Implementation of CIR (EU) 2021/808

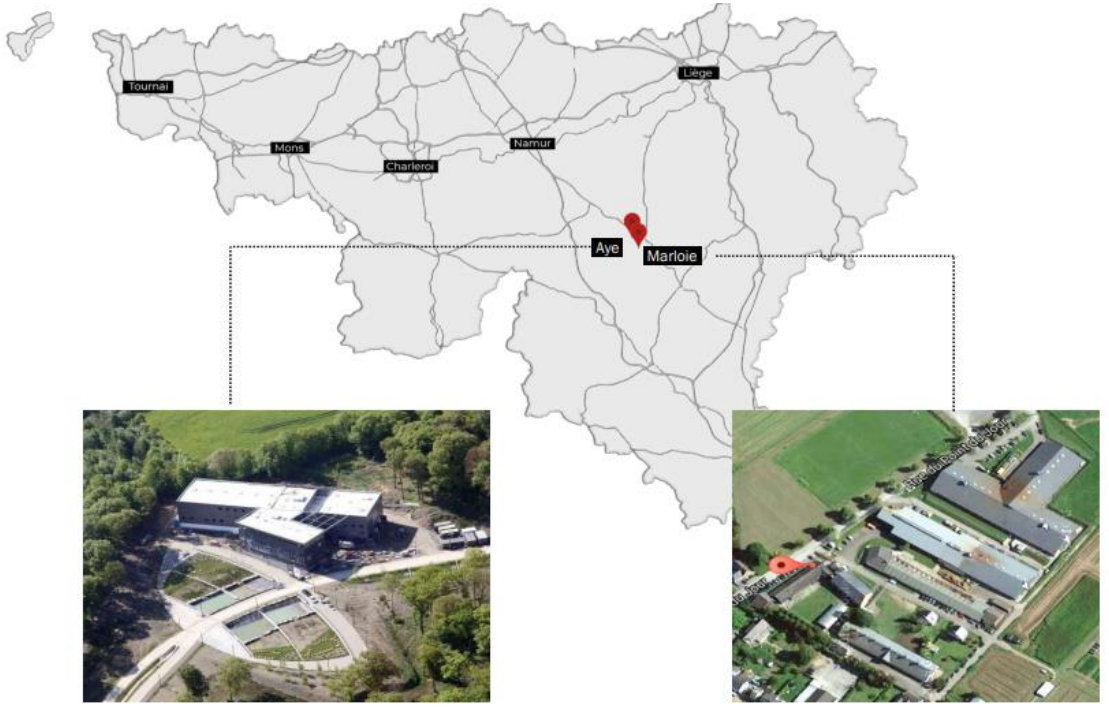
Illustration on forbidden/non authorized substances

SARAF webinar, 20 December 2023

SARAF
School for Advanced Residue Analysis in Food

Dr Nathalie Gillard

CER Groupe is a collective research center supporting human and animal health by providing products and services to companies active in the biotech, pharma, food safety and agriculture sectors.



