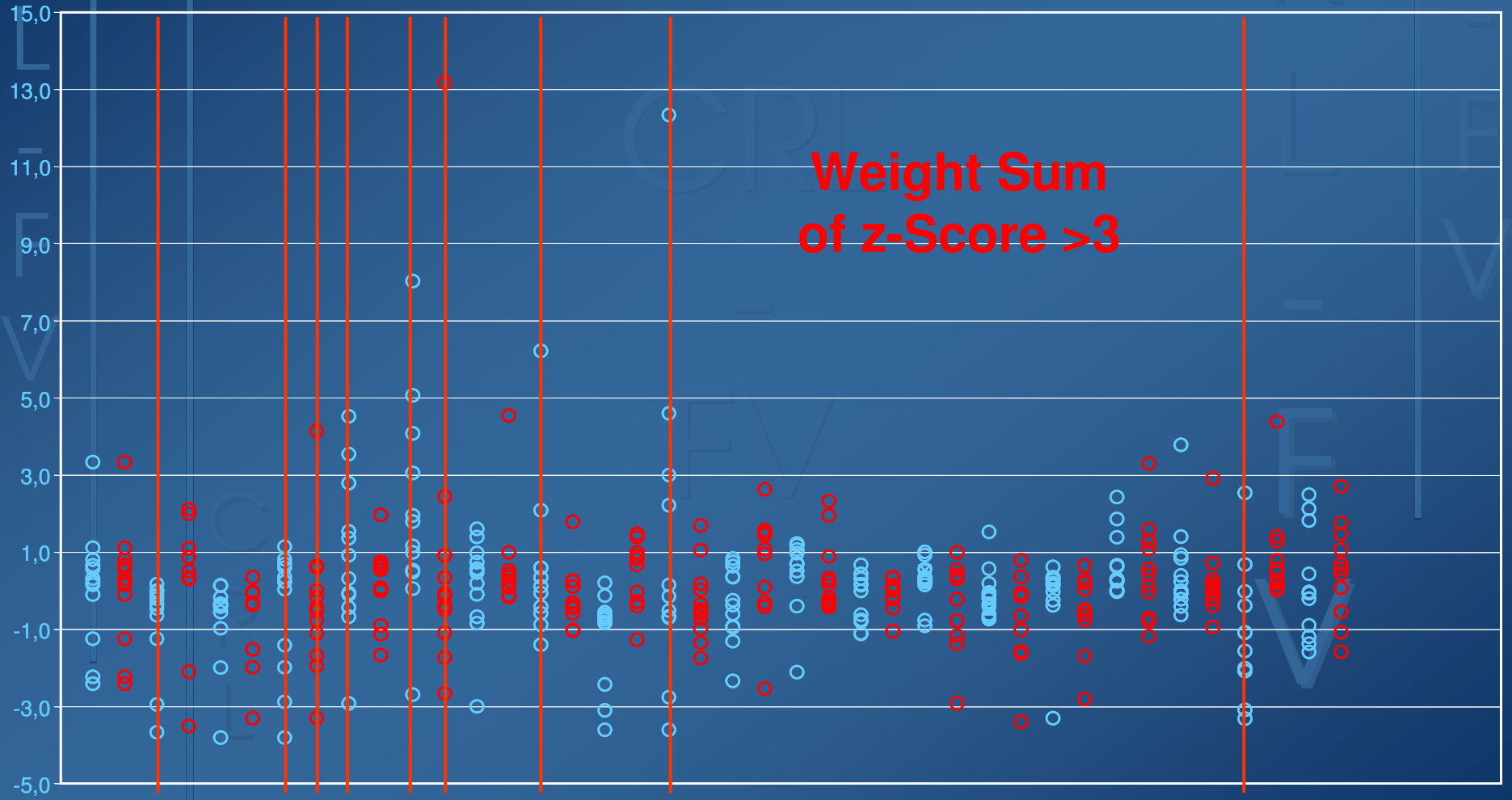
$$WSZ = \frac{\sum_i |Z_{accep.}| \times 1 + \sum_i |Z_{quest}| \times 3 + \sum_i |Z_{unaccep.}| \times 5}{n}$$

| | |
|---|---|
| $\|z\| \leq 2$ | Good |
| $2 < \|z\| \leq 3$ | Satisfactory |
| $\|z\| > 3$ | Unsatisfactory |

