European Food Safety Authority

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SCIENTIFIC OPINION

Proposal for MRLs and withdrawal period for Cycostat®66G for chickens and turkeys for fattening[1]

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2007-180)

Adopted on 16 September 2008

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority was asked to deliver a scientific opinion on the proposal made by the applicant for the addition of MRLs in chickens for fattening and turkeys to the current authorisation of the additive Cycostat®66G and for the adaptation of the withdrawal period if applicable.

Cycostat®66G containing 6.6 % robenidine hydrochloride is already authorised for use in chickens for fattening, turkeys and rabbits for fattening. Efficacy and safety of the product have been assessed by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) in two former opinions, establishing also an ADI.

Consumer exposure from the consumption of tissues from chickens administered robenidine at the maximum authorised dose represents without withdrawal only a limited fraction of the ADI. Therefore, based on safety considerations, the FEEDAP Panel does not see the necessity for setting a withdrawal period for chickens for fattening and consequently of MRLs. The same conclusion applies to turkeys for fattening.

The FEEDAP Panel considers robenidine as the marker residue in chickens and turkeys. Ratios for the marker residue to total residue could be established for a zero withdrawal time in both species. If MRLs are required, the FEEDAP Panel proposes:

[1] For citation purposes: Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on a proposal for MRLs and withdrawal period for Cycostat®66G for chickens and turkeys for fattening. *The EFSA Journal* (2008) 798, 1-15

- chicken – per kg wet tissue: 0.80 mg for liver, 0.35 mg for kidney, 0.20 mg for muscle and 1.30 mg for skin/fat,
- turkey – per kg wet tissue: 0.40 mg for liver and skin/fat and 0.20 mg for kidney and muscle.

The residue intake calculated from the above MRLs is below the ADI (95 % for chickens and 48 % for turkeys). These values overestimate the real exposure due to the insensitivity of the analytical method for the determination of tissue residues.

Off-flavours were observed in edible tissues of chickens for fattening until three days after withdrawal. Despite the absence of data on the impact of robenidine on the sensory properties of turkey products, the FEEDAP Panel considers that chickens and turkeys should be regarded similarly. As a consequence, the FEEDAP Panel proposes a five-day withdrawal period for chickens and turkeys for fattening, to avoid off-flavours in edible tissues from poultry treated with robenidine. To guarantee the quality of the product, a Maximum (food product) Processing Compatible Residue (MPCR) should be introduced which would be considerably lower than the MRL. Due to the insufficient sensitivity of the available method of analysis and the absence of data at five-day withdrawal, the FEEDAP Panel cannot propose such an MPCR for robenidine.

Key words: coccidiostats, Cycostat®66G, robenidine hydrochloride, chickens for fattening, turkeys for fattening, consumer safety, ADI, MRLs, product quality, MPCR, withdrawal time

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BACKGROUND

Regulation (EC) No 1831/2003[2] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States.

The European Commission received a proposal from the company Alpharma Belgium BVBA[3] for setting MRLs for chickens for fattening and turkeys and modifying the withdrawal periods. Cycostat®66G (E 758) is already authorised under Regulation (EC) No 1800/2004[4] for use in chickens for fattening, turkeys and rabbits for fattening until 2014.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 13(3) (modification of the authorisation of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[5] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 13 May 2008.

EFSA issued an opinion on the revaluation of Cycostat®66G in accordance with article 9G of Council Directive 70/524/EEC (adopted on 8 June 2004) and an opinion on the safety of Cycostat®66G based on robenidine hydrochloride, as feed additive in accordance with Council Directive 70/524/EEC (adopted on 1 October 2004).

TERMS OF REFERENCE

On a request from the European Commission, the European Food Safety Authority (EFSA) shall deliver an opinion on the proposal made by the applicant for the addition of MRLs in chickens for fattening and turkeys to the current authorisation of the additive Cycostat®66G and for the adaptation of the withdrawal period if applicable.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Standing Working Group on Coccidiostas for the preparation of this opinion.