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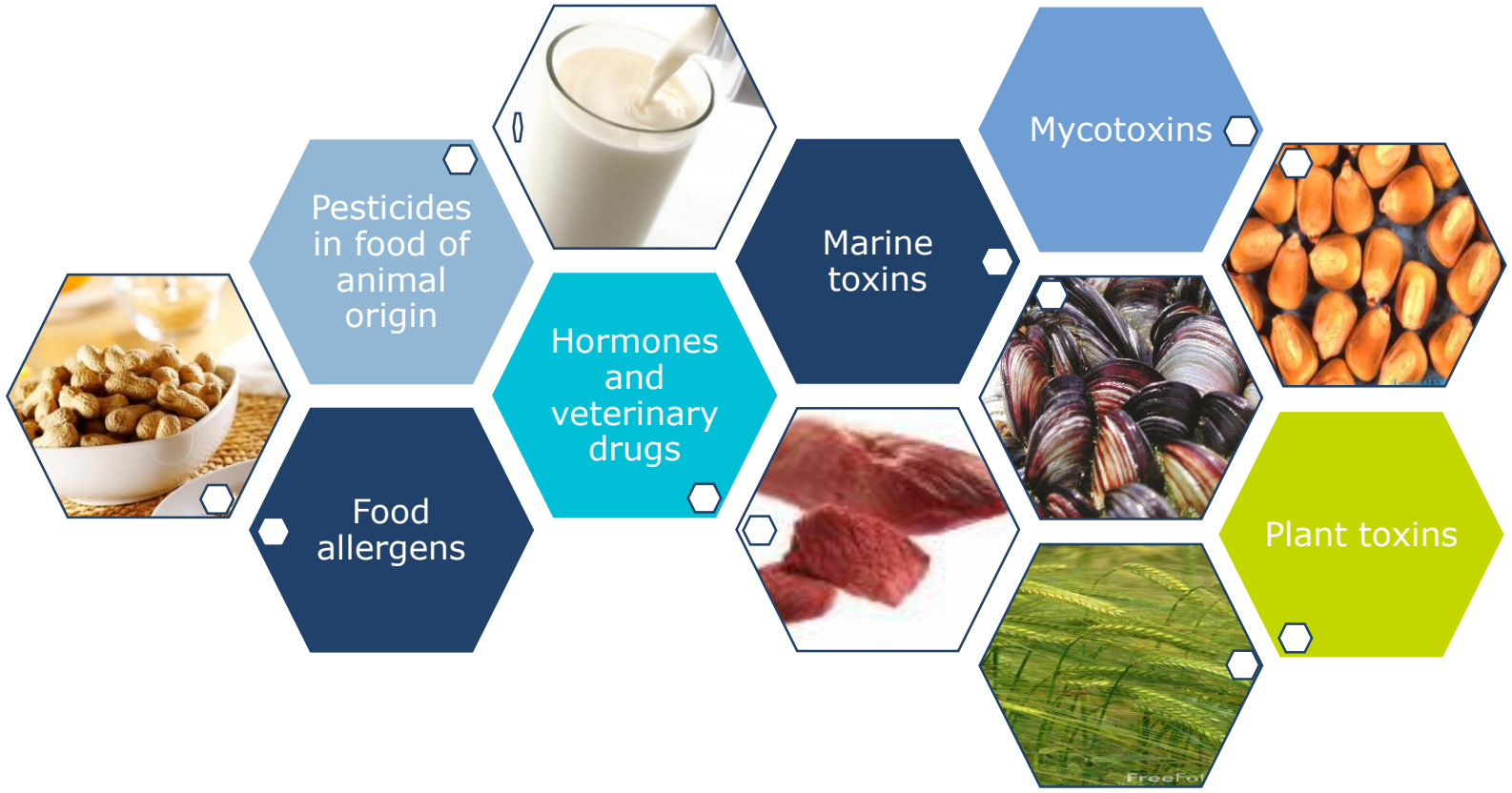
> 200 p. incl. 60 DVM/PhD/MSc

2 sites / 15000 sqm

CER & Food Safety : belgian NRL activities



CER & Food Safety : official control laboratory activities (ISO17025)

Organisme belge d'Accréditation
Belgische Accreditatieinstelling
Belgische Akkreditierungsstelle
Belgian Accreditation Body

EA MLA Signatory

Accreditation Certificate No. 073-TEST

In compliance with the provisions of the Royal Decree of 31 January 2006 setting up BELAC, the Accreditation Board hereby declares to have granted accreditation conform the requirements of the standard EN ISO/IEC 17025:2017 to:

CER-Groupe
Rue de la Science 8
6900 Marche-en-Famenne

The body demonstrated the competence to perform the activities in the activity sites, as described in the scope of accreditation 073-TEST which is an integral part of the present certificate.

The current version of the scope of accreditation is available at www.belac.be.

This certificate remains valid as long as the body continues to meet the accreditation conditions.

The Chair of the Accreditation Board BELAC,

Maureen LOGGHE

Version : **8**

Validity period : **2020-09-07 - 2024-04-16**

Original version of this certificate is in French.

.be

BELAC BELAC BELAC

BELAC 6-319 RO 2020

Flexible scope

- Veterinary drugs, hormones, forbidden substances
- Non-dioxin like PCBs
- Unauthorized dyes
- Food allergens, lactose, sulfite
- Mycotoxins, plant toxins, marine toxins
- Pesticides
- Proteins of animal origin

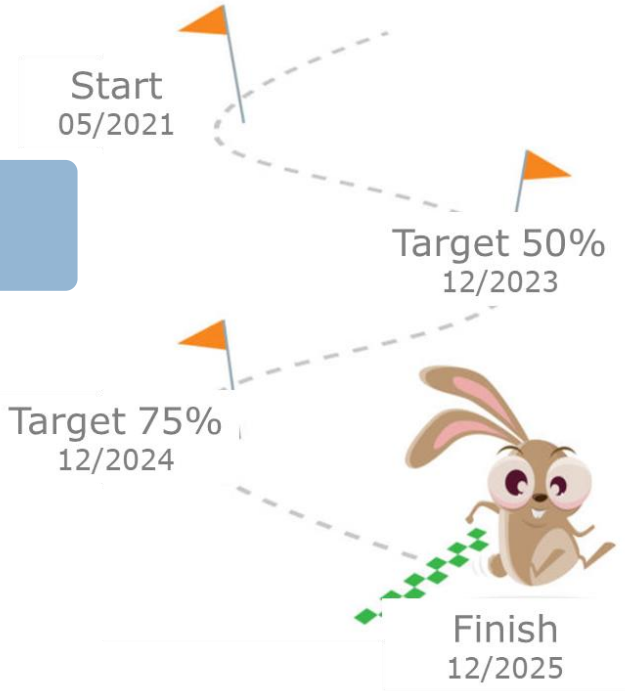
Revalidation of existing methods at CER Groupe according to 2021/88

