EURL GUIDANCE ON MINIMUM METHOD PERFORMANCE REQUIREMENTS (MMPRs) FOR SPECIFIC PHARMACOLOGICALLY ACTIVE SUBSTANCES IN SPECIFIC ANIMAL MATRICES

The purpose of this technical guidance is to improve and harmonise the performance of analytical methods used for the analysis of residues of unauthorised or prohibited pharmacologically active substances and for authorised pharmacologically active substances in matrices for which no use is authorised, taking into account state of the art analytical methods. This guidance applies to national residue control plans established in accordance with Council Directive 96/23/EC and to all other official controls carried out on residues of pharmacologically active substances in live food-producing animals, their body parts and fluids, excrements, tissues, products of animal origin, animal by-products, animal feed and water[1].

For specific prohibited[2] or unauthorised pharmacologically active substances[3], reference points for action (RPAs) in food have been set under Regulation (EU) 2019/1871. Food of animal origin, containing residues of a pharmacologically active substance in a concentration at or above the reference point for action, shall be considered not to comply with Union legislation and shall not enter the food chain. By consequence, the RPAs also define the minimum method performance requirements (MMPRs) for these substances in food. However, when laboratories are able to reliably identify these substances at lower concentrations, also these concentrations can lead to follow-up investigations for verifying whether an illegal treatment took place (Art. 6 of Regulation (EU) 2019/1871). For substances for which RPAs are established in food, this document defines the MMPRs in non-food matrices.

For authorised pharmacologically active substances[4], for which no MRL has been set in a specific matrix or species, for those matrices/species the MMPR is $1/10^{th}$ of the cascade MRL, established under Regulation (EU) 2018/470 for the concerned substance, where analytically feasible.

For prohibited or unauthorised pharmacologically active substances, for which no RPA has been set in food, this document specifies MMPRs for specific substances in specific food and non-food matrices.

Laboratories should ensure that the CCβ for screening methods or the CCα for confirmatory methods[5] is lower than the MMPR.

It needs to be emphasised that this document is a technical guidance for analytical methods in residues control. The MMPRs should by no means be considered as enforcement limits. They represent the minimum concentrations that official laboratories should be able to reliably identify. In case certain laboratories can identify lower concentrations in accordance with the method requirements established in Commission Decision 2002/657/EC, competent authorities can also enforce lower concentrations of residues of prohibited or unauthorised pharmacologically active substances.

[1] Council Directive 96/23/EC remains applicable for the national residue control plans until 14 December 2022. For the period thereafter, new legislation is under preparation.
[2] Substances within the scope of Directive 96/22/EC and substances included in Table 2 of the Annex to Regulation (EU) No 37/2010.
[3] Pharmacologically active substances, which are not included in Table 1 of the Annex to Regulation (EU) No 37/2010 or substances that are not authorised as a feed additive under Regulation (EC) No 1831/2003, with the exception of substances essential for the treatment of equidae and substances bringing added clinical benefit compared to other treatment options available for equidae, as laid down in Regulation (EC) No 1950/2006.
[4] Substances included in Table 1 of the Annex to Regulation (EU) No 37/2010 or pharmacologically active substances that are authorised as a feed additive under Regulation (EC) No 1831/2003.
[5] As defined in Decision 2002/657/EC, currently under revision.

For practical reasons µg/kg and µg/l have been replaced by ppb in all tables.

For further technical assistance on how to improve analytical methods in order to reach the recommended concentrations the EURL responsible for the substance should be consulted.

