2. Discussion on an amendment of Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC

1. Article 1 of CIR (EU) 2021/808 – rephrasing of the scope

A draft Commission Delegated Regulation supplementing Regulation (EU) 2019/4 by establishing specific maximum levels of cross-contamination of antimicrobial active substances in non-target feed and methods of analysis for these substances in feed is under preparation.

The issue is related to the official control of the presence of the antibiotics in non-target feed whereby compliance with low levels (close to or at the LOQ) has to be controlled should be achieved (cross-contamination level in the non-target feed of 1% or at the LOQ of the active substance in the medicated feed). The EURL for feed additives have identified and elaborated methods of analysis that can be used for the control of compliance and has established LOQ for these methods for the different antibiotics. Given that feed is a more complex matrix, the criteria for validation (trueness, precision) as laid down in Regulation (EU) 2021/808 are not complied with for these established LOQs as well as for the control of very low levels of antibiotics in feed.

Given that Commission Implementing Regulation (EU) 2021/808 has explicitly feed in the scope and is therefore applicable to feed also for the control of these very low levels of antibiotics in non-target feed this would result in an inconsistency in EU legislation. Therefore, it is necessary to limit the scope of Commission Implementing Regulation (EU) 2021/808 to avoid such inconsistency in EU legislation.

The scope of Reg. 2021/808 has to be limited to the samples taken in the frame of control plans as this was the case of Decision 2002/657, that means to limit the scope to follow-up investigations in feed and other "non-food" matrices.

Scope of Decision 2002/657:

This Decision provides rules for the analytical methods to be used in the testing of official samples taken pursuant to Article 15(1), second sentence, of Directive 96/23/EC and specifies common criteria for the interpretation of analytical results of official control laboratories for such samples.

Second sentence of Art. 15(1) of 96/23: *The detailed rules for the taking of official samples and the routine and reference methods to be employed for the analysis of such official samples shall be specified by the Commission.*

The scope of Dir. 96/23: *This Directive lays down measures to monitor the substances and groups of residues listed in Annex I.*

The proposed change of Article 1 of CIR (EU) 2021/808:

This Regulation lays down rules concerning the methods of analysis used for sampling and for laboratory analyses for samples taken in the frame of national plans as defined in Article 3 of Commission Implementing Regulation (EU) 2022/1646 ~~in relation to residues of pharmacologically~~

~~*active substances in live food-producing animals, their body parts and fluids, excrements, tissues, products of animal origin, animal by products, feed and water*~~. *It also lays down rules for the interpretation of analytical results of these laboratory analyses.*

This Regulation applies to official controls aimed at verifying compliance with the requirements on the presence of residues of pharmacologically active substances.

2. **Change of the references to ISO standards**

Currently, there are references to ISO standards in the form of the number of the standard and the edition or year of publication. As a good practice in referring to standards, only the number of the standard has to be mentioned. Therefore, all references to ISO standards will be amended accordingly.

3. Article 3 – addition of sentence concerning deviations from established criteria

Any deviations from the established technical criteria shall be spotted and analysed with traceable evidence kept. Therefore, it is suggested to add a sentence in Article 3.

The proposed change of Article 3:

Member States shall ensure that the samples taken in accordance with Article 34 of Regulation (EU) 2017/625 are analysed using methods that comply with the following requirements:

(1) they are documented in test instructions, preferably according to Annexes of ISO 78-2:1999 Chemistry-Layouts for standards – Part 2: Methods of chemical analysis (15);

(2) they comply with the performance criteria and other requirements for analytical methods laid down in Chapter 1 of Annex I to this Regulation;

(3) they have been validated in accordance with the requirements laid down in Chapters 2 and 4 of Annex I to this Regulation;

(4) they allow enforcement of the reference points for action laid down in Regulation (EU) 2019/1871, the identification of the presence of prohibited and unauthorised substances and the enforcement of maximum levels (MLs), which have been set on the basis of Regulation (EEC) No 315/93 and Regulation (EC) No 124/2009 and maximum residue limits (MRLs), which have been set on the basis of Regulations (EC) No 1831/2003 and (EC) No 470/2009.

When deviations from the criteria established in Tables 1 and 2 have been observed during validation, impact of those deviations on the outcome of the validation shall be analysed in a documented traceable manner.

4. **Article 7 – transitional measures**

The last sentence in Article 7 can be deleted as the transitional period has already expired (by 27 November 2022).

The proposed change:

Decisions 2002/657/EC and 98/179/EC are repealed from the date of entry into force of this Regulation.

However, until 10 June 2026, the requirements laid down in points 2 and 3 of Annex I to Decision 2002/657/EC shall continue to apply to methods, which have been validated before the date of entry into force of this Regulation.