[2] OJ L 268, 18.10.2003, p.29
[3] Alpharma Belgium BVBA, Laarstraat 16, B-2610 Antwerp, Belgium)
[4] OJ L317, 15.10.2004, p. 37
[5] Dossier reference: FAD-2007-0035

Table 1. Register entry as proposed by the applicant

Additive	Robenidine hydrochloride 66g/kg
Registration number/EC No/No (if appropriate)	E 758
Category of additive	Coccidiostat
Functional group of additive	

Description			
Composition, description	**Chemical formula**	**Purity criteria (if appropriate)**	**Method of analysis (if appropriate)**
Active substance: Robenidine hydrochloride Additive composition: Robenidine hydrochloride: 66 g/kg Calcium lignosulfonate: 40 g/kg Calcium sulphate dihydrate: 894 g/kg	$C_{15}H_{13}Cl_2N_5$.HCl, 1,3-bis[(p-chlorobenzylidene)amino] guanidine hydrochloride CAS number: 25875-50-7	Robenidine hydrochloride>97% Related impurities: N,N',N''-Tris(p-chlorobenzylideneamino) guanidine: <0.5 % Bis-(4-chlorobenzylidene) hydrazine: <0.5 % N'-(4-chlorobenzylidene)-1,2-hydrazinedicarboxamide: <0.5 %	HPLC method

Trade name (if appropriate)	Cycostat® 66G
Name of the holder of authorisation (if appropriate)	Alpharma Belgium BVBA

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period (if appropriate)**
		mg kg^{-1} of complete feedingstuffs		
Chickens for fattening	-	30	36	No withdrawal needed
Turkeys	-	30	36	No withdrawal needed
Rabbits for fattening	-	50	66	5 days before slaughter

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	
Specific conditions or restrictions for handling (if appropriate)	-
Post market monitoring (if appropriate)	Post market monitoring will be performed in accordance with the available scientific techniques
Specific conditions for use in complementary feedingstuffs (if appropriate)	-

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**
Robenidine	Chickens for fattening	Muscle Skin/Fat Liver Kidney	200 µg/kg 2000 µg/kg 1250 µg/kg 750 µg/kg
Robenidine	Turkeys	Muscle Skin/Fat Liver Kidney	200 µg/kg 1000 µg/kg 1000 µg/kg 200 µg/kg

ASSESSMENT

1. Introduction

Cycostat®66G (E 758) is already authorised under Regulation (EC) No 1800/2004[6] for use in chickens for fattening, turkeys and rabbits for fattening until 2014. Cycostat®66G contains 6.6 % of the active ingredient robenidine hydrochloride.

In its former opinions, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) re-assessed the product in accordance with article 9G of Council Directive 70/524/EEC (EFSA, 2004a and EFSA, 2004b).

In 2004, the FEEDAP Panel, taking a conservative approach, considered all robenidine-derived residues (total residues) as toxicologically relevant and representing a risk at most equal to an equivalent quantity of robenidine (EFSA, 2004a).

In a later opinion (EFSA, 2004b), the FEEDAP Panel established an ADI of 0.0375 mg kg^{-1} bw day^{-1} (2.25 mg per person per day). It also established the similarity of the metabolic fate of robenidine in chicken and rat which makes the ADI based on laboratory animals studies a suitable basis for MRL calculations. MRLs were not proposed.

The applicant now proposes to set MRLs in chickens for fattening and turkeys amending the current authorisation of the additive Cycostat®66G and to adapt the five-day withdrawal period, if necessary.

2. Maximum Residue Limit (MRL)

The maximum residue limit (MRL) concept was introduced in the assessment of feed additives by Directive 2001/79/EC.

In Directive 2001/79/EC, the proposal for MRLs of the additive states that: 'To establish an MRL the chemical nature of the drug-related material which is intended to be used to specify the tissue residue levels must be defined. This is termed the marker residue. This residue constituent must not necessarily be the toxicologically relevant residue but has to be chosen as a suitable indicator to represent the total significant residue. The ratios of the marker residue/total residues in connection with the ADI (i.e., ratio of the marker residue/total radioactive residues, marker residue/all biologically active residues) should be established at all the time points during the depletion studies. In particular, this ratio should be known at the time point retained to elaborate MRLs. A suitable analytical method for this marker residue must also be available to ensure compliance with the MRL.'

Individual MRLs should be set for the different tissues of relevance; in the case of poultry, for liver, kidneys, meat and skin/fat. The individual MRLs in different tissues should reflect the depletion kinetics and variability of the residue levels in those tissues. Variability should normally be reflected by the 95 % confidence limit of the mean.

2.1. Total residues

Robenidine total residue data for chickens and turkeys for fattening have been taken from the opinion on Cycostat (EFSA, 2004a) and completed with the standard deviation values. They correspond to the maximum dose of robenidine approved for use, 36 mg kg^{-1} feed for chickens and turkeys for fattening. Those data are presented in Tables 2 and 3.

