TILMICOSIN

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Addendum to the monographs prepared by the 47th meeting of the Committee and published in the FAO Food and Nutrition Paper 41/9

BACKGROUND

The forty-seventh meeting of the Committee (FAO/WHO, 1998) reviewed tilmicosin and established an ADI of 0-40 µg/kg body weight (0-2400µg per day for a 60 kg person). The following MRLs (µg/kg) for cattle, sheep and pigs were recommended:

Species	Food commodity				
	Muscle	Liver	Kidney	Fat	Milk
Cattle	100	1000	300	100	
Sheep	100	1000	300	100	50 (T)
Pigs	100	1500	1000	100	

The temporary MRL of 50µg/kg for sheep milk was not extended by the Committee at the fifty-fourth meeting as results of a study with radioactively labeled drug in lactating sheep to establish the relationship between total residues and parent drug in milk was not available. The present addendum addresses both new and relevant previously submitted data.

The sponsor has requested MRLs for tilmicosin in chicken, turkey and rabbit tissues and chicken eggs in addition to a MRL for sheep milk. In this submission the sponsor explains the reasons for not having provided a total residue study in sheep milk using ^{14}C-tilmicosin as requested by the forty-seventh meeting of Committee.

IDENTITY

IUPAC Name: (5S,6S,7R,9R,11E,13E,15R,16R)-6-[(2R,3R,4S,5S,6R)-4-dimethylamino-3,5-dihydroxy-6-methyloxan-2-yl]oxy-7-[2-(3,5-dimethylpiperidin-1-yl)ethyl]-16-ethyl-4-hydroxy-15-[[(2R,3R,4R,5R,6R)-5-hydroxy-3,4-dimethoxy-6-methyloxan-2-yl]oxymethyl]-5,9,13-trimethyl-1-oxacyclohexadeca-11,13-diene-2,10-dione

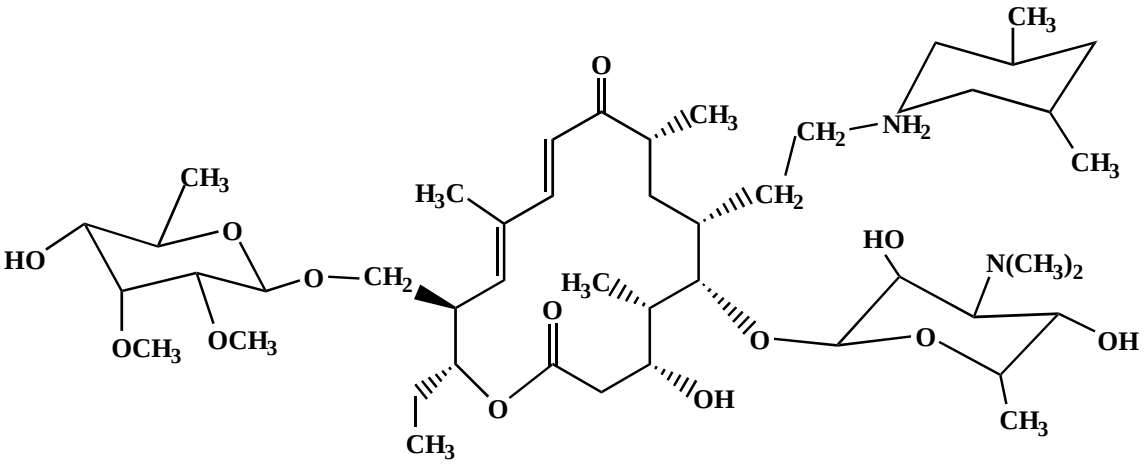
CAS Name: Tylosin,A-O-de(2,6-dideoxy-3-C-methyl-alpha-L-ribo-hexopyranosyl)-20-deoxy-20-(3,5-dimethyl-1-piperidinyl)-(20(cis: trans))

Other names: 20-dihydro-20-deoxy-20-(cis-3,5- dimethylpiperidin-l-yl)-desmycosin

CAS Number: 108050-54-0

Synonyms: NCBI PubChem Compound lists 19 synonyms
(Examples: Tilmicosin, Micotil, Micotil (TN), Micotil 300)

Structural formula:



Molecular formula: $C_{46}H_{80}N_2O_{13}$ (tilmicosin)
$C_{46}H_{83}N_2O_{17}P$ (tilmicosin phosphate)

Molecular weight: 869.133 [g/mol] (tilmicosin)
967.128 [g/mol] (tilmicosin phosphate)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Active ingredient: Tilmicosin is a white to off-white solid comprised of a *cis* isomer and a diastereomeric pair of *trans* isomers. The ratio of *cis* to *trans* isomers is about 85:15, respectively.

Melting point: Melting points of commercial products are not regularly given. 107-112°C and 143-149 °C can be found for certain commercial products of tilmicosin and tilmicosin phosphate, respectively.

