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Retour d’expérience sur la conduite des études de Toxicologie pour les substances naturelles « novel food »

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Substances Naturelles
&
Toxicologie

- CRL Hungary & Experiences with Natural Substances
- Novel Foods, Tests & Types of Substance
- Practical Issues for Testing
- Diet Design
- Diet Analysis, including NIR

Substances Naturelles
&
Toxicologie

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Charles River Hungary

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Charles River Hungary

- Centre of excellence for toxicology
- Two main buildings – Building A (rodent & laboratories) and Building B (large animal and avian suites)
- Latest Accreditation inspections:
  - GLP Certification –2022
  - ISO 9001, ISO 14001, ISO 50001 - 2022
  - AAALAC - 2022
- 110+ animal rooms
- Facilities extend to over ~12 000 m^2
- ~200 employees
- Non-pharma testing (~70% of activities)
- Inhalation, Diet/Gavage/Dermal
- Acute, Repeat dose, Reprotox, Ecotox

Testing Natural Substances

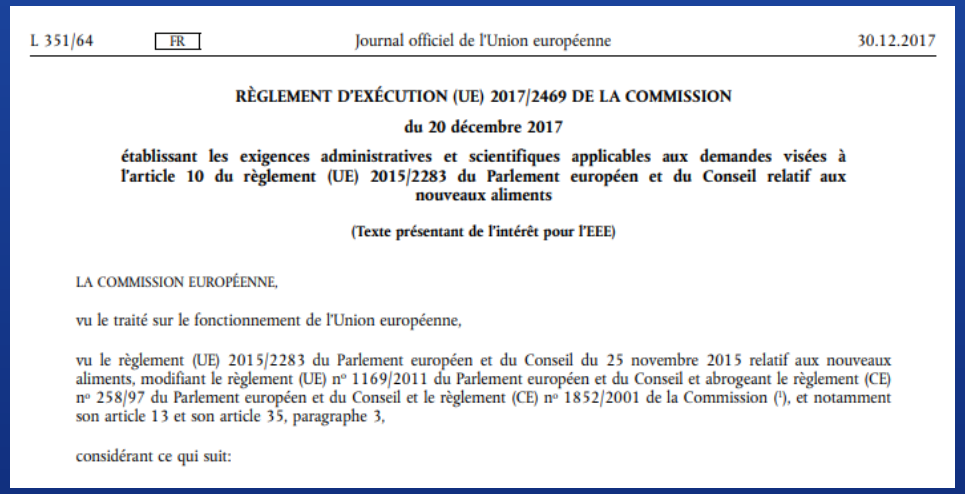
Natural Substances we work with include:

- **Novel Foods (to expand…)**
- **Natural products used in Agro: Plant extracts, Microbials, RNAs (Biopesticides vs Biostimulants)**
- **Natural products in pharma**
- **Natural products used in personal care (*usually in vitro only unless* ECHA *require worker protection under* REACH)**
- **Natural products used as chemicals (REACH, K-REACH, USA-EPA)**
- **Natural nanoparticles**
- **Cannabinoid products**

Charles River Hungary – Novel Food Testing

Regulations

- In the EU, EFSA
- In USA, FDA (GRAS is the most common USA approach)
- Other regions usually follow similar processes and requirements



L 351/64 FR Journal officiel de l'Union européenne 30.12.2017

RÈGLEMENT D'EXÉCUTION (UE) 2017/2469 DE LA COMMISSION

du 20 décembre 2017

établissant les exigences administratives et scientifiques applicables aux demandes visées à l'article 10 du règlement (UE) 2015/2283 du Parlement européen et du Conseil relatif aux nouveaux aliments

