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(Co)Rapporteurs' referral assessment report

For Doxycycline 50% WSP and associated names

Inn/active substance: Doxycycline hyclate

Procedure no: EMEA/V/A/059

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This document is an unredacted copy of part of the CVMP rapporteur and co-rapporteur referral assessment report of 13 April 2011 for Doxycycline 50 % WSP and associated names (Procedure no: EMEA/V/A/059).

Below is the section of the assessment report dealing with the residue depletion study supporting a meat withdrawal period of 5 days when chicken are administered at 25 mg doxycycline hyclate per kg body weight for 5 days.

2.2.4 Withdrawal Periods

The following MRLs have been established for doxycycline:

Pharmacologically active substance(s)	Marker residue	Species	MRLs (µg/kg)	Target tissue	Other provisions
Doxycycline	Doxycycline	Bovine,	100	Muscle	Table 1 of Reg. 37/2010.
			300	Liver	
			600	Kidney	
		Porcine, poultry	100	Muscle	Not for use in animals from which milk/eggs is produced for human consumption

CHICKENS

A residue depletion study for Doxycycline 50% WSP in broilers was provided (1993, GLP, Study No. 30943).

GLP	Yes	**Study No.:**	30943
Dose (mg/kg)	25.3 mg doxycycline hyclate /kg bw/day for 5 days (mean)		
Dose (volume)	50 mg product/kg bw/day for 5 days		
Route of administration	Oral (in drinking water)		
Breed	Ross	**Species**	Broilers
Age	40 days	**Weight**	1.83 kg (mean)
Batch No.	93015	**Certificate of analysis**	submitted
No. of animals per time point	6	**Sex**	Not sexed
Slaughter time points	0, 1, 2, 4, 6, 10, 14 and 20 days		
Analytical method	Microbiological assay (SOP No. 830/90/0175)		
Method validated	Yes	**LOQ**	50 µg/kg in kidney and liver; 20 µg/kg in muscle and skin; 10 µg in fat

About 20 gram of muscle, kidney and liver tissues (or as much as possible kidney) was cut in small pieces and homogenised in a Waring blender (muscle) or stomacher (kidney, liver) with the same weight phosphate buffer (0.2 M). The homogenates were deep frozen (:s-20°C) in PPN tubes until analysis. Muscle samples were taken from the breast area muscle. Skin samples were taken from the breast and all feathers were removed followed by a thorough cleaning with hand warm water. The skin samples were homogenised in a Waring blendor for 1 minute with the same weight phosphate buffer (0.2 M) and deep frozen (<20°C) until analysis. Abdominal fat was collected and deep frozen (<-20°C) until analysis.

The quantitative determination of doxycycline in medicated water, plasma and tissue specimens was carried out by microbiological methods. The agar large-plate method was used utilising *B. subtilis* BGA or *B. cereus* var. mycoides spores as test strains. For quality control reasons spiked control samples (QC-samples) at 2 different levels were prepared with fresh blank material at the time of slaughter of the first broilers and deep frozen (<-20°C). The QC-samples were analysed simultaneously with the samples. Also for quality control reasons a number of experimental samples were subjected to replicate analysis (22% of the samples).

After a withdrawal period of four days the average doxycycline concentrations in the tissues were below the MRL. Within six days after medication the mean doxycycline concentrations were below 100 µg/kg in all matrices. A withdrawal period of 4 days would be advisable after water medication of 25 mg doxycycline/kg/day to broilers.

Validation of the method

The validation of the microbiological methods for determination of doxycycline in medicated water, plasma and various tissue specimens are summarised in the table below. The calibration curves for doxycycline in various matrices were linear in the range tested and revealed correlation coefficient >0.99. The limits of quantifications (LOQs) were 10 ppb for fat, 20 ppb for skin and muscle, and 50 ppb for plasma, kidney and liver. The methods were reproducible with respect to replicate analysis of QC-samples and experimental samples (mean precision coefficient of variation < 8.1 %). From the fat tissue the mean doxycycline recovery was 52.5 % (n= 23).

MATRIX	PRECISION CV%	% ACCURACY *
PLASMA LOQ 0.050 µg/ml		
Reproducibility calibration	4.3	101
Reproducibility quality controls	5.3-3.6	105
Reproducibility replicate analyses	4.2	
KIDNEY LOQ 0.050 µg/g		
Reproducibility calibration	3.9	100
Reproducibility quality controls	2.3-8.1	94.7
Reproducibility replicate analyses	5.5	
LIVER LOQ 0.050 µg/g		
Reproducibility calibration	3.2	100
Reproducibility quality controls	5.7-3.4	100
Reproducibility replicate analyses	4.6	
MUSCLE LOQ 0.020 µg/g		
Reproducibility calibration	3.9	101
Reproducibility quality controls	5.7-3.6	100
Reproducibility replicate analyses	6.6	
SKIN LOQ 0.020 µg/g		
Reproducibility calibration	3.7	100
Reproducibility quality controls	6.5-3.1	100
Reproducibility replicate analyses	3.7	
FAT LOQ 0.010 µg/g		
Reproducibility calibration	1.0	101
Reproducibility quality controls	1.2-0.9	96.4
Reproducibility replicate analyses	6.4	

* : In case of quality control samples, expressed as % of the spiked concentration.