[6] OJ L317, 15.10.2004, p. 37

Table 2. Kinetics of robenidine-derived total residues[7] in tissues of chickens (average of three males and three females) administered 36 mg radio-labelled robenidine kg^{-1} feed until steady state and slaughtered at different withdrawal times (expressed as mg robenidine equivalents kg^{-1} wet tissue)

Withdrawal (days)	Liver	Kidney	Skin/fat	Muscle
0	2.516 ± 0.732	0.885 ± 0.251	1.372 ± 0.364	0.169 ± 0.043
1	1.569 ± 0.468	0.540 ± 0.131	0.821 ± 0.349	0.070 ± 0.025
3	0.633 ± 0.137	0.161 ± 0.024	0.211 ± 0.042	0.007 ± 0.004

Table 3. Kinetics of robenidine-derived total residues[8] in tissues of turkeys (average of three males and three females) administered 36 mg radio-labelled robenidine kg^{-1} feed until steady state and slaughtered at different withdrawal times (expressed as mg robenidine equivalents kg^{-1} wet tissue)

Withdrawal (days)	Liver	Kidney	Skin/fat	Muscle
0	1.610 ± 0.332	0.570 ± 0.052	0.829 ± 0.126	0.065 ± 0.012
1	0.814 ± 0.282	0.279 ± 0.099	0.389 ± 0.214	0.023 ± 0.013
3	0.498 ± 0.082	0.145 ± 0.024	0.250 ± 0.072	0.010 ± 0.003

Total residues in liver, kidney and muscle are higher in chicken compared to turkey, which supports the view that chickens offer a worst-case situation. Moreover, the FEEDAP Panel considers that given the physiological proximity of chicken and turkey and the similar qualitative metabolic fate of robenidine in both species, it is sufficient to rely on the chicken data.

2.2. Consumer exposure

The exposure of the consumer has been calculated according to daily consumption values of animal products set in Directive 87/153/EEC and amended by Directive 2001/79/EC, and the average values of the residual levels plus 2SD (95 % confidence limit) measured in the different tissues and at different withdrawal times. The results of the calculation are presented in Table 4.

Table 4. Human consumer daily exposure to robenidine total residues from relevant chicken tissues after zero-day, one-day and three-day withdrawals, and corresponding percentages of the acceptable daily intake (ADI)

	Withdrawal (days)	Liver	Kidney	Muscle	Skin/fat	Sum	% ADI
Total residues[(a)] (mg kg^{-1} tissue)	0	3.980	1.386	0.255	2.100	-	-
	1	2.505	0.802	0.120	1.519	-	-
	3	0.905	0.209	0.015	0.295	-	-
DITR[(b)] (mg person^{-1} day^{-1})	0	0.398	0.014	0.077	0.189	0.678	30
	1	0.251	0.008	0.036	0.137	0.432	19
	3	0.091	0.002	0.005	0.027	0.125	6

(a): average values + 2SD
(b): DITR = daily intake of total residues

[7] Technical Dossier. 2004. Section IV, Reference 4
[8] Technical Dossier. Section IV, Reference 10

Consumer exposure from the consumption of tissues from chickens administered robenidine at the maximum recommended dose and at steady state (zero withdrawal), represents only a limited fraction of the ADI. The FEEDAP Panel concludes that even without any withdrawal period, no risk for the consumer is expected to occur. Therefore, based on safety considerations, the FEEDAP Panel does not see the necessity for establishing MRLs and consequently for setting a withdrawal period for chicken.

At the maximum recommended dose of robenidine from Cycostat®66G proposed for turkey, the respective total robenidine-derived residues in tissues are lower than in chicken. Therefore, the corresponding exposure of the consumer would be lower and the same conclusions apply.

2.3. Quality of animal produce

Several experiments to study the effect of robenidine hydrochloride on the flavour of meat and organs of broilers treated with 33 mg robenidine hydrochloride kg^{-1} have been carried out. Trained panels could distinguish treated from non-treated meat when robenidine hydrochloride was applied up to 12 hours before slaughter. The incidence of off-flavours decreased as the period of withdrawal increased. After a withdrawal of three days, only a few panellists were able to detect the off-flavour.[9]

No studies have been performed in turkeys, but no adverse effects on carcass quality have been reported.