✓ Current ISO17025 scope of CER Groupe :

Full scope : 13179 combinations method/matrix/species/compounds

VMPR : 9050 combinations (4845 for group A)

VMPR : 336 combinations method/matrix/species



✓ <u>Revalidation Priorities :</u>

1. Methods already fit-for-purpose (MMPRs already OK)
2. Most frequently used methods (high number of samples)
3. Methods requiring modifications for lower MMPRs
4. Methods requiring inclusion of new compounds/matrices regarding MMPRs
5. Other methods

New EU regulation for VMPR : reference documents

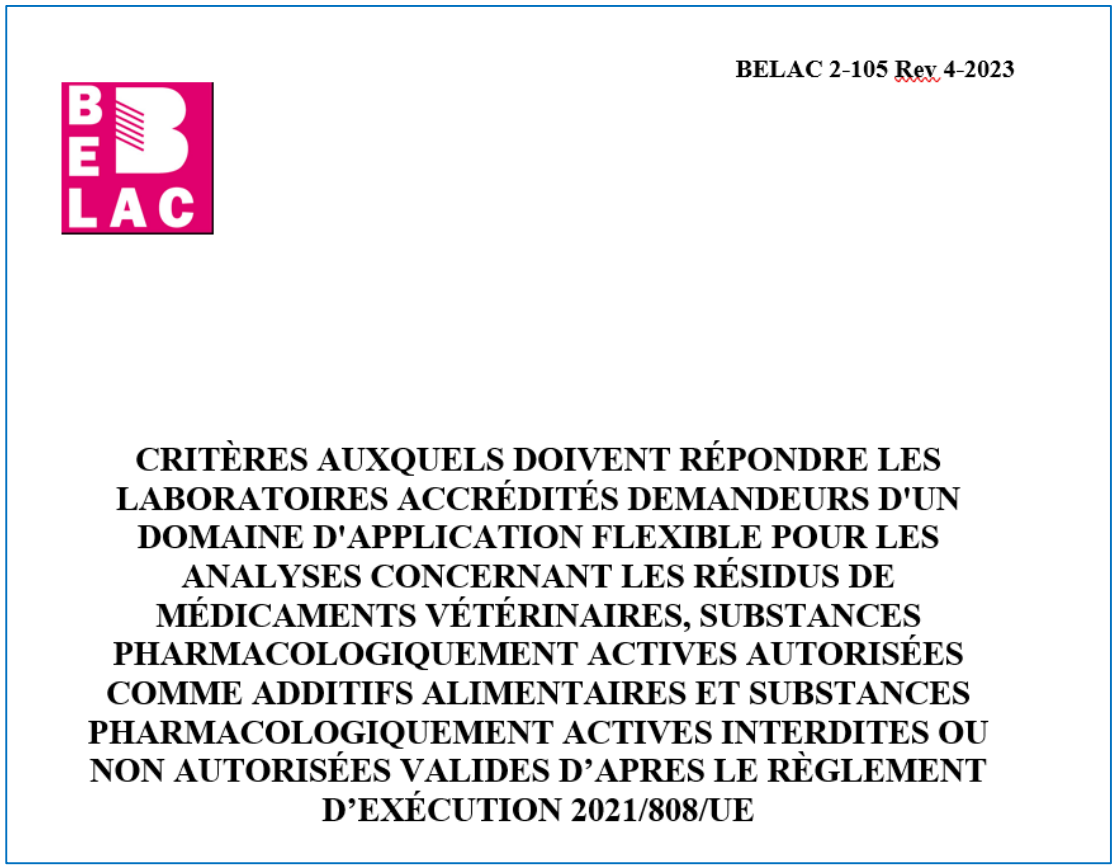
- Regulation 2021/808 regarding method validation
- Regulation 2022/1644 regarding new classification of substances
- Technical EURL Guidance Documents were planned:
  1. *on the quality control during routine analysis (ongoing method performance verification) (finalized, version 1.1)*
  2. *on confirmation method validation (finalized, version 1.1)*
  3. *on the extension of methods (finalized, version 1.0)*
  4. *on validation of screening methods (not yet finalized)*
  5. *EURL Guidance Document on the validation of HRMS methods*
  6. *Standard addition*

https://eurl-residues.eu/eurl-portal/portal-guidance-documents/

In Belgium, additional guideline for flexible scope management of VMPRs

BELAC 2-105 R3-2015 F (fgov.be)

Belgian guideline for flexible scope management of VMPR (BELAC 2-105)