~~*For the purposes referred to in the second paragraph of Article 8 of Regulation (EU) 2019/1871, Annex II to Decision 2002/657/EC shall continue to apply until 27 November 2022.*~~

5. 1.2.2.2 Precision (EURL)

An issue is in the sentence below table 2: "For analyses carried out under repeatability conditions, the coefficient of variation under repeatability conditions shall be equal or below two thirds of the values listed in Table 2."

For example: a perfect method (validation data for confirmation method for metronidazole in the low ppb range) –would not fulfill the requirements if the repeatability condition is "shall be equal or below two thirds of the in-house reproducibility" (but "shall be below two thirds of the values listed in Table 2" is OK):
Reproducibility is 8.5 % - repeatability is 7.2 % (Horwitz in table 2: 30%)

The repeatability is always part of the within-lab reproducibility – but the only relevant parameter for the calculation of the important method characteristics is the within-lab reproducibility. It is good to know the repeatability (e.g., for stability studies under repeatability conditions). However, it does not matter if it is 10 % or 90 % of the within-lab reproducibility – the within-lab reproducibility itself is the critical parameter.

Dec. 2002/657/EC: For analyses carried out under repeatability conditions, the intra-laboratory CV would typically be between one half and two thirds of the above values. For analyses carried out under within-laboratory reproducibility conditions, the within-laboratory CV shall not be greater than the reproducibility CV.

The proposed change:

1.2.2.2. Precision

The coefficient of variation (CV) for the repeated analysis of a reference or fortified material, under within-laboratory reproducibility conditions, shall not exceed the level calculated by the Horwitz Equation. The equation is:

$CV = 2^{(1 - 0{,}5 \log C)}$

where C is the mass fraction expressed as a power (exponent) of 10 (e.g. 1 mg/g = 10^{-3}). For mass fractions below 120 µg/kg the application of the Horwitz equation yields unacceptably high values. Therefore, the allowed maximum coefficient of variation shall not be greater than the values presented in Table 2.

Table 2

Acceptable coefficient of variation

Mass fraction	***Reproducibility CV (%)***
> 1 000 µg/kg	*16 (adapted from Horwitz equation)*
> 120 µg/kg – 1 000 µg/kg	*22 (adapted from Horwitz equation)*
10 – 120 µg/kg	*25 ([1])*
< 10 µg/kg	*30 ([1])*
([1]) The CV (%) presented is a guideline and should be as low as reasonably possible.	

For analyses carried out under repeatability conditions, the coefficient of variation under repeatability conditions is usually below two thirds of the values listed in Table 2 and shall be lower than the coefficient of variation under reproducibility conditions.

6. 2.1 Performance characteristics to be determined for analytical methods – request for "definition" of semi-quantitative screening method (to be inserted under Table 5)

The proposed change:

A semi-quantitative screening method is a screening method which gives quantitative results but does not fulfil the precision requirements given in Table 2 of Annex I to this Regulation.

7. 2.10 Relative matrix effects (EURL)

An issue is in the last sentence: "The coefficient of variation shall not be greater than 20 % for the MF (standard normalised for IS)."

The proposed change:

2.10. Relative matrix effects

The relative matrix effect shall be determined in all cases. This can be done either as part of the validation or in separate experiments. The calculation of the relative matrix effect shall be done for at least 20 different blanks lots (matrix/species), according to the scope of the method e.g. different species to be covered.

The blank matrix should be fortified after extraction with the analyte at the RPA, MRL or ML and should be analysed together with a pure solution of the analyte.

The relative matrix effect or matrix factor (MF) is calculated as:

$$\text{MF (standard)} = \frac{\text{peak area of MMS standard}}{\text{peak area of solution standard}}$$

$$\text{MF (IS)} = \frac{\text{peak area of MMS IS}}{\text{peak area of solution IS}}$$

$$\text{MF (standard normalised for IS)} = \frac{\text{MF (standard)}}{\text{MF (IS)}}$$

IS : internal standard

MMS : matrix-matched standard

The coefficient of variation shall not be greater than the values listed in table 2 ~~20 %~~ for the MF (standard normalised for IS).

Additional comment: It should be clarified in the EURL validation guidance in which cases the validation procedure itself includes already automatically the examination and evaluation of the matrix effect.

8. 2.5.2 Stability in matrix (DK, EURL)

Points 3 and 4 should be corrected: the reference has to be made to matrix and not solutions.

The proposed change:

2.5.2. Determination of the stability of analyte(s) in matrix

1. Use, where possible, incurred samples. When no incurred matrix is available, a blank matrix fortified with the analyte shall be used.

2. When incurred matrix is available, determine the concentration in the matrix, while the matrix is still fresh. Store further aliquots of the homogenised incurred matrix at minus 20 °C or lower if required, and determine the concentrations of the analyte periodically as long as the sample is retained in the laboratory.