2.4. Withdrawal time

With regard to consumer safety, the FEEDAP Panel does not see the necessity to establish MRLs and consequently to set a withdrawal period for chickens and turkeys for fattening.

Off-flavours were observed in edible tissues of chickens for fattening until three days after withdrawal. To avoid impairment of the distinctive features of the animal product,[10] a withdrawal period of five days would be required.

In the absence of data on the impact of robenidine on the sensory properties of turkey products, the FEEDAP Panel considers that chickens and turkeys should be similarly regarded.

As a consequence, the FEEDAP Panel proposes a five-day withdrawal period for chickens and turkeys for fattening to avoid off-flavours in edible tissues from poultry treated with robenidine.

3. Background data for MRL calculation

3.1. Marker Residue

According to the opinion on Cycostat®66G (EFSA, 2004a), metabolic pathways of robenidine as well as metabolic tissue profiles are similar in chicken and turkey. Robenidine is considered as the marker residue.

Robenidine residues have been determined at the same time as total residues in tissues of chickens and turkeys administered radio-labelled robenidine at the maximum dose and applying 0, 1 and 3 days withdrawal. The analytical method used for the quantification of robenidine had a limit of quantification (LOQ) of 0.1 mg kg^{-1}. The results are reported in Table 5.

[9] Original dossier. Section IV, Volume 12, Reference 45
[10] Article 5.2, Regulation (EC) No 1831/2003

Table 5. Kinetics of robenidine residues in chicken and turkey tissues (average of three males and three females) administered 36 mg radio-labelled robenidine kg^{-1} feed until steady state and slaughtered at different withdrawal times (mg robenidine kg^{-1} wet tissue)

	Withdrawal (days)	Liver	Kidney	Muscle	Skin/fat
Chicken	0[a]	0.507	0.229	0.060	0.823
	1[b]	0.232	0.134	<LOQ	0.420
	3[b]	<LOQ	<LOQ	<LOQ	<LOQ
Turkey	0[a]	0.261	0.030	0.006	0.257
	1[b]	0.131	<LOQ	<LOQ	0.197

(a): determined by radio-HPLC; average values from pooled samples (males and females separately)
(b): determined by HPLC in individual samples; average values, LOQ = 0.1 mg kg^{-1} for all tissues

Due to the limitation of the analytical method, no robenidine could be quantified, if still present, at three-day withdrawal time in any chicken tissue.

3.2. Ratio marker/total residues

The setting of an MRL is based on the ratio of the marker residue to total residue at the proposed withdrawal time.

The ratios marker residue (robenidine) to total residue used have been calculated for the different tissues and withdrawal periods (Table 6).

Table 6. Ratios marker residue (robenidine) to total residue in chicken and turkey tissues measured at different withdrawal period (0-, 1-and 3-day)

	Withdrawal (days)	Liver	Kidney	Muscle	Skin/fat
Chicken	0	0.2	0.26	0.04	0.60
	1	0.15	0.23	ND	0.61
	3	ND	ND	ND	0.42
Turkey	0	0.16	0.05	0.09	0.31
	1	ND	ND	ND	ND

Because the marker residue could not be determined after a three-day withdrawal in liver, kidney and muscle, no ratio of marker/total residue is available.

3.3. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the report submitted by the Community Reference Laboratory (CRL) concerning the analytical method(s) for Cycostat®66G. The executive summary of the report can be found in the Appendix.

4. Proposal of MRLs

4.1. MRLs in chickens for fattening

Considering the zero-day withdrawal period, the following MRLs could be used for control purposes: liver, 0.80; kidney, 0.35; muscle, 0.20; skin/fat, 1.30 mg kg^{-1} wet tissue.

The safety of the proposed MRLs has been evaluated on the basis of the normalised template presented in Table 7.

Table 7. Consumer safety of the proposed MRLs for edible chicken tissues

	Liver	Kidney	Muscle	Skin/fat	Sum
Ratio Marker/Total residue	0.20	0.26	0.04	0.60	
Proposed MRL (mg kg^{-1} tissue)	0.80	0.35	0.20	1.30	
Consumption (kg day^{-1})	0.100	0.010	0.300	0.090	
DITR[(a)] (mg day^{-1})	0.400	0.013	1.500	0.195	**2.108**
Consumption (% ADI)	18	1	67	9	**95**

[(a)] Dietary intake calculated from MRLs of individual tissues/products

The DITR derived from the MRLs, which overestimates the residue in muscle due to the insensitivity of the analytical method, is still below the ADI (95 %).