BELAC 2-105 Rev 4-2023

CRITÈRES AUXQUELS DOIVENT RÉPONDRE LES LABORATOIRES ACCRÉDITÉS DEMANDEURS D'UN DOMAINE D'APPLICATION FLEXIBLE POUR LES ANALYSES CONCERNANT LES RÉSIDUS DE MÉDICAMENTS VÉTÉRINAIRES, SUBSTANCES PHARMACOLOGIQUEMENT ACTIVES AUTORISÉES COMME ADDITIFS ALIMENTAIRES ET SUBSTANCES PHARMACOLOGIQUEMENT ACTIVES INTERDITES OU NON AUTORISÉES VALIDES D'APRES LE RÈGLEMENT D'EXÉCUTION 2021/808/UE

Publication planned for 01/2024
(french & dutch, english version later in 2024)

✓ Historical content :

- how to obtain a flexible scope
- validation concepts

✓ Modifications linked to :

1. Update to fit with new Regulations/guidance
   - new classification of substances
   - new classification of matrices
   - modification of validation requirements
2. News issues to be implemented
   - On-going validation
   - Standard addition
   - Target concentration based on ALARA principle for not authorized compounds
   - Analysis of dual use substances

Belgian guideline for flexible scope management of VMPR (BELAC 2-105)

- ✓ Flexible scope could be requested for matrices or parameters

- ✓ Classification of matrices
- ✓ Flexibility possible at different levels (if ≥ 50% already validated/accredited)
    - sub-matrices
    - principal matrices
    - groups of matrices

- ✓ Classification of parameters
- ✓ Flexibility possible at different levels (if ≥ 50% already validated/accredited)
    - principal parameters
    - sub-groups

BELAC 2-105 guideline : groups of matrices

Flexibility on Matrices :

Main matrix	Sub-matrix	Typical representative species and/or matrices
Group of matrices: **1. BIOLOGICAL MATRIXES INCLUDING FOOD AND WATER**		
1. Matrices of animal origin	Muscle	Cattle, sheep, goats, cervids (red deer, roe deer, reindeer, etc.) Pigs, wild boar Poultry, small game birds (duck, goose, pigeon/dove, pheasant, partridge) Equidae Lagomorphs (rabbits, hares) Fish, molluscs, crustaceans Reptiles
	Liver	
	Kidney	
	Grease	
	Injection site (meat containing -)	
	White offal (head, feet, ears, guts, etc.)	
	Red offal (heart, cheek, marrow, brain, tongue, sweetbread, lung, etc.)	
	Blood, plasma, serum	
	Thyroid gland	
	Eggs	Chickens, ducks, geese, ostriches, insects, amphibians, fish, reptiles
	Insects	Larvae, adult insects, Insect meal
	Products processed from animal tissues[***]	Pâté, minced meat and other preparations, egg products Preparation for animal feed, fish meal
2. Milk and dairy products	Milk	Cattle, goats, sheep, horses
	Processed milk products[***]	Milk powder, beaten milk, ice cream, butter, cheese, cream, yoghurt Milk replacer (animal feed)
3. Animal excretion products and water	Urine	Cattle, sheep, goats, pigs, horses, etc. **4/7**
	Faeces	
	Bile	
	Saliva	
	Water	Drinking water
4. Hair, fleece, eyes and products containing keratin	Hair	Cattle, sheep, goats, pigs, horses, etc.
	Fleece (smears, swabs)	
	Eye and retina	
	Products containing keratin	

BELAC 2-105 guideline : groups of matrices

5. Animal feed, foodstuffs of plant origin and raw materials	Plant matrices with a high water content	Pome fruit (apples, pears and by-products for animal feed) Stone fruit (apricots, cherries, peaches and by-products for animal feed) Other fruit (Bananas and by-products for animal feed) Bulb vegetables (onions, leeks and by-products for animal feed) Fruiting vegetables/cucurbits (tomatoes, peppers, cucumbers, melons and by-products for animal feed) Brassicaceae (Cauliflower, Brussels sprouts, cabbage, head cabbage, broccoli and by-products for animal feed) Fresh leafy vegetables and herbs (Lettuce, spinach, basil and by-products for animal feed) Stem vegetables (Celery, asparagus and by-products for animal feed) Fresh pulses (snow peas, peas, broad beans, princess beans, bush beans, flageolet and by-products for animal feed) Root and tuber vegetables (sugar and fodder beet, carrots, potatoes, sweet potatoes and by-products for animal feed) Mushrooms (Mushrooms, chanterelles and by-products for animal feed) Fodder crops (grasses, lucerne, clover, rape and by-products for animal feed) Silage (Silage made from maize, clover, grasses and by-products for animal feed)
	High-acid matrices	Citrus fruit (lemons, mandarins, clementines, oranges and by-products for animal feed) Berries and small fruit (strawberries, blueberries, raspberries, redcurrants (black, red, white), grapes and by-products for animal feed)
	Matrices with a high oil content and very low water content	Vegetable oil (palm oil, rapeseed oil, soya oil) Nuts (walnuts, hazelnuts, chestnuts) Oilseeds (turnip rape, sunflower, cotton, soya and peanuts) Nuts and oilseed pastes (peanut butter, tahina, hazelnut paste) Compound feedingstuffs with a high oil content and very little water (Compound feedingstuff with a high lipid content)
	Matrices with high oil content and intermediate water content	Fruit and oilseed products (olives, avocados and their pastes) Oilseed cake and meal (olive, rapeseed, sunflower, cottonseed, soya cake)