3. If no incurred matrix is available, take some blank matrix and homogenise it. Divide the matrix into five portions ~~aliquots~~. Fortify each portion ~~aliquot~~ with the analyte, which should preferably be prepared in a small quantity of aqueous solution. Analyse one aliquot of each portion immediately. Store the remaining portions ~~aliquots~~ at least minus 20 °C or lower if required and analyse 5 aliquots of each portion ~~them~~ after short term, mid-long term and long term storage taken into account the applied analytical methods.

4. Record the maximum acceptable storage time and the optimum storage conditions.

The mean value of the five aliquots of one portion ~~replicate solutions~~, which was ~~were~~ stored, shall not differ by more than the within-laboratory reproducibility of the method from the mean value of the five freshly prepared aliquots ~~replicate solutions~~. The mean value of the five freshly prepared aliquots ~~solutions~~ shall be used as the basis for calculating the percentage difference.

9. 2.7.1(b) Detection capability for screening (CCβ), Method 2 for unauthorised or prohibited pharmacologically active substances (COM, EURL)

The current phrasing could be misleading as regards ‘at and above’. It can be interpreted as a requirement for several sets of 20 x fortified samples at and above the chosen STC, which is not needed if the lowest concentration is found to work effectively with the screening method applied. The correct interpretation is that it is only necessary to test fortified blank material at the concentration levels above the initial STC if the level initially chosen is not performing in respect of 5% false positives.

The proposed change:

Method 2: Investigation of fortified blank material at the concentration level~~s at and above~~ of the initially chosen STC. At this ~~For each~~ concentration level 20 fortified blanks shall be analysed in order to ensure a reliable basis for this determination. If at this ~~The~~ concentration level~~, where only~~ ≤ 5 % false compliant results remain, the level equals the detection capability of the method. If > 5 % false

compliant results are obtained, the selected STC shall be increased, and the investigation repeated to verify compliance with the ≤ 5 % false compliant results requirement.

10. 2.7.2 Detection capability for screening (CCβ) – the last sentence (EURL)

It is suggested to delete the last sentence in 2.7.2.: "For pharmacologically active substances for which the MRL is established for the sum of different substances, the CCβ of the substance with the highest concentration in the sample shall be used as the CCβ to assess the sum of substances in the measured sample."

For the screening methods, usually the CCβ for the individual substances is reported (and not a "sum CCβ"). Therefore, this additional instruction is not necessary (in contrary to the sum CCα, where it affects the decision about sample compliance).

11. Other comments

a. A definition for 'combined standard measurement uncertainty' should be included. In section 2.6 is written: "The within-laboratory reproducibility and the trueness are suitable to define the (combined) standard measurement uncertainty, if determined by taking into account all relevant influencing factors." It should be clarified with an example.

EURL reply: This is clarified in the guidance. If the guidance is not clear on this point, it needs to be revised.

b. Section 1.2.4.1: For other areas (pesticides and contaminants) the criterion for ion ratio is 30 %. 40 % seems to be quite wide, as it gives a higher risk for a false positive result.

EURL reply: In principle it is correct. However, this has been discussed in detail when the revision has been introduced. Extensive data (examples of veterinary medicines and pesticides) showed that there is a negligible increase in risk for increasing the ratio from 30 % to 40 %.

c. Section 2.4 Ruggedness: Reference for Youden or another test and an example should be included.

EURL reply: This is clarified in the guidance. If the guidance is not clear on this point, it needs to be revised.

d. Section 2.5.1 Stability, Table 7: It is quite time consuming to conduct the stability testing. It seems irrelevant to include stability at +20 °C, as this will never be the condition for storing standard solutions. Instead of it should be stated, that the stability testing should be conducted at relevant storing temperatures.

EURL reply: The aim of the stability tests is not only to prove the stability under the subsequent storage conditions, but also to prove whether degradation occurs at all by storing under more extreme conditions. This information can also be used to extrapolate storage periods for other lower temperatures or longer periods of time.

e. Section 2.6: “The CCα shall be determined for confirmatory methods. The CCα shall be established under conditions complying with the requirements for identification or identification plus quantification as defined under ‘Performance criteria and other requirements for analytical methods’ as laid down in Chapter 1.”

It should be clarified in how many cases (%) the criteria for identification at CCα should be fulfilled at the validation. In a former guideline “GUIDELINES FOR THE IMPLEMENTATION OF DECISION 2002/657/EC” (**SANCO/2004/2726-rev 4-December 2008)** it was clarified as below:

“For confirmation, the method should also be able to identify the analyte in 50 % of all cases at the CCα. Where the percentages obtained are significantly lower than these theoretical ones, it can be concluded that the calculated values for CCα are too low, necessitating further investigation of these performance characteristics.

EURL reply: It is a good suggestion; this should be included/clarified in the guidance.