EURL Name and Substance Groups	**Address**
ANSES EURL	
Antibacterial substances, including sulphonamides and quinolones Dyes Carbadox and olaquindox Chloramphenicol Dapsone Nitrofurans	ANSES - Laboratoire de Fougères La Haute Marche – Javené – BP 90203 F-35302 Fougères, France Phone: +33(0)2 99 17 27 47 Director: Dr. Eric Verdon email: eurl-vmpr-fougeres@anses.fr eric.verdon@anses.fr http://eurl-veterinaryresidues.anses.fr
BVL-EURL	
Beta-agonists Nitroimidazoles Anthelmintics Anticoccidials Non-steroidal anti-inflammatory drugs (NSAIDs)	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit Diedersdorfer Weg 1 12277 Berlin, Germany Phone: + 49(0)30 18445-8210 Fax: + 49(0)30 18445-8099 Director: Dr. Joachim Polzer email: eurlvetdrug@bvl.bund.de https://www.bvl.bund.de/EN/Tasks/09_Laboratories/01_Tasks/02_reference_laboratories/01_reference_laboratories_EURL/reference_laboratories_EU_node.html password protected webpage, also in English: https://fis-vl.bvl.bund.de/share/page/
WFSR-EURL	
Stilbenes, stilbene derivatives and their salts and esters Antithyroid agents Steroids Resorcylic acid lactones (RALs) including zeranol Sedatives Mycotoxins	Wageningen Food Safety Research (former RIKILT) P.O. Box 230 6700 AE Wageningen, The Netherlands Phone: + 31(0)317 480256 Fax: + 31(0)317 417717 Director: Dr. Saskia Sterk email: eurl.growthpromoters@wur.nl Saskia.sterk@wur.nl https://www.wur.nl/en/Research-Results/Research-Institutes/food-safety-research/Reference-laboratory/European-Union-Reference-Laboratory-1/EURL-growth-promoters.htm

1. A1 Stilbenes[6] (EURL WFSR Wageningen)

For the purpose of control the matrices of choice are urine followed by liver.
Muscle has been included for the control of imports and for imported aquaculture products but it is not the matrix of choice for routine plans as the concentrations of residues are very low in muscle.

Substances	Matrix	MMPR*
Diethylstilbestrol (DES) **Dienestrol (DE)** **Hexestrol (HEX)** Benzestrol (BENZ)	Urine	0.5 ppb for DES 1 ppb for DE, HEX, BENZ
	Liver	1ppb (for all substances)
	Meat (including fish)	1 ppb (for all substances)

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*

2. A2 Thyrostats (EURL WFSR Wageningen)

For the purpose of control the matrices of choice are urine and thyroid gland. Muscle has been included for the control of imports and for imported aquaculture products but it is not the matrix of choice for routine plans as the concentrations of residues are very low in muscle.
It should be noted that low concentrations of thiouracil (maximum 30 ppb) have been detected in animals fed with a diet containing cruciferous plants.

Substances	Matrix	MMPR*
Thiouracil **Methylthiouracil** **Propylthiouracil** **Tapazole** Benzylthiouracil Mercaptobenzimidazol	Urine Thyroid	10 ppb for all[&]

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*
[&]Low concentrations of thiouracil have been detected in bovine animals fed with cruciferous plants, however there is scientific evidence showing that levels above 30 ppb in urine have a low chance of being linked to natural origin due to this contamination. There are however cases where 30 ppb is exceeded for thiouracil and no exogenous source could be found. Research into discrimination is on-going. For the latest strategies consult EURL Reflection Paper.

[6] Classification according to Directive 96/23/EC remains applicable until 14 December 2022. For the period thereafter, new legislation in which a new classification is proposed, is under preparation.

3. A3 Steroids (EURL WFSR Wageningen)

For control purposes matrices of choice are urine followed by liver.
For 17ß-oestradiol, testosterone and esters of oestrogens, androgens and progestagens serum and for gestagens such as MPA kidney fat is the matrix of choice, as indicated in the table. The matrix hair can be used when controlling esters of oestradiol, testosterone, nortestosterone, boldenone and other steroid esters. Muscle has been included for control purposes of imports and for aquaculture products. For A3 steroids not all steroids are mentioned by name in this guidance paper. Generally 0.5 ppb for the marker of the steroid in urine is an acceptable MMPR.