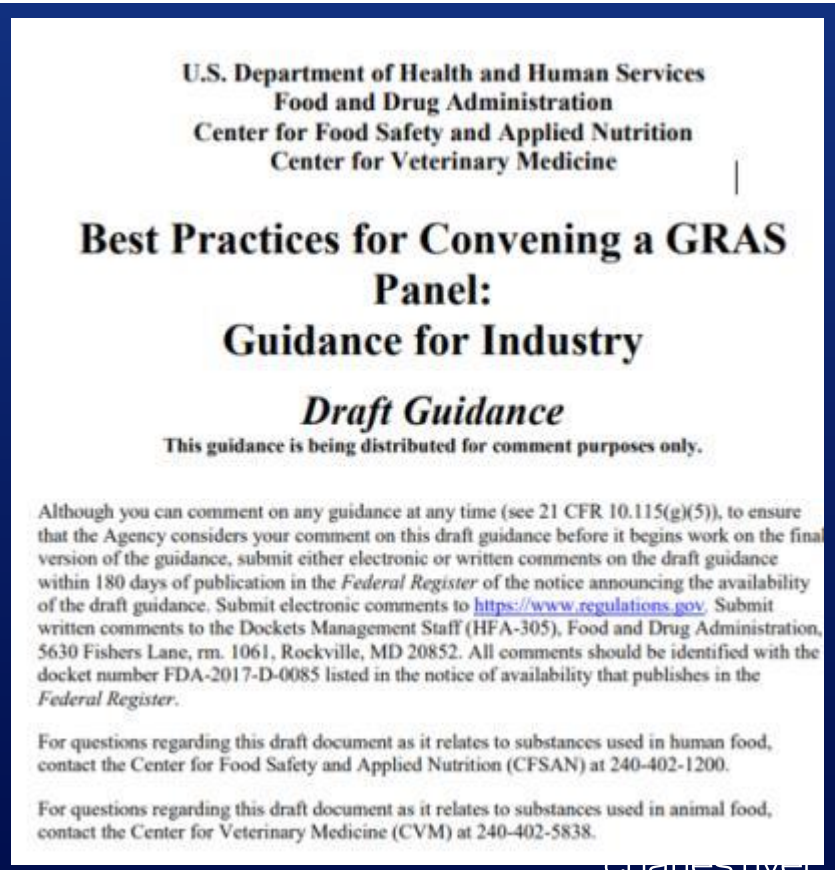
(Texte présentant de l'intérêt pour l'EEE)

LA COMMISSION EUROPÉENNE,

vu le traité sur le fonctionnement de l'Union européenne,

vu le règlement (UE) 2015/2283 du Parlement européen et du Conseil du 25 novembre 2015 relatif aux nouveaux aliments, modifiant le règlement (UE) nº 1169/2011 du Parlement européen et du Conseil et abrogeant le règlement (CE) nº 258/97 du Parlement européen et du Conseil et le règlement (CE) nº 1852/2001 de la Commission (1), et notamment son article 13 et son article 35, paragraphe 3,

considérant ce qui suit:



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Food Safety and Applied Nutrition
Center for Veterinary Medicine

Best Practices for Convening a GRAS Panel:
Guidance for Industry

Draft Guidance

This guidance is being distributed for comment purposes only.

Although you can comment on any guidance at any time (see 21 CFR 10.115(g)(5)), to ensure that the Agency considers your comment on this draft guidance before it begins work on the final version of the guidance, submit either electronic or written comments on the draft guidance within 180 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number FDA-2017-D-0085 listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document as it relates to substances used in human food, contact the Center for Food Safety and Applied Nutrition (CFSAN) at 240-402-1200.

For questions regarding this draft document as it relates to substances used in animal food, contact the Center for Veterinary Medicine (CVM) at 240-402-5838.

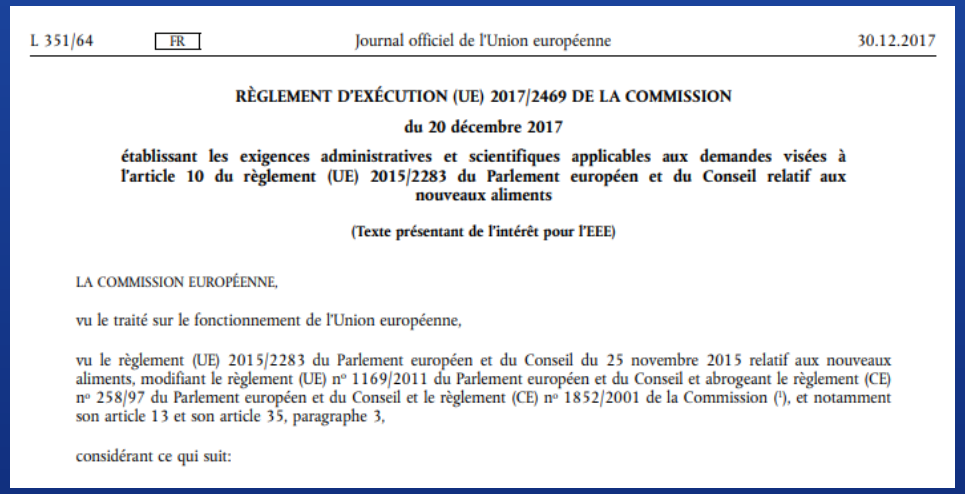
Charles River Hungary – Novel Food Testing

Regulations – Testing Requirements

Typically, as a MINIMUM:

- Genotoxicity (bacterial & mammalian *in vitro*)
- Address ADME
- 90 day rodent test (OECD 408)

With Preliminary Testing as appropriate, and analysis of formulation/diets for Concentration, Homogeneity & Stability with a suitably specific method