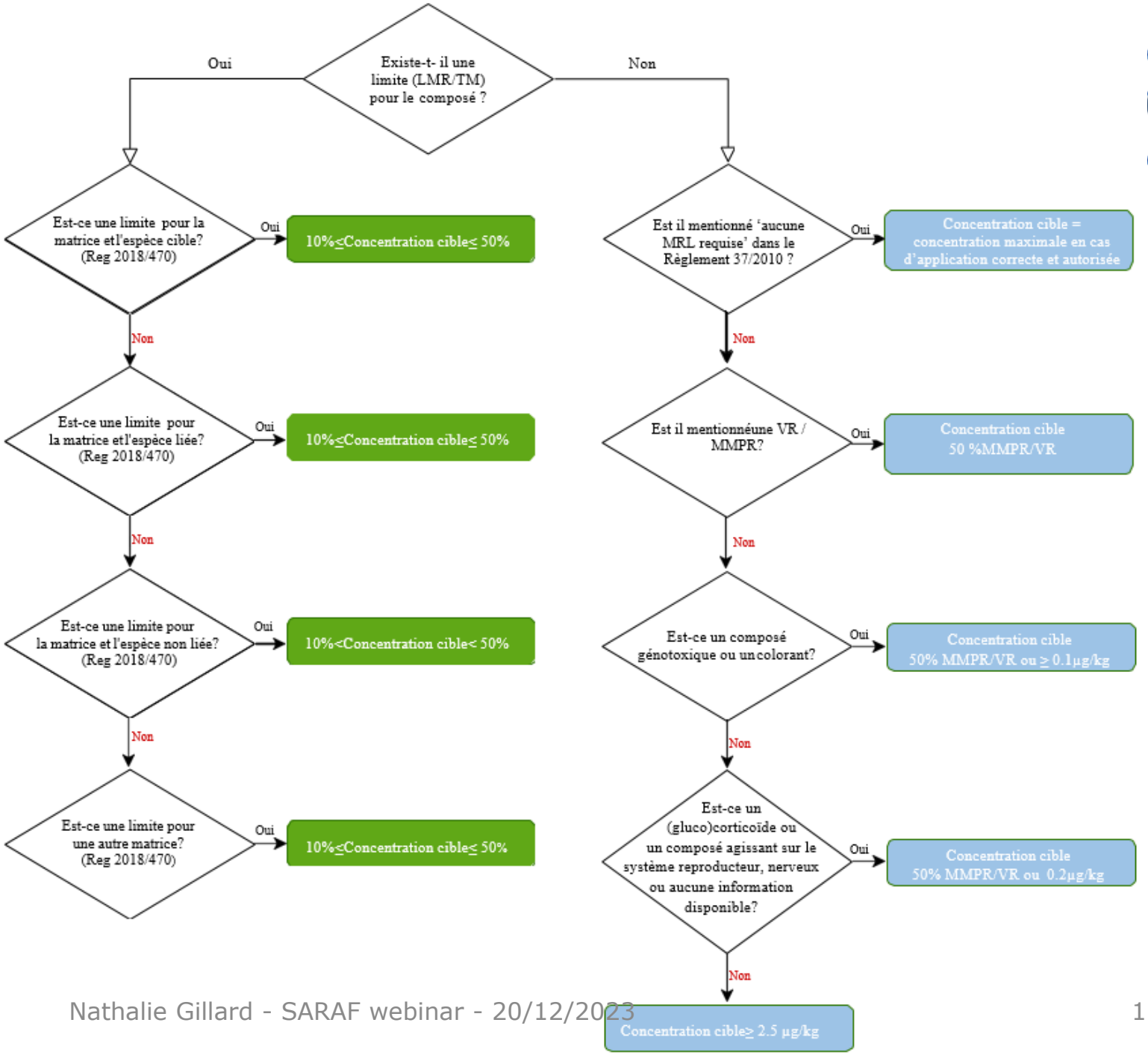
BELAC 2-105 guideline : groups of matrices