Solubility: Tilmicosin base has a solubility of 1500 mg/L in n-hexane and solubility of up to >5000 mg/L in other organic solvents, e.g., acetone, acetonitrile, chloroform, dichloromethane, ethyl acetate, methanol, and tetrahydrofuran. Solubility in water and distribution between aqueous and organic phases is strongly pH-dependent (Xu, et al., 2006). The pKa values of tilmicosin *cis* and *trans* isomers are 7.4 and 8.5, respectively, in 66% dimethylformamide. At pH 9, the solubility is 7.7 mg/mL at 25°C and 72.5 mg/mL at 5°C. At pH 7 and 25°C, the solubility is 566 mg/mL.

Purity: Commercial products are of variable purity and isomeric composition (Stoev and Nazarov, 2008). Typical products may consist of 82-88 % *cis* isomer and 12-18% *trans* isomer

UV-absorbance: Tilmicosin exhibits a UV absorbance maximum at 284 nm. When in solution, tilmicosin is light sensitive.

RESIDUES IN FOOD AND THEIR EVALUATION

Condition of use

Tilmicosin is a macrolide antibiotic developed for veterinary use. The following are examples of recommended uses for the prevention and treatment of diseases caused by tilmicosin sensitive microorganisms:

- Pigs: treatment and prevention of pneumonia caused by *Actinobacillus pleuropneumoniae, Mycoplasma hyopneumoniae* or *Pasteurella multocida.*
- Cattle: Treatment and metaphylaxis of respiratory diseases caused by *Mannheimia haemolytica* und *Pasteurella multocida*. Tilmicosin is not to be used in cattle producing milk for human consumption.
- Sheep: For the treatment of pneumonia associated with *Mannheimia haemolytica* und *Pasteurella multocida*; for the treatment of ovine mastitis associated with *Staphylococcus aureus* and *Mycoplasma agalactiae* and as an aid in the control of enzootic abortion in ewes caused by *Chlamydia psittaci*.
- Rabbits: therapy of respiratory tract infections caused by *Pasteurella multocida* and *Bordetella bronchiseptica* and of bacterial enteritis caused by *Clostridia*.
- Chickens: For the treatment of respiratory infections in chicken flocks, associated with *Mycoplasma gallisepticum, M. synov*iae and other organisms sensitive to tilmicosin.
- Turkeys: For the treatment of respiratory infections in turkey flocks, associated with *Mycoplasma gallisepticum*. Tilmicosin is currently not to be used in chickens and turkeys producing eggs for human consumption.

Dosage

On the request of the Committee the sponsor provided copies of approved labels from a several countries. The information given in table 1 was extracted from the label instructions. Species not subject to a detailed review in the present monograph are given in squared brackets and no further details are included in the table. In summary: the currently recommended modes of administration include (examples only) subcutaneous injection in pigs, cattle and sheep, oral administration via feed in rabbits and pigs, and administration via drinking water in pigs, calves, chickens and turkeys and via milk, milk replacer in calves.

Table 1: Conditions of registered uses of tilmicosin in selected countries.

Country	Product	Target species	Treatment	Daily dose [mg/kg bw]	Withdraw time days]	Warnings and related texts
Austria	PULMOTIL ® Premix 20%, granulate	**Rabbit** [pigs]	Respiratory diseases: 100-200 ppm in feed, 7 days	10-12	5	
			Bacterial enteritis: 40-80 ppm in feed, 7 days	5-6		
Ireland	Pulmotil AC tilmicosin	**Chicken**	75 mg/L in drinking water, 3 days	10-25	12	Not to be used in chickens and turkeys producing eggs for human consumption.
		Turkey		6-30	15	
		[pigs]				
France	PULMOTIL AC Usage veterinaire Tilmicosine, Formulation aqueuse	**Chicken**		15-20	12	Not to be administered to hens producing eggs for human consumption
		Turkey		10-27	19	
Switzerland	Pulmotil AAC ad us.vet. liquid premix	**Chicken** [calves, pigs]	30-40 mL Pulmotil/100 mL of drinking water, 3 days	15-20	12	At the exception of laying hens producing eggs destined for consumption
Philippines	TILMICOSIN PHOSPHATE Pulmotil AC	**Chicken** [pigs]	75 mg/L in drinking water, 3-5 days for prevention, 5-7 days for treatment		10	Contraindication: Should not be used in birds producing eggs for human consumption.
Ireland	Micotil	**Sheep** [cattle]	Single dose of 10 mg/kg bw (1 ml Micotil /30kg)			Not for use in cattle producing milk for human consumption.

PHARMACOKINETICS AND METABOLISM

A number of studies provided information on pharmacokinetics, metabolism and on tissue residue depletion in target animal species. In such cases major pharmacokinetic findings are briefly summarized in this section and more details and data evaluations are given below in the section on tissue residue depletion studies.

Ruminants

A published study (