Rapporteur's comments: *The study above is an old one but appears to have been conducted in a reasonable manner. The right number of animals were used and all of the other study details were in accordance with current requirements. The main shortcoming of the above study was the use of a microbiological assay. Although, it was generally validated well, storage stability was not addressed in the above study. The QC samples were not stored with the test samples to allow the effect of storage on the levels of the marker residue to be evaluated. This could have affected the results that were reported.*

It is noted that the applicant now wishes to propose a chicken meat withdrawal period of 4 days, whereas 6 days was proposed during the Article 35 referral. Using the 'Alternative approach', the first time point where residues in all tissues fell below their respective MRLs was 4 days (muscle and fat being the determining tissues). A 'safety span' greater than is normally used for this type of product is required as storage stability has not been accounted for. Hence, addition of a 30% 'safety span' gives a meat withdrawal period of 6 days. The 'Statistical approach' could not be applied as the statistical assumptions were not met. Therefore, a chicken meat withdrawal period of 6 days is proposed when chicken are administered 25 mg doxycycline/kg bw/day for 5 days.

Co-rapporteur´s comments

The co-rapporteur agrees with conclusion of rapporteur. In addition the influence of QC samples on results could be done by the earlier preparation (090793) in comparison with sampling (150793 –

040893). The part of or whole picture with levels of residue is unreadable therefore we decided to put these tables on this place again:

TABLE 7a. Doxycycline concentrations (μg/ml or μg/g) in plasma and edible tissues

Animal code	Date of slaughter	Days after cessation of medication	Kidney	Liver	Muscle	Skin	Fat	Plasma
Controls								
C1	150793	0	<0.050	<0.050	<0.020	0.062	0.017	<0.050
C2	150793	0	<0.050	<0.050	<0.020	<0.020	<0.010	<0.050
C3	150793	0	<0.050	<0.050	<0.020	<0.020	<0.010	<0.050
C4	150793	0	<0.050	<0.050	<0.020	<0.020	<0.010	<0.050
C5	150793	0	<0.050	<0.050	<0.020	<0.020	<0.010	<0.050
C6	150793	0	<0.050	<0.050	<0.020	<0.020	<0.010	<0.050
medicated	broilers							
I1	150793	0	1.36	0.97	0.98	0.91	0.073	1.50
I2	150793	0	2.91	1.25	1.02	0.98	0.17	2.34
I3	150793	0	2.15	0.77	0.70	0.78	0.038	1.05
I4	150793	0	2.53	1.37	0.98	0.96	0.092	2.43
I5	150793	0	1.97	0.79	0.77	0.82	0.090	1.38
I6	150793	0	2.61	2.00	0.93	0.75	0.17	3.33
mean			2.26	1.19	0.90	0.87	0.11	2.01
s.d.			0.55	0.46	0.13	0.10	0.054	0.85
II1	160793	1	0.76	0.25	0.17	0.22	0.026	0.66
II2	160793	1	0.60	0.20	0.13	0.29	0.016	0.33
II3	160793	1	0.26	0.10	0.11	0.20	0.014	0.34
II4	160793	1	0.53	0.14	0.17	0.23	0.033	0.29
II5	160793	1	0.47	0.20	0.13	0.21	0.021	0.18
II6	160793	1	0.65	0.18	0.13	0.24	0.061	0.66
mean			0.55	0.18	0.14	0.23	0.029	0.41
s.d.			0.17	0.052	0.025	0.03	0.017	0.20
III1	170793	2	0.39	0.16	0.094	0.25	0.079	0.19
III2	170793	2	0.26	0.088	0.059	0.37	0.020	0.15
III3	170793	2	0.25	0.079	0.058	0.34	0.026	0.14
III4	170793	2	0.57	0.10	0.12	0.53	0.026	0.24
III5	170793	2	0.34	0.092	0.098	0.37	0.029	0.20
III6	170793	2	0.26	0.073	0.077	0.37	0.059	0.24
mean			0.35	0.099	0.084	0.37	0.040	0.19
s.d.			0.12	0.032	0.024	0.09	0.024	0.04
IV1	190793	4	0.16	<0.050	0.037	0.10	0.016	0.095
IV2	190793	4	0.23	0.055	0.038	0.10	<0.010	0.062
IV3	190793	4	0.15	<0.050	0.038	0.087	<0.010	0.066
IV4	190793	4	0.20	0.058	0.027	0.17	<0.010	0.067
IV5	190793	4	0.14	0.059	0.046	0.091	<0.010	<0.050
IV6	190793	4	0.24	0.073	0.046	0.16	0.014	0.12
mean			0.19	0.061	0.039	0.12	<0.020	0.082
s.d.			0.043	0.008 (n=4)	0.007	0.037		0.025 (n=5)