Substances	Marker residue-metabolite[$]	Matrix	MMPR*
Boldenone[#]	17ß-boldenone glucuronide (young bovine)	Urine	1 ppb
		Liver	2 ppb
		Muscle	1 ppb
	17α-boldenone (bovine, sheep, goat, horse)	Urine	0.5 ppb
		Liver	2 ppb
		Muscle	1 ppb as β-boldenone
17ß-19-Nortestosterone[##] (nandrolone)	17α-19-Nortestosterone[###] (epi-nandrolone)	Urine	0.5 ppb
		Liver	2 ppb
		Muscle	1ppb
Ethinylestradiol		Urine	0.5 ppb
		Liver	2 ppb
		Muscle	1 ppb
17ß-oestradiol	17β-oestradiol	Plasma/Serum	0.1 ppb
		Muscle	1 ppb
17β-oestradiol ester		Hair	20 ppb
		Plasma/Serum	0.1 ppb
17β-testosterone	17β-testosterone	Serum	Male < 6 months: 10 ppb
			Male 6 - 18months: 30 ppb
			Female < 18 months: 0.5 ppb
17β-testosterone ester		Hair	10 ppb
		Serum	0.1 ppb
Methyltestosterone Methylboldenone		Urine	0.5 ppb
		Liver	2 ppb
		Muscle	1ppb
Chlorotestosterone	**17α-clostebol Chlorandrostenedione (CLAD)**	Urine	0.5 ppb
		Liver	2 ppb
		Muscle	1 ppb
17ß-trenbolone	**17α-trenbolone**	Urine	0.5 ppb
		Liver	2 ppb
		Muscle	1 ppb
		Hair	10 ppb (ester)
Stanozolol	**16ß-hydroxystanozolol**	Urine	0.5 ppb
		Liver	2 ppb
		Muscle	1 ppb
		Hair (stanozolol)	10 ppb

Dexamethasone		Urine	0.5 ppb
		Liver, Muscle	MRL when there has been authorised treatment
Megestrol	Megestrol (acetate)	Kidney fat	5 ppb
		Muscle	1.0 ppb
Melengestrol	Melengestrol (acetate)	Kidney fat	5 ppb
		Muscle	1.0 ppb
Chlormadinone	Chlormadinone (acetate)	Kidney fat	2 ppb
		Muscle	1 ppb
Medroxy-progesterone	Medroxy-progesterone (acetate)	Kidney fat	1 ppb
		Muscle	1.0 ppb

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*
[#]Boldenone as described in expert group paper of 2003, reference: Presence and metabolism of the anabolic steroid boldenone in various animal species(A review. July 2004, Food Additives and Contaminants 21(6):515-25).
[$]Porcine animals do not metabolise into α-isomers. For porcine animals the administered steroid is the marker.
[##]17β-19-nortestosterone occurs naturally in non-castrated pigs and horses. For the latest strategies consult EURL Reflection Paper.
[###]17α-19-nortestosterone occurs naturally in pregnant cows and newborn calves. For the latest strategies consult EURL Reflection Paper.

4. A4 Resorcylic acid lactones and derivates (EURL WFSR Wageningen)

For the purpose of control matrices of choice are urine followed by liver. Muscle has been included for control purposes of imports and for imported aquaculture products.

Substances	Marker residue-metabolite	Matrix	MMPR*
Zeranol**	**Taleranol**	Urine	1 ppb
		Liver	2 ppb
		Muscle	1 ppb
Zearalenone		Urine	2 ppb
		Liver	2 ppb
α-zearalenol		Urine	2 ppb
		Liver	2 ppb
β-zearalenol		Urine	2 ppb
		Liver	2 ppb

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*

***In case both zeranol and zearalenone are present, the presence of zeranol is considered as the result of mycotoxin contamination. Screening can be done on zeranol and its marker metabolites taleranol. When one of these compounds is detected a full resorcylic acid lactone (RAL) profile is needed to decide on noncompliance. For the latest strategies consult EURL Reflection Paper.*

5. A5 Beta-agonists (EURL BVL Berlin)

For control purposes the matrices of choice are urine and liver and especially retina since here higher concentrations of residues can be found for a longer time period. The analysis of complete eyes is the second choice compared to retina which is the first choice. Hair is also a recommendable matrix however the risk of external contamination has to be considered. When taking hair it is always recommended to sample also urine at the same time from the same animal. Muscle has been included for control purposes of imports but concentrations in muscle are significantly lower than in previously mentioned matrices.

Substances	Matrix	MMPR*
Clenbuterol: *MRL (for bovine and equidae**:* *0.1 µg/kg in muscle* *0.05 µg/kg in milk (only bovine)* *0.5 µg/kg in liver and kidney* **Brombuterol**, Bromchlorbuterol Cimaterol	**Urine** **Liver** Lung Muscle Kidney Faeces Plasma Drinking water	0.1 ppb
Cimbuterol Clenpenterol Clenproperol Hydroxymethylclenbuterol Mabuterol Mapenterol Tulobuterol	Retina **Hair** (Screening)	1 ppb
Carbuterol Clencyclohexerol **Isoxsuprine** **Ractopamine** Ritodrin **Salbutamol** Terbutaline **Zilpaterol**	**Urine** **Liver** Lung Muscle Kidney Faeces Plasma Drinking water	0.5 ppb
	Retina **Hair** (Screening)	5 ppb
Fenoterol, Salmeterol	**Urine** **Liver** Lung Muscle Kidney Faeces Plasma Drinking water	1 ppb
	Retina **Hair** (Screening)	5 ppb
All analytes	Feed	50 ppb