# z-Scores de Lab 1-30 en Categoría A

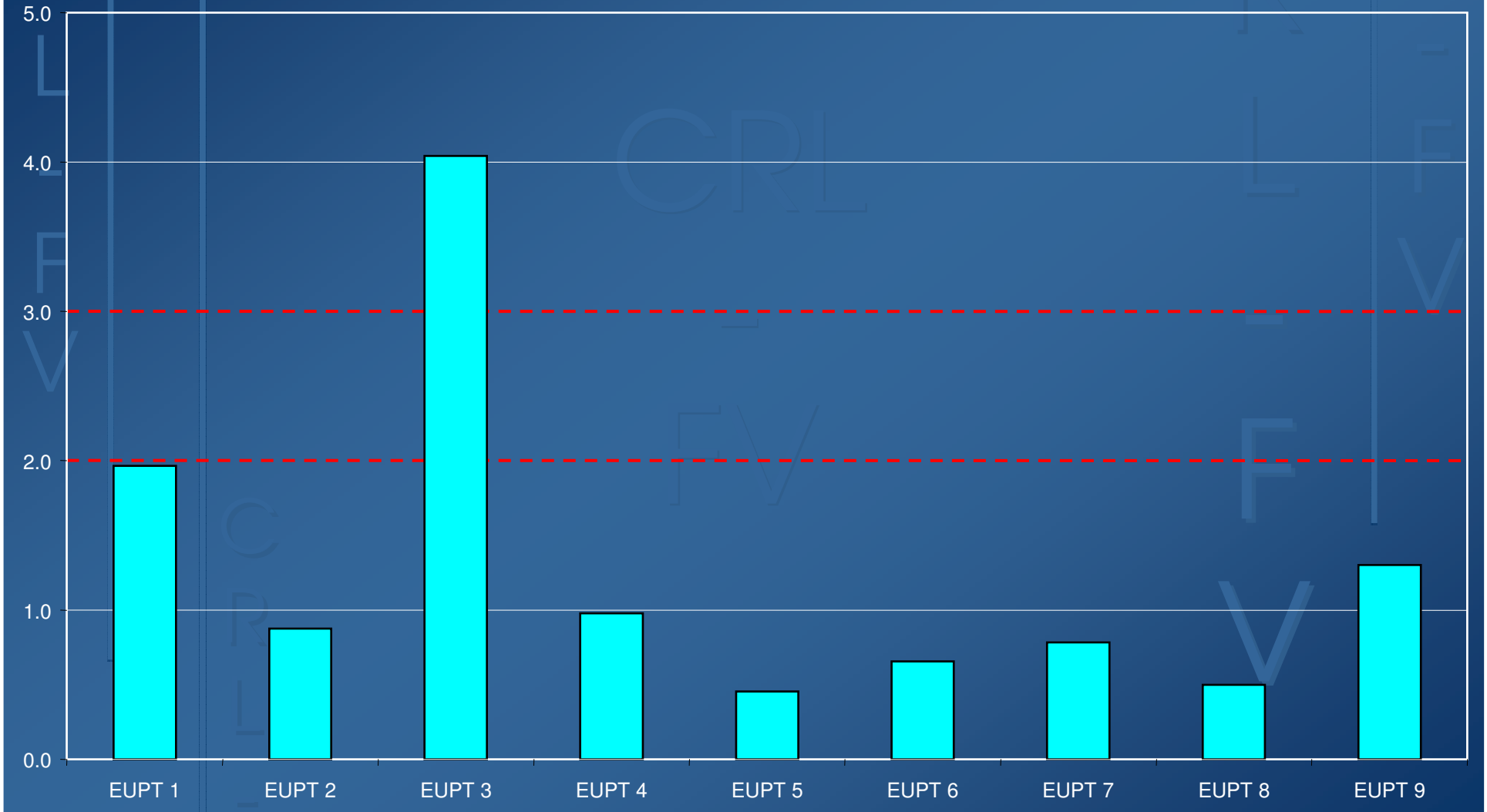


# z-Score Lab 31-130 en Categoría A

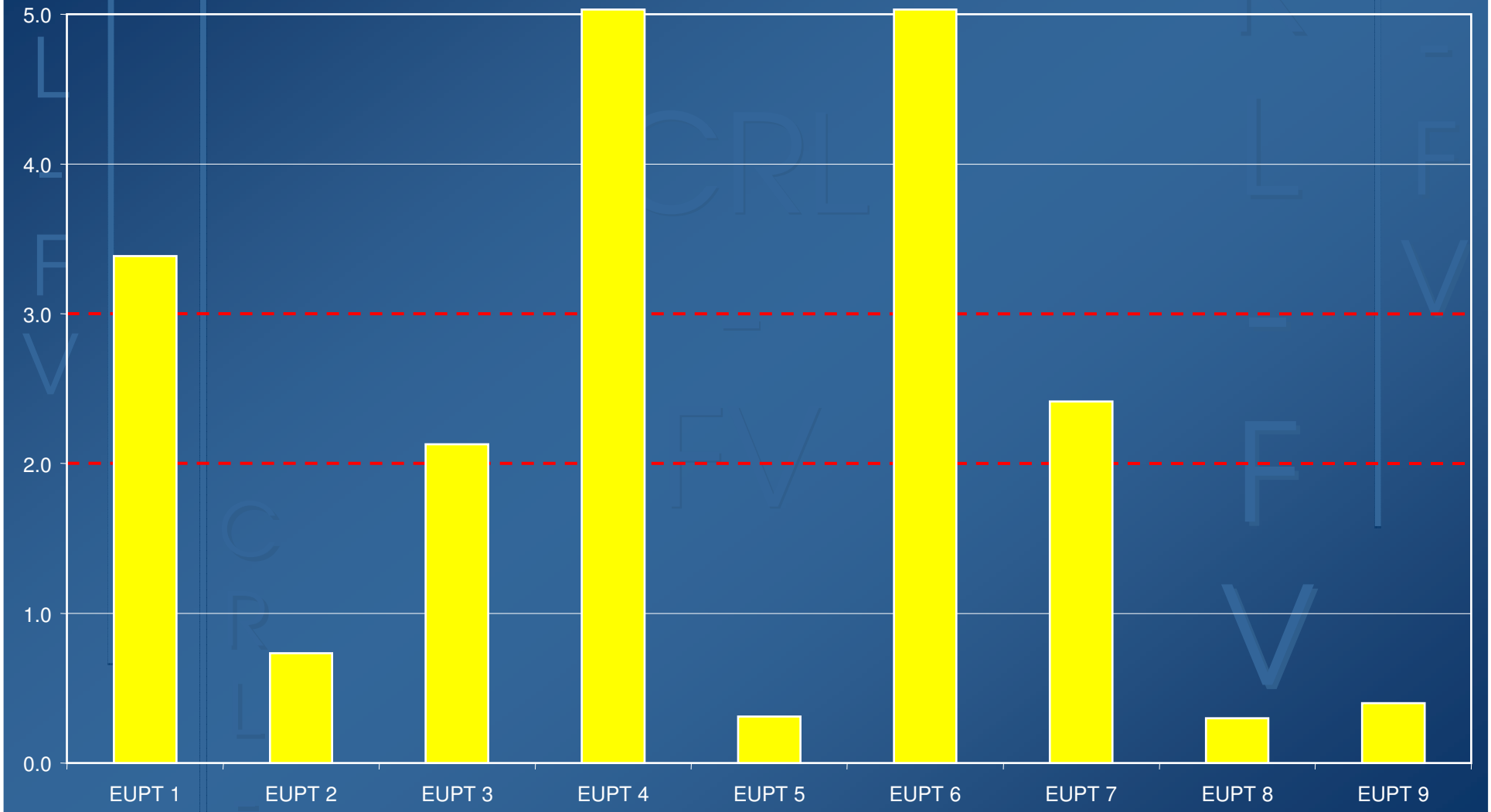