TABLE 7b. Doxycycline concentrations (μg/ml or μg/g) in plasma and edible tissues

Animal code	Date of slaughter	Days after cessation of medication	Kidney	Liver	Muscle	Skin	Fat	Plasma
V1	210793	6	0.062	<0.050	0.021	0.037	<0.010	<0.050
V2	210793	6	0.075	<0.050	0.020	0.084	0.015	<0.050
V3	210793	6	0.066	<0.050	0.021	0.062	<0.010	<0.050
V4	210793	6	0.068	<0.050	0.020	0.063	0.017	<0.050
V5	210793	6	0.19	<0.050	0.021	0.062	<0.010	<0.050
V6	210793	6	0.070	<0.050	0.020	0.067	<0.010	<0.050
mean			0.089		0.021	0.063	<0.020	
s.d.			0.050		0.001	0.015		
VI1	250793	10	0.053	<0.050	<0.020	0.064	0.018	<0.050
VI2	250793	10	0.065	<0.050	<0.020	0.065	0.016	<0.050
VI3	250793	10	0.053	<0.050	<0.020	0.10	<0.010	<0.050
VI4	250793	10	0.051	<0.050	<0.020	0.083	0.018	<0.050
VI5	250793	10	<0.050	<0.050	<0.020	0.075	<0.010	<0.050
VI6	250793	10	0.053	<0.050	<0.020	0.11	<0.010	<0.050
mean			0.055			0.083	<0.020	
s.d.			0.006			0.019		
			(n=5)					
VII1	290793	14	<0.050	<0.050	<0.020	0.038	<0.010	<0.050
VII2	290793	14	<0.050	<0.050	<0.020	0.046	<0.010	<0.050
VII3	290793	14	<0.050	<0.050	<0.020	0.047	0.011	<0.050
VII4	290793	14	<0.050	<0.050	<0.020	0.058	0.014	<0.050
VII5	290793	14	<0.050	<0.050	<0.020	0.057	0.011	<0.050
VII6	290793	14	0.050	<0.050	<0.020	0.060	<0.010	<0.050
mean			<0.050			0.051	<0.020	
s.d.						0.009		
VIII1	040893	20	<0.050	<0.050	<0.020	0.036	<0.010	<0.050
VIII2	040893	20	<0.050	<0.050	<0.020	0.030	<0.010	<0.050
VIII3	040893	20	<0.050	<0.050	<0.020	0.030	<0.010	<0.050
VIII4	040893	20	<0.050	<0.050	<0.020	0.031	<0.010	<0.050
VIII5	040893	20	<0.050	<0.050	<0.020	0.032	<0.010	<0.050
VIII6	040893	20	<0.050	<0.050	<0.020	0.037	<0.010	<0.050
mean						0.033		
s.d.						0.003		

TABLE 8. Summary of the doxycycline (mean ± s.d.) concentrations (μg/ml or g) in plasma and edible tissues.

Group	Date of slaughter	Days after cessation of medication		Kidney	Liver	Muscle	Skin	Fat	Plasma
C	150793	0	mean s.d.	<0.050	<0.050	<0.020	<0.020	<0.010	<0.050
I	150793	0	mean s.d.	2.26 0.35	1.19 0.46	0.90 0.13	0.87 0.10	0.11 0.054	2.01 0.85
II	160793	1	mean s.d.	0.55 0.17	0.18 0.052	0.14 0.025	0.23 0.03	0.029 0.017	0.41 0.20
III	170793	2	mean s.d.	0.35 0.12	0.099 0.032	0.084 0.024	0.37 0.09	0.040 0.024	0.19 0.04
IV	190793	4	mean s.d.	0.19 0.043	0.061 0.008 (n=4)	0.039 0.007	0.12 0.037	<0.020	0.082 0.025 (n=5)
V	210793	6	mean s.d.	0.089 0.050	<0.050	0.021 0.001	0.063 0.015	<0.020	<0.050
VI	250793	10	mean s.d.	0.055 0.006 (n=5)	<0.050	<0.020	0.083 0.019	<0.020	<0.050
VII	290793	14	mean s.d.	<0.050	<0.050	<0.020	0.051 0.009	<0.020	<0.050
VIII	040893	20	mean s.d.	<0.050	<0.050	<0.020	0.033 0.003	<0.020	<0.050

Rapporteur's conclusions:

Given that QC samples were prepared at the same time as test samples and analysed together, the peer reviewer proposed that a 25% 'uncertainty factor' can be used. The rapporteur agrees that with a 25% 'uncertainty factor', **a chicken meat withdrawal period of 5 days can be accepted.**