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*
***According to Council Directive 96/22/EC beta-agonists may be authorised for very exceptional and restrictive therapeutic treatments.*

6. A6 (EURL ANSES Fougeres, BVL Berlin and WFSR Wageningen)

For nitroimidazoles the matrices of choice are eggs, plasma/serum and retina, followed by – depending on the species - muscle. Then Milk can also be chosen if relevant. For aquaculture products muscle is the relevant matrix, furthermore crustacean and fish eggs.

Substances	Marker residue-metabolite	Matrix	MMPR*
Nitroimidazoles: **Ronidazol** **Dimetridazol** **Metronidazol** + other 5-nitroimidazoles	**Hydroxy-metabolites**	**Poultry:** Plasma, Serum, Retina**, Eggs **Pigs (and other species):** Plasma, Serum, Muscle, Retina** **Aquaculture products:** Muscle Milk (Drinking water)	1 ppb
		Feed	50 ppb
Chloramphenicol		**Meat, milk, eggs, aquaculture products, urine**	**0.15 ppb RPA**
Nitrofurans	**Metabolites AMOZ, AHD, SEM, AOZ, DNSH**	**Poultry Meat, Aquaculture products, Muscle/meat, Milk, Eggs**	**0.5 ppb RPA**
Dapsone		Muscle/meat Milk	5 ppb
Chlorpromazine		Kidney	5 ppb

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*
***For retina it is not possible yet to give a recommended concentration since it is not defined so far to which part of the eye (or the whole eye) the concentration should refer.*

7. B2d Sedatives (EURL WFSR Wageningen)

Matrix of choice is kidney.

Substances	Matrix	MMPR*
Carazolol **Acepromazine** **Propiopromazine Haloperidol** **Azaperon/Azaperol**	Kidney	5 ppb

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*

8. B2e NSAIDs (EURL BVL Berlin)

For control purposes matrices of choice are muscle and milk, followed by kidney, liver and plasma.

Substances	Matrix	MMPR*
Phenylbutazone **Oxyphenbutazone**	**Muscle** **Milk** Kidney Liver Plasma	5 ppb
Ibuprofen **Naproxen** Mefenamic acid Niflumic acid Flufenamic acid		10 ppb

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*

9. Other substances (EURL ANSES)

Substances	Marker residues	Matrix	MMPR*
Malachite green	**Malachite green and Leucomalachite green**	**Muscle fish**	**Sum: 0.5 ppb RPA**
Crystal violet *(Gentian violet)*	**Crystal violet and Leucocrystal violet *(Leucogentian violet)***	**Muscle fish**	**Sum: 0.5 ppb**
Brilliant green	**Brilliant green and Leucobrilliant green****	**Muscle fish**	**Sum: 0.5 ppb**
Carbadox	QCA (quinoxaline- 2-carboxylic acid) and/or DCBX (Desoxycarbadox)	Muscle, liver	5 ppb
Olaquindox	MQCA (3-methylquinoxaline-2-carboxylic acid)	Muscle, liver	5 ppb

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*

***Control of this substance is not mandatory until certified reference standard is available.*

10. Honey (EURL ANSES Fougeres for Antimicrobials and BVL Berlin for Nitroimidazoles)

Group	Substances to be included	MMPR*
A6	**Chloramphenicol**	**0.15 ppb RPA**
	Nitroimidazoles	**1 ppb**
	Nitrofurans	**0.5 ppb RPA**
B1**	Tetracyclines	10 ppb
	Sulfonamides	10 ppb
	Streptomycin	20 ppb
	Macrolides: Erythromycin Tylosin	 20 ppb 10 ppb

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*

***MMPRs for B1 substances in honey are related to control in the absence of a signified cascade use in line with Regulation (EU) 2018/470. They have to be controlled at 1/10th to the lowest MRL in other species according to Regulation (EU) 2018/470.*