	Matrices with high starch or protein content and low water and fat content	Dried pulses (broad beans, dried fava beans, dried beans (yellow, white, brown, spotted), lentils and by-products for animal feed) Cereals and derived products (wheat, rye, barley and millet, maize, rice (kernels, flakes, by-products), bread, biscuits, breakfast cereals, spaghetti, flour and animal feed by-products (hulls and bran, brewing and distilling grains), cereal-based compound feed for animals)
6. High-carbohydrate animal and plant matrices[**]	Honey and beekeeping products	Honey, wax, propolis, royal jelly, pollen
	Products processed from fruit, root and tuber vegetables, cereals or other plant matrices	Sultanas, dried apricots, dried plums, fruit jams; molasses; sugar syrup (starch-based, agave-based, etc.), supplementary food for bees.
7. Singular matrices [*] [****]	Food supplements	
	Food additives and animal feed additives	Enzymes
	Plant matrices and unique by-products	Hops, cocoa beans and by-products, coffee, tea, spices, straw, hay, fatty acid distillate, potato protein
Matrix group: **2. MATERIAL SAMPLES**		
1. High concentration samples	Preparations, smears, syringes and other materials	

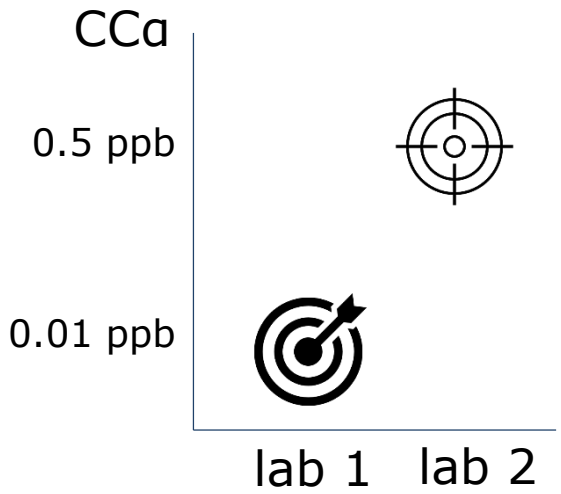
BELAC 2-105 guideline : groups of matrices Flexibility on parameters :

Parameter group	Subgroup	Main parameters
Group A : Prohibited or unauthorised substances	1. Substances having a hormonal or thyrostatic action and beta-agonists whose use is prohibited by Directive 96/22/EC	a. Stilbenes, c. Steroids, d. Resorcylic acid lactones, including zeranol b. Antithyroid agents e. Beta-agonists **2/3**
	2. Prohibited substances listed in table 2 of the annex to regulation (EU) 37/2010	a. Chloramphenicol b. Nitrofurans c. Dimetridazole, metronidazole, ronidazole and other nitroimidazoles d. Other substances **2/4**
	3. Pharmacologically active substances not listed in Table 1 of the Annex to Regulation (EU) 37/2010 or substances not authorised for use in food-producing animals in accordance with Regulation (EU) 1831/2003	a. Dyes b. Plant protection products within the meaning of Regulation (EU) 1107/2009 (Pesticides) and biocides within the meaning of Regulation (EU) 528/2012 which may be used in the rearing of food-producing animals; d. Coccidiostats, histomonostats and other antiparasitic agents. (dual-use substance) c. Antimicrobial substances e. Protein and peptide hormones f. Anti-inflammatory substances, tranquillisers and any other pharmacologically active substance g. ***Antiviral*** substances **3/6**
Group B Authorised substances *if there are restrictions such as "do not use in aquaculture" or "only for poultry", the classification as substance B does not change.	1. Pharmacologically active substances listed in table 1 of the annex to regulation (EU) 37/2010.	a. Antimicrobial substances b. Insecticides, fungicides, anthelmintics and other pest control agents. c. Tranquilizers d. Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and glucocorticoids. **3/5** e. Other pharmacologically active substances
	2. Coccidiostats and histomonostats authorised under Regulation (EU) 1831/2003, for which MRLs are set under EU legislation and for which maximum levels are set under Regulation (EC) 124/2009.	

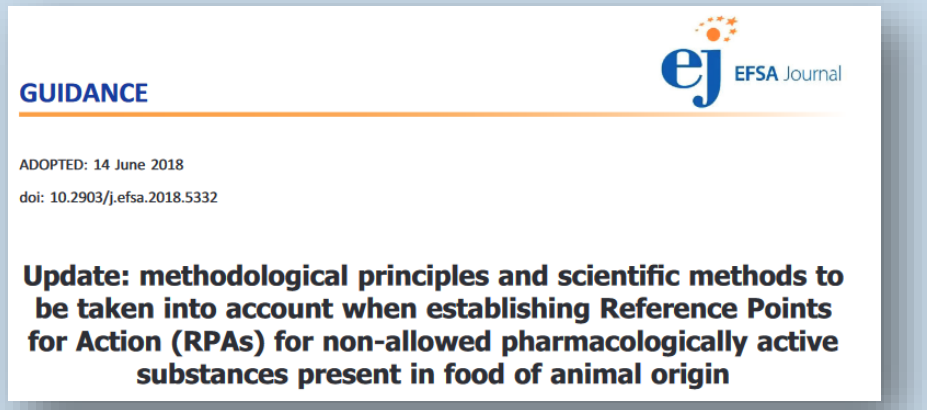
Levels of validation : general decision tree



Levels of validation: harmonization for Group A substances



Harmonisation : set a reasonable lowest limit



GUIDANCE

EFSA Journal

ADOPTED: 14 June 2018

doi: 10.2903/j.efsa.2018.5332

Update: methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin

Strategy to determine Target Concentration (TC) based on EFSA methodology : Step-wise approach applies toxicological screening values (TSVs), based on genotoxic potential, pharmacological activity, as well as other effects of the substance.