4.2. MRLs in Turkeys

Considering the zero-day withdrawal period, the following MRLs could be used for control purposes: liver, 0.4; kidney, 0.2; muscle, 0.2; skin/fat, 0.4 mg kg^{-1} wet tissue.

The safety of the proposed MRLs has been evaluated on the basis of the normalised template presented in Table 8.

Table 8. Consumer safety of the proposed MRLs for edible turkey tissues.

	Liver	Kidney	Muscle	Skin/fat	Sum
Ratio Marker/Total residue	0.16	0.05	0.09	0.31	
Proposed MRL (mg kg^{-1} tissue)	0.40	0.20	0.20	0.40	
Consumption (kg day^{-1})	0.100	0.010	0.300	0.090	
DITR[(a)] (mg day^{-1})	0.250	0.040	0.667	0.116	**1.073**
Consumption (% ADI)	11	2	30	5	**48**

[(a)] Dietary intake calculated from MRLs of individual tissues/products

The DITR derived from the MRLs, which overestimates the residue in muscle and kidney due to the insensitivity of the analytical method, is still below the ADI (48 %).

4.3. Maximum (food product) Processing Compatible Residue (MPCR)

In case of additives in which residues below the MRL interfere with food quality, Directive 2001/79/EC[11] states that it may be appropriate to consider a Maximum (food product) Processing Compatible Residue (MPCR) in addition to establishing MRLs values. In the case of Cycostat®66G, it has been identified that the product quality is adversely affected by concentrations of residue significantly lower than the MRL and therefore a longer withdrawal time (five days) is considered necessary to provide satisfactory product quality.

Due to the insufficient sensitivity of the available method of analysis, no data are provided on the marker residue after three- and five-day withdrawals. Therefore, the FEEDAP Panel cannot propose a MPCR that would guarantee the quality of the product.

[11] OJ L 267, 06.10.2001, p.1

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Consumer exposure from the consumption of tissues from chickens administered robenidine at the maximum authorised dose represents without withdrawal only a limited fraction of the ADI. Therefore, based on safety considerations, the FEEDAP Panel does not see the necessity for setting a withdrawal period for chickens for fattening and consequently for MRLs. The same conclusion applies to turkeys for fattening.

The FEEDAP Panel considers robenidine as the marker residue in chickens and turkeys. Ratios for the marker residue to total residue could be established for a zero-withdrawal time in both species. If MRLs are required, the FEEDAP Panel proposes:

- chicken - per kg wet tissue: 0.80 mg for liver, 0.35 mg for kidney, 0.20 mg for muscle and 1.30 mg for skin/fat,
- turkey - per kg wet tissue: 0.40 mg for liver and skin/fat and 0.20 mg for kidney and muscle.

The residue intake calculated from the above MRLs is below the ADI (95 % for chickens and 48 % for turkeys). These values overestimate the real exposure due to the insensitivity of the analytical method for the determination of tissue residues.

Off-flavours were observed in edible tissues of chickens for fattening until three days after withdrawal. Despite the absence of data on the impact of robenidine on the sensory properties of turkey products, the FEEDAP Panel considers that chickens and turkeys should be similarly regarded. As a consequence, the FEEDAP Panel proposes a five-day withdrawal period for chickens and turkeys for fattening, to avoid off-flavours in edible tissues from poultry treated with robenidine. To guarantee product quality a Maximum (food product) Processing Compatible Residue (MPCR) should be introduced which would be considerably lower than the MRL. Due to the insufficient sensitivity of the available method of analysis and the absence of data at five-day withdrawal, the FEEDAP Panel cannot propose such an MPCR for robenidine.

RECOMMENDATION

The FEEDAP Panel recommends a refinement of the analytical method for detection of robenidine residues to allow the setting of an MPCR for chicken and turkey edible tissues.

DOCUMENTATION PROVIDED TO EFSA

1. Original dossier Cycostat 66G 6.6 % robenidine hydrochloride. Submitted by Alpharma.
2. Supplementary dossier. June 2002. Submitted by Alpharma.
3. Supplementary dossier. Responses to questions on application for brand specific approval. April and May 2003. Submitted by Alpharma.
4. Cycostat 66G. Additional documentation in support of comments raised by FEEDAP. July 2004. Submitted by Alpharma.
5. Technical dossier on the proposal for the addition of MRLs in chickens for fattening and turkeys to the current authorisation of the additive Cycostat®66G and for the adaptation of the withdrawal period if applicable. September 2007. Submitted by Alpharma.