# WSZ results comparison for the same laboratory throughout the EUPTs

# Lab 1


5.0
4.0
3.0
2.0
1.0
0.0
EUPT 1
EUPT 2
EUPT 3
EUPT 4
EUPT 5
EUPT 6
EUPT 7
EUPT 8
EUPT 9

# Lab 3

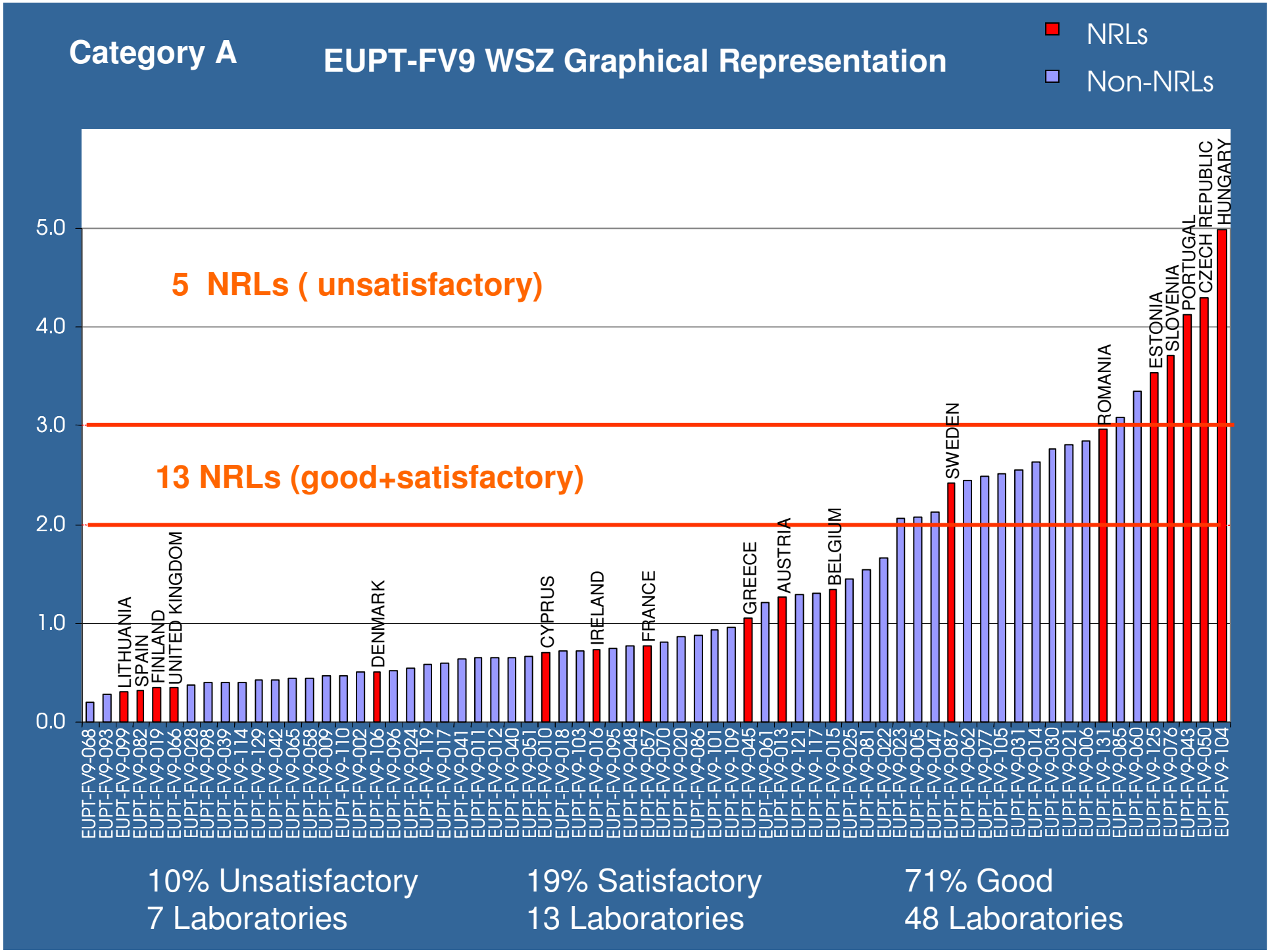





**For WSZ < 2.0, it was not enough!**

## Category A

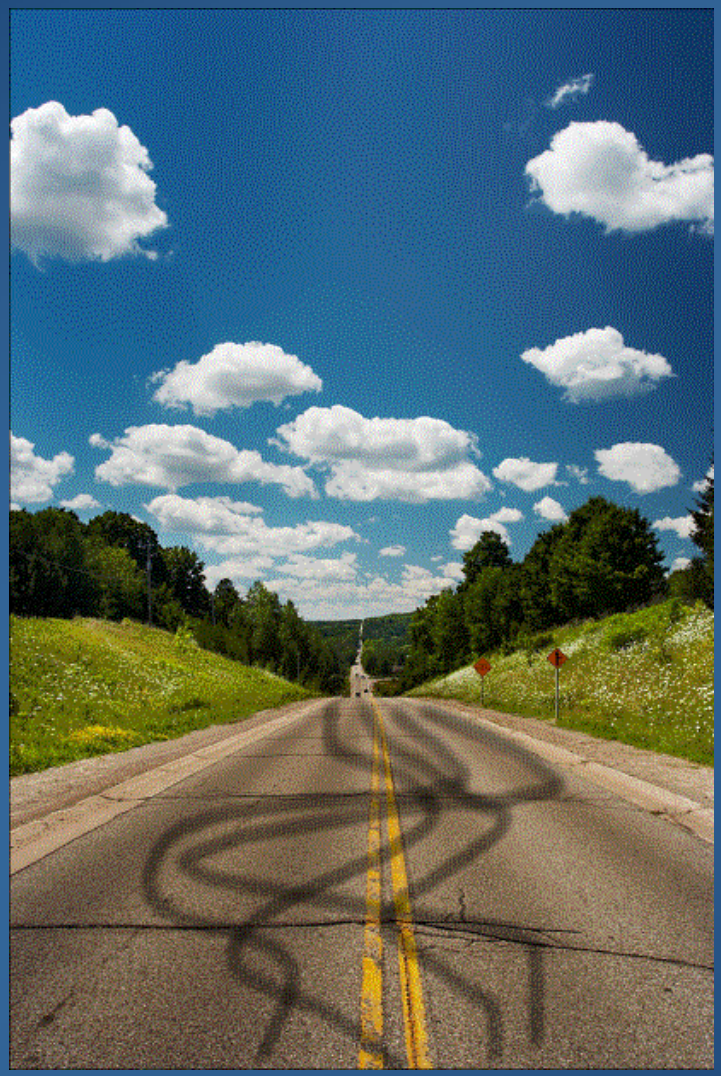
# EUPT-FV9 WSZ Graphical Representation




10% Unsatisfactory
7 Laboratories

19% Satisfactory
13 Laboratories

71% Good
48 Laboratories




CLIO

# Thanks for your attention