Levels of validation: harmonization for Group A substances

Groupes toxicologiques	Groupe de composés	Toxicological screening value (TSV) (µg/kg poids corporel) / jour	(µg/kg poids corporel) / jour	µg/kg d'aliment	VR* / MMPR (µg/kg)	Niveau TC (en deçà duquel il ne faut pas descendre)
Groupe I (substances génotoxiques)	Nitroimidazoles (A2c)	0.0025	0.03	0.15	1*	50% VR / MMPR ou 0.1 µg/kg si pas de VR / MMPR
	Nitrofuranes (A2b)				0.5*	
	Chlorpromazine (A2d)				5*	
	Vert de malachite (A3a)				0.5*	
	Carbadox.Olaquindox (A3c)				5	
	Chloramphenicol (A2a)				0.15*	
	Composés Table II -2010/37 (A2d)				5	
Groupe II (substances agissant sur le système nerveux, reproducteur et corticostéroïdes)	Stilbènes (A1a)	0.0042	0.0504	0.252	0.5	50% MMPR ou 0.2 µg/kg si pas de MMPR
	Stéroïdes (sauf 17β-oestradiol) (A1c)				0.1-20	
	RALs (A1d)				1	
	Sédatifs (A3f)				5	
	Hormones (A3e)				-	
	β agonistes (A1e)				0.1-50	
Groupe III (anti-infectieux, anti-inflammatoires et anti-parasitaires, diurétiques)	Thyreostatiques (A1b)	0.22	2.64	13.2	10	50% MMPR ou 2.5 µg/kg si pas de MMPR
	AINS (A3f)				0.5-10	
	Antibiotiques (A3c)					
	Coccidiostatiques / histomonostatiquess (A3d)					
	Diurétiques et agents masquants (A3f)					
	Antiparasitaires (A3d)		-	-	-	
	Pesticides et biocides (A3b)	-	-	-	-	Voir MRL reg 396/2006 ou valeur par défaut de 10 µg/kg
Autres composés	Colorants interdits (A3a)	-	-	-	0.5	50% MMPR ou 0.1 µg/kg si pas de MMPR
	Antiviraux (A3g)	-	-	-	-	2.5 µg/kg°
	Protéines et hormones peptidiques (A3e)	-	-	-	-	2.5 µg/kg°

Validation at 50 % of RPA or MMPR :

- CCα<RPA/MMPR

Validation for Group A substances : CER strategy

- Use of screening or qualitative confirmatory methods

Classification of analytical methods by performances characteritics that have to be determined					
Method	Confirmation		Screening		
	Qualitative	Quantitative	Qualitative	Semi-Quantitative	Quantitative
Substances	A	A/B	A/B	A/B	A/B
Identification in accordance with 1.2	x	x			
CCa	x	x			
CCb			x	x	x
Trueness		x			x
Precision		x		(x)	x
Relative Matrix effect/absolute recoveyr		x			x
Selectivity/Specificity	x	x	x	x	x
Stability	x	x	x	x	x
Ruggedness	x	x	x	x	x

A substances in screening or qualitative confirmatory methods

Option 1 : full/primary validation

- New method
- Revalidation after modification of the sample prep

Validation in one day (several day for on-going validation) with:

- 20 blanks
- X QCs at STC (screening method) or LCL (qualitative confirmation method)

X varying between 20 and 60 depending on the ration STC/RL

Ratio STC/RL	Number of QCs (X)
STC ≤ 0.5 RL STC ≤ RPA, MMPR LCL ≤ 0.5 RPA, 0.5 MMPR*	20
0.5 RL < STC < 0.9 RL	40
0.9 RL ≤ STC ≤ RL	60

*: for not authorised or forbidden substances, the target concentrations has to be as low as practically achievable and the CCβ/CCα have to be ≤ RPA and ideal < MMPR

Combined validation for different species/matrices if the extraction is the same

A substances in screening or qualitative confirmatory methods

Option 1 : full/primary validation

Example of ANA28 – qualitative screening method for antiectoparasitics compounds (A3f) in food commodities by UPLC-MS/MS