6. Evaluation report of the Community Reference Laboratory feed additives on the methods(s) of analysis for Cycostat®66G. July 2008.

REFERENCES

EFSA (European Food Safety Authority), 2004a. Opinion of the scientific panel on additives and products or substances in animal feed on a request from the Commission on the re-evaluation of coccidiostats Cycostat 66G in accordance with article 9G of Council Directive 70/524/EEC. <http://www.efsa.europa.eu/EFSA/Scientific_Opinion/opinion_feedap20_ej69_cycostat_en1.pdf>

EFSA (European Food Safety Authority), 2004b. Opinion of the scientific panel on additives and products or substances in animal feed on a request from the Commission to update the opinion on the safety of "Cycostat 66G" based on robenidine hydrochloride, as feed additive in accordance with Council Directive 70/524/EEC (article 9g). <http://www.efsa.europa.eu/EFSA/Scientific_Opinion/feedap_opinion29_ej98_cycostat66g_en1.pdf>

APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory Feed Additives on the Method(s) of Analysis for Cycostat®66G for chickens and turkeys for fattening

Cycostat 66G is a coccidiostat already authorised as feed additive for use in rabbits for breeding by Commission Regulation (EC) No 2430/1999 and for use in rabbits for fattening, chickens for fattening and turkeys by Commission Regulation (EC) No 1800/2004.

The active agent of *Cycostat 66G* is robenidine hydrochloride. The authorised inclusion level is ranging from 30 to 66 mg active substance/kg complete feedingstuffs, depending on the species or category of animal.

In the current application a modification of the conditions of authorisation is sought for *Cycostat 66G* according to Article 13(3) of Regulation (EC) No 1831/2003. Specifically Maximum Residue Limits (MRLs) in chickens for fattening and turkeys are proposed. The provisional MRLs proposed for chickens for fattening are (1) 200 μg kg^{-1} in muscle, (2) 2000 μg kg^{-1} in skin/fat, (3) 1250 μg kg^{-1} in liver and (4) 750 μg kg^{-1} in kidney. For turkeys the MRLs proposed are (1) 200 μg kg^{-1} in muscle, (2) 1000 μg kg^{-1} in skin/fat, (3) 1000 μg kg^{-1} in liver and (4) 200 μg kg^{-1} in kidney.

Since robenidine hydrochloride belongs to group B of Annex I to Council Directive 96/23/EC[12], the confirmatory methods for the detection of residues in target matrices that are suitable for official control have to comply with the criteria specified in Commission Decision 2002/657/EC[13].

For the determination of the residues of robenidine in tissues of all target species the applicant proposed a High Pressure Liquid Chromatography (HPLC) method with *single* wavelength Ultraviolet (UV) detection adjusted at 317 nm. A limit of quantification (LOQ) of 100 μg kg^{-1} has been established for all tissues and animal species which is well below the proposed MRLs. Also acceptable values for the precision and accuracy have been obtained and therefore the method is considered suitable for *quantification* of robenidine in target tissues at concentrations below or at the proposed MRLs. However, the method does not comply with the required criteria for the *confirmation* of the presence of robenidine in the case of exceeding the proposed MRLs, since Commission Decision 2002/657/EC requires that for LC/UV, *two* different chromatographic systems or a *second, independent detection method* are used.

For confirmatory purposes a method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has been developed and is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety, Berlin[14]. This method was successfully in–house validated in accordance with the requirements of Commission Decision 2002/657/EC. The decision limit (CC_α) for robenidine was 2.79 μg kg^{-1} and the detection capability (CC_β) was 4.64 μg kg^{-1}.

Another confirmatory method for detection of robenidine in muscle validated according Commission Decision 2002/657/EC is available and published in literature (*Dubois M. et al., (2004). Journal of Chromatography B, 813: 181-189*). Also this method is based on liquid

[12] Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC, OJ 125, 23.05.1996, p. 10

[13] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, OJ L 221, 17.08.2002, p. 8

[14] Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Berlin, Germany

chromatography coupled to low resolution tandem mass spectrometry (LC-MS/MS). The CC_α was 0.2 μg kg^{-1} and the CC_β was 0.5 μg kg^{-1}.

The CRL concludes that both LC-MS/MS method can be applied for confirmatory purposes of robenidine in animal tissue.

Further testing or validation is not considered necessary.