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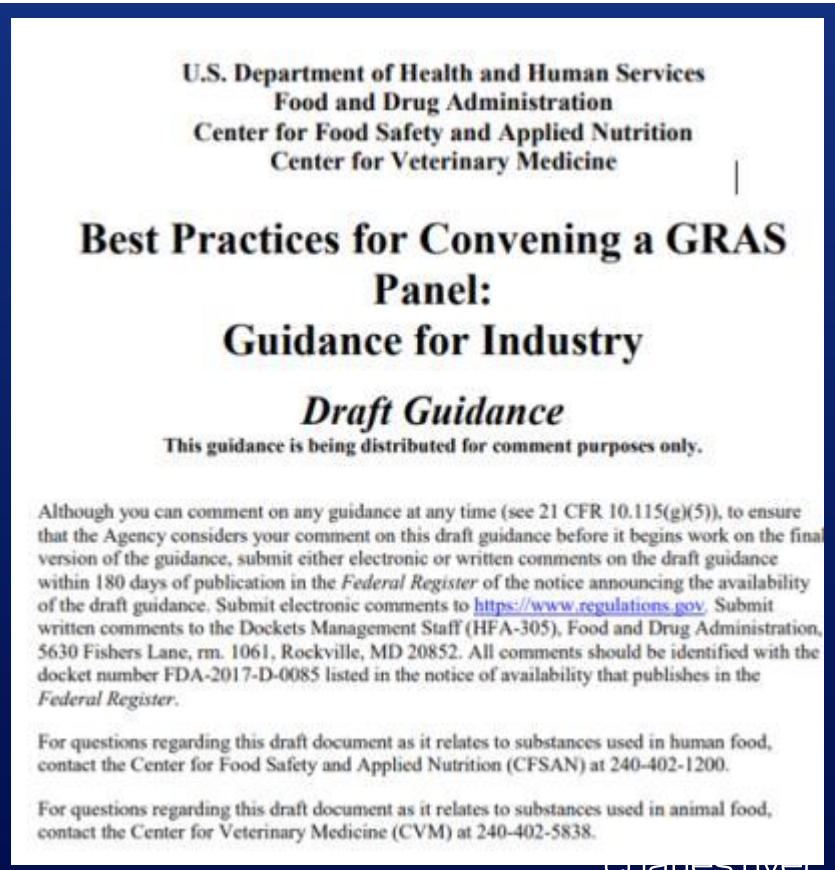

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What Types of Novel Foods are Evaluated?

Charles River Hungary – What Types of Novel Foods?

Major Food Ingredients vs Minor Food Ingredients

Charles River Hungary – What Types of Novel Foods?

Major Food Ingredients

(where a large quantity will be consumed in human or animal diet)

- e.g. ‘Replacement product’ for meat or a significant protein source.
  Commonly one of the following:
- Cell-Culture origin (lab-grown meat)
- Plant origin
- Insect protein products
- Bacterial origin
- Algae origin
- Seaweed origin

Charles River Hungary – What Types of Novel Foods?

Minor Food Ingredients

(where a small quantity, or ~zero, will be consumed in human or animal diet)

- e.g. Food Additives

  Commonly one of the following:
- Food processing enzymes
- Amino Acids or Peptides
- Oil type structures
- Sugar type structures
- Other plant products considered healthy or beneficial (e.g. CBD)

Natural Products not requiring testing

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Charles River Hungary – Practical Issues

Genotox Testing

- Ames Test – Histidine Content
- Organisms that grow on agar or in media
- How to formulate 3D cell products
- Testing nano-materials

Charles River Hungary – Practical Issues

Rodent Testing - Minor Food Ingredients

- Consider the Safety Margin with the Sponsor, typically a factor of 200 is considered appropriate. Often this will be no more than ~ 1000 mg/kg/day in rats
- Decide on Gavage vs Dietary incorporation
- Analysis of Gavage or Diet formulations for defined chemicals – LC/MS, GC, HPLC etc.
- Enzymes used in food processing are typically not analysed by specific methods in Tox labs. There are suitable approaches to measure test item (as TOS)
- Take care to define the 'Test Item' and the required dose levels – to avoid retesting
- Consider nutritional imbalance

Charles River Hungary – Practical Issues

Rodent Testing - Major Food Ingredients

- **Consider the Safety Margin with the Sponsor, typically a factor of 200 is considered appropriate for an EFSA submission (according to consultants).**
- **Consider a human consumption of 10 g/day of a novel food ingredient:**

Charles River Hungary – Practical Issues

Rodent Testing - Major Food Ingredients

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- **Consider a human consumption of 10 g/day of a novel food ingredient:**

  In a 70 kg person, that is ~140 mg/kg/day

  With a 200 fold factor, the High dose would be ~30000 mg/kg/day

Charles River Hungary – Practical Issues

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Charles River Hungary – Practical Issues

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Charles River Hungary – Practical Issues

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  OECD 408 indicates not to use more than 5% in diet

What is the Intake in humans?

e.g. A meat replacement for burgers ….

Burgers range widely in size, but 50 to 200g is not uncommon

(for one burger – some people might eat more than one a day)

A rat can not eat 200 times ≅ 2x200g/day !

Charles River Hungary – Practical Issues

Rodent Testing - Major Food Ingredients

- Consider the Safety Margin with the Sponsor, typically a factor of 200 is considered appropriate, but need to be pragmatic!
- Decide on Gavage vs Dietary incorporation
- Consider nutritional imbalance
- Diets across the groups should be ~ isocaloric
- Imbalance of essential amino acids may have adverse outcomes
- Some natural products can be high in fibre, salt (NaCl) or Iron (Fe) for example

Charles River Hungary – Practical Issues

So what do we do if >5% in diet is required to approach the maximal intake?

One approach we use is to make specific diets for a test item:

For example:

- For lipid test items, match the control diet by adding corn oil (~isocaloric)
- For a protein substance (typically they are ~17% protein) then make a diet where the sole protein source for the High dose group is the test item. Then make an equivalent diet for the controls with a 'safe' common protein such a casein. [Low & Mid diets are a mixture of these].
- BUT verify the amino acid balance, and check the other components.
- Usually the diet design requires a specialist nutrition expert, to avoid unexpected problems with study result interpretation.
- Additional control groups for high fibre, high salt, high iron etc. may be highly desirable.

Charles River Hungary – Practical Issues

The next issue is how to analyse the diets to show Concentration, Homogeneity & Stability?

For an Algae or Insect based powder intended for use as a protein replacement in human food, the dietary dose levels will be ~ 20%, 10% and 5% and one or two control groups.

- HPLC or LC/MS methods are generally unsuitable to measure these types of product. Sometimes a ‘chemical marker’ can be found for establishing concentration, but often it represents <2% of the test item. So any stability assessment is not valid.
- We have developed InfraRed technology, using the type of device which is now the default in most continuous-flow production facilities (for everything from chocolate to petroleum products).
- Every organic product has distinct IR ‘fingerprint’. Historically, IR could only be used for qualitative assessments, but the latest devices combined with computer statistical methods do allow a fully quantitative analysis to be made for very complex mixture like rodent diet with test item.

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Basics of IR Analytics – A Model system

semisynthetic diet mix with additional L-casein

+ Nutrients:
  - 17.4% crude protein
  - 38.5% starch
  - 9.4% sugar
  - 5.0% crude fat
  - 5.0% crude fibre
  - 4.4% crude ash

Calibration range: 1-10 w/w% casein added

+ 10 calibration samples + 2 quality check samples

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Instrumentation

Hardware:

Viavi MicroNIR Pro
(miniaturized Near Infrared Spectrophotometer)

Diffuse reflectance mode
Glass cuvettes
200 ms spectral recording
Spectrum recording in triplicates

Chemometrics Analysis Software:

Camo Unscrambler 11

Unscrambler
by Camo Analytics

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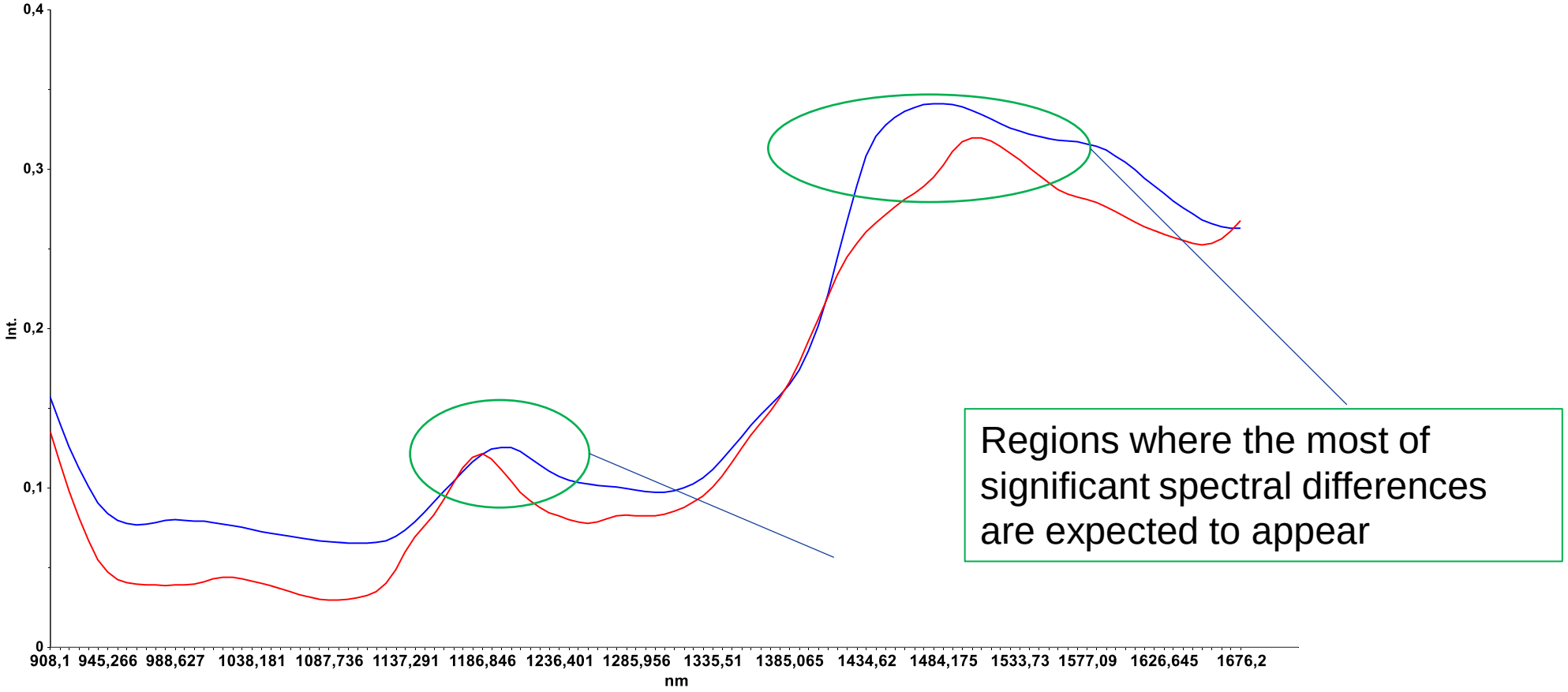
Charles River Hungary – Novel Food IR Analytics

Casein vs Diet



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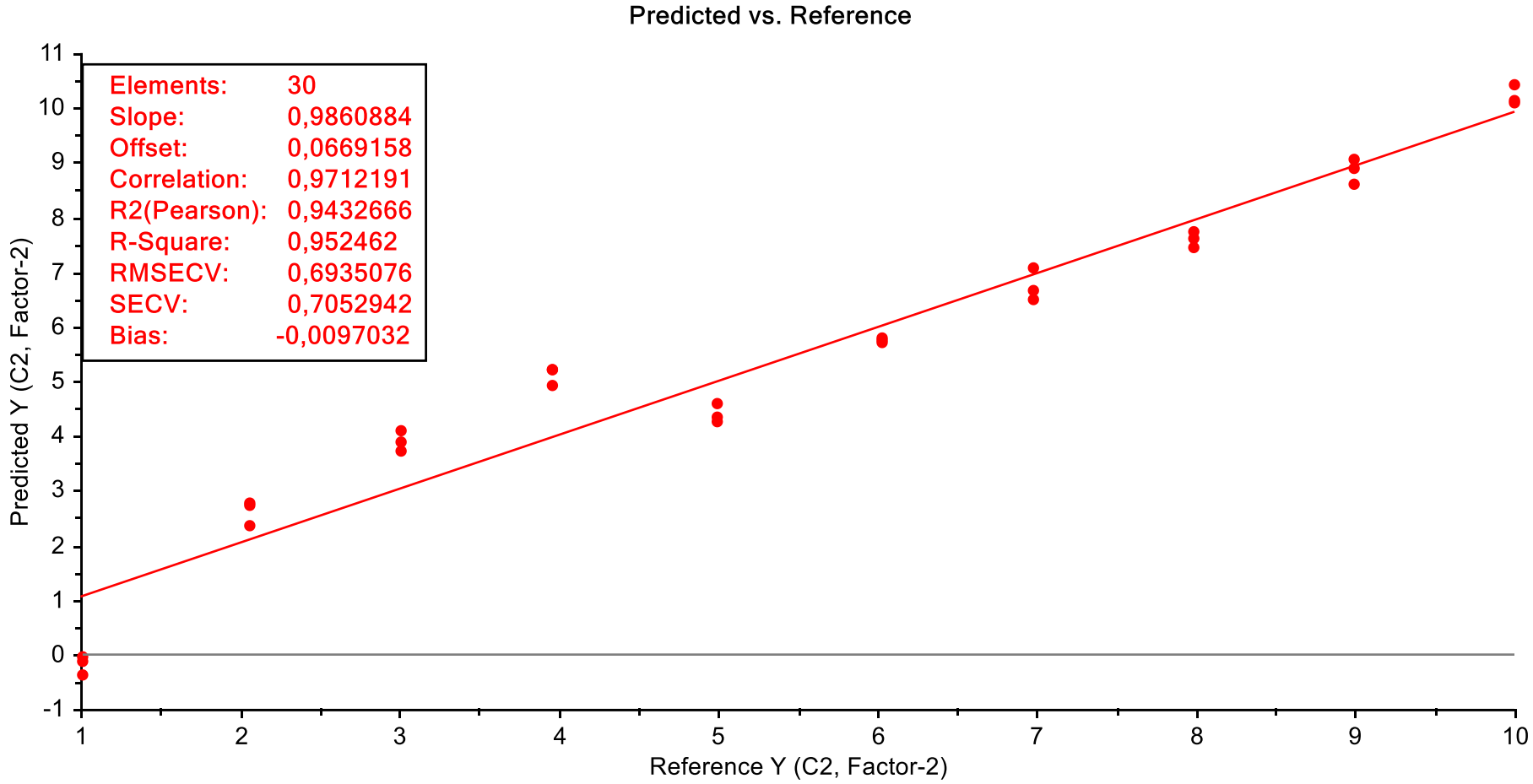
Charles River Hungary – Before Data Evaluation

Raw regression model (10 concentrations)



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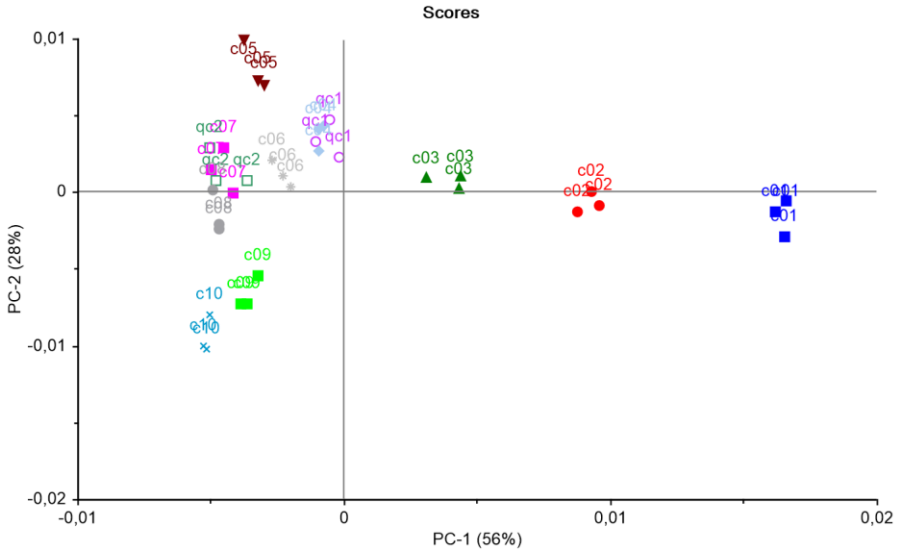
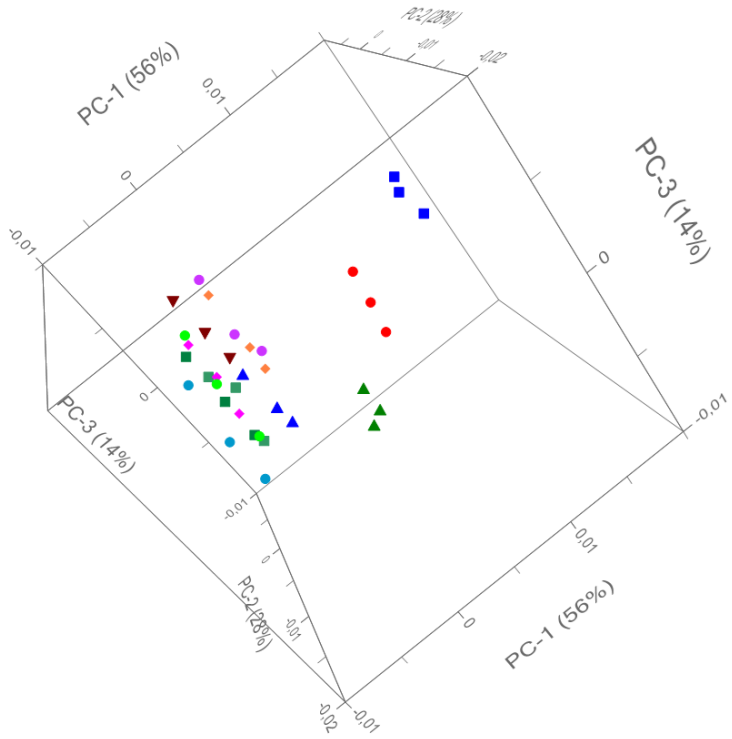
Statistical analysis: PCA (principal component analysis)





The first few calibration points form distinct groups ➡ the calibration series must be split to subsets

c01 c02 c03 c04 c05 c06 c07 c08 c09 c10 qc1 qc2

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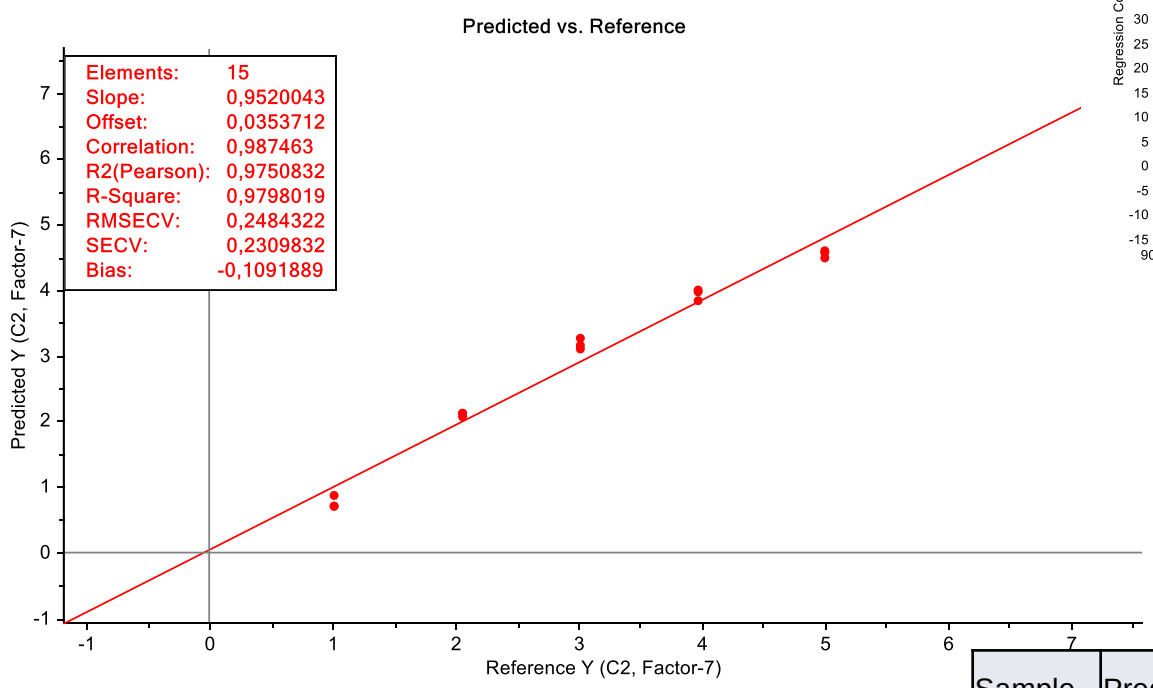
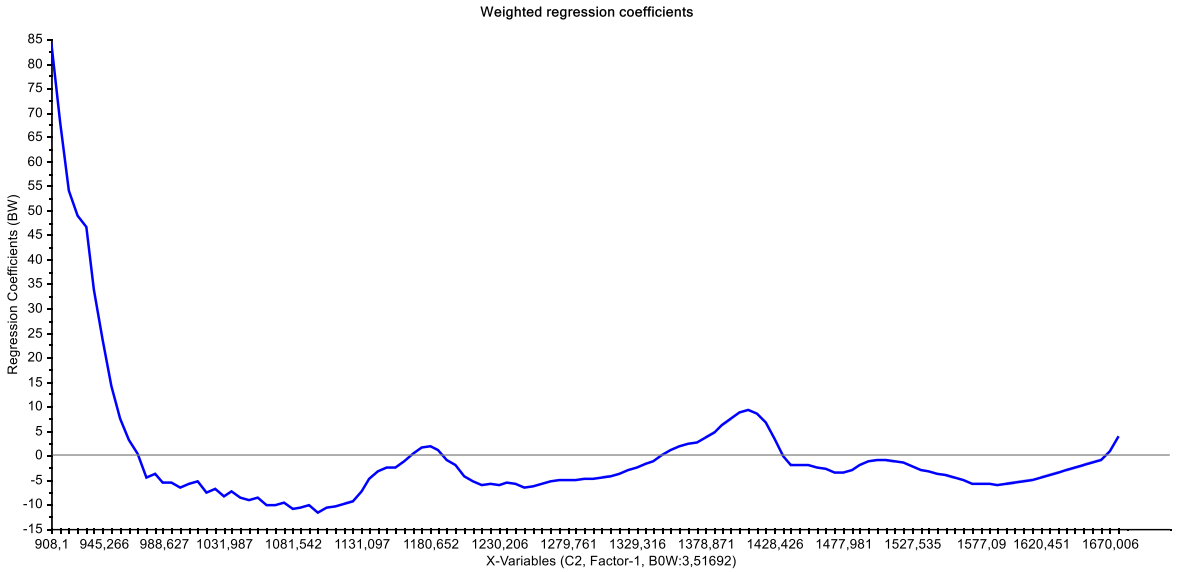
Low calibration domain





Sample	Predicted (%)	Nominal (%)	Recovery
QC1	3,67	3,50	105
	3,69		106
	3,69		105

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Summary for NIR analysis

-Successful quantitation of casein in semi-synthetic diet

-PCA pre-screening for data visualization and subset definition

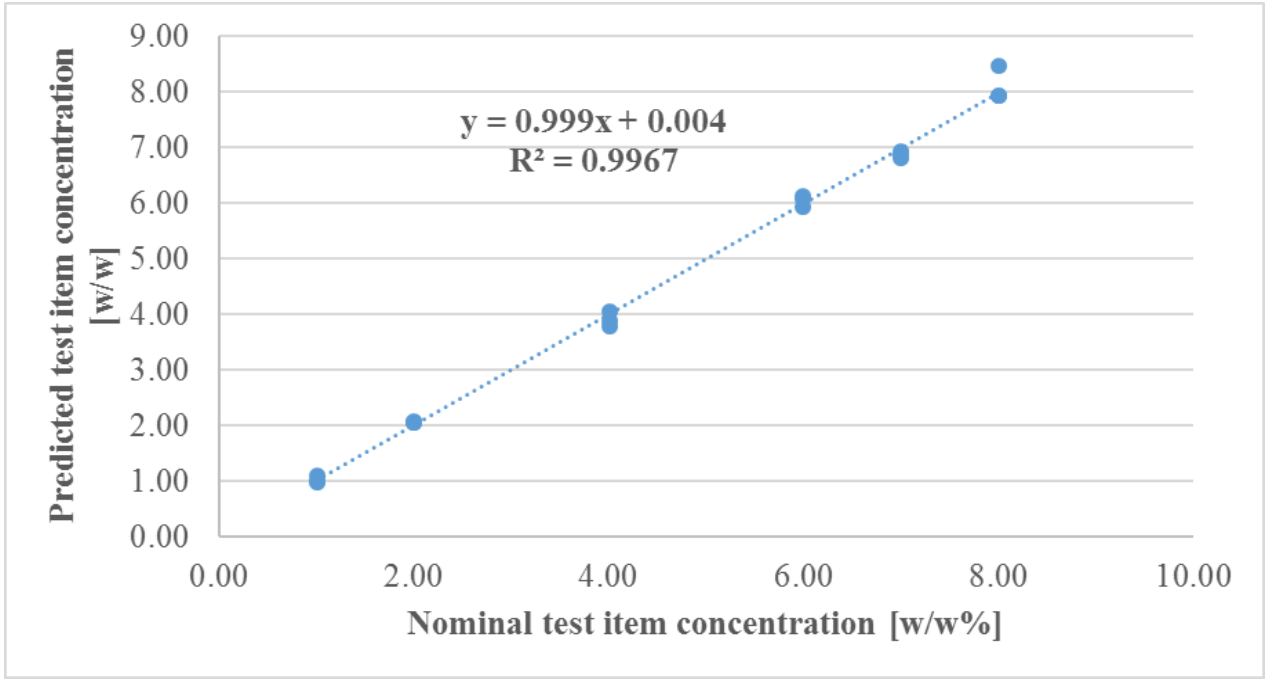
-Analysis time 0.3-2 seconds per sample.

-Improved results by use of concentration domain dependent models, based on careful use of QCs

-Note that sample handling & preparation is very important

NOTE: Other IR approaches may be necessary, e.g. MIR or pastille analysis.

Charles River Hungary – Some Real NIR Results



Repetition	Conc. [%]
1	5.6740
2	5.4580
3	5.5369
4	5.6401
5	5.5086
6	5.7428
7	5.4475
average	5.5768
RSD%	2.0

Parameter	Measured
Mean recovery at LOQ	100.9%
RSD% at LOQ	3.6%
Min mean recovery above LOQ	99.2%
Max mean recovery above LOQ	101.2%
Slope	0.9953
Intercept	0.0231
R^2	0.9985

SUMMARY

- Background of CRL Hungary
- Experiences with Natural Substances
- Novel Foods - Requirements
- What Types of Novel Foods are Tested?
- What products do not require testing?
- Practical Issues (Genotox)
- Practical Issues for Food Additives
- Practical Issues for Major Food Ingredients
- Design of Diets for Major Ingredients
- Need for Reference Controls
- Diet/Formulation Analysis for Natural Substances
- NIR Technology to analyse Natural Substances

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