→ validation of several matrices with the same extraction protocol

Validation scheme : 1 day
- 4 blank liver
- 4 QC liver
- 5 blank poultry muscle
- 5 QC poultry mucle
- 3 blank fatty fish
- 3 QC fatty fish
- 3 blank lean fish
- 3 QC lean fish
- 5 blank egg
- 5 QC egg

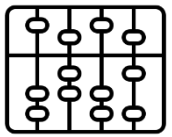
Performance parameters :
- RRT
- specificity
- ruggedness
- Ccbeta(=target concentration)

Challenges : sensitivity of the method, workload of the operators

A substances in screening or qualitative confirmatory methods

Option 2 : light/secondary validation

- Extension to new parameters
- Extension to new matrices (within same group) or species
- Revalidation at lower concentration (by example for lower MMPRs)



Validation in one day (several day for on-going validation) with:

- 6 blanks
- 6 QCs at STC (screening method) or LCL (qualitative confirmation method)

X varying between 20 and 60 depending on the ration STC/RL

Combined validation for different species/matrices if the extraction is the same

A substances in screening or qualitative confirmatory methods

Option 2 : light/secondary validation

Example of ANA34 – qualitative confirmatory method for beta-agonists in bovine and porcine urine by UPLC-MS/MS

- decrease of target concentrations for alignement with new MMPRs → light validation

<u>Validation scheme : 1 day</u>
- 3 blank bovine urine
- 3 QC bovine urine
- 3 blank porcine urine
- 3 QC porcine urine

→

<u>Performance parameters :</u>
- RRT
- ion ratio
- specificity
- ruggedness
- CCalpha

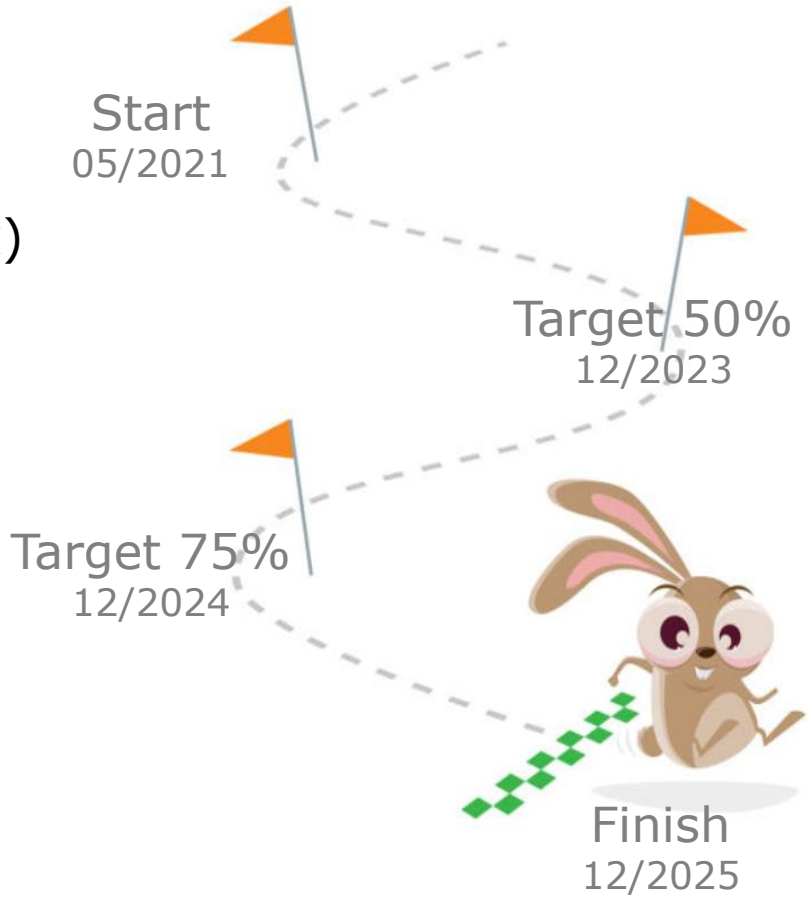
Challenges : sensitivity of the method, workload of the operators

Challenges:	Solutions:
Workload	Alternative validation scheme (light validation, combination of matrices/species)
Complexicity of new legislation	- Decision tree to determine target concentration + ALARA approach - new group of parameters : list of compounds + target matrices ?
Flexible scope	Revalidated method under accreditation after project completion
Application of new verification criteria	*Applicable only for revalidated method*

Revalidation of existing methods at CER Groupe according to 2021/88

- Actual status for re-validation/re-interpretation
  - 56 % (5071 combinations) (Group A&B)
  - 65% (3175 combinations) (Group A)

- Remaining validations to be performed (Group A):
  - 162 combinations method/matrix/species
  - 50 combinations method/matrix (regrouping species/matrix)
    - ✓ 12 quantitative confirmatory methods
    - ✓ 31 qualitative confirmatory methods
    - ✓ 7 screening methods



Still a lot to do….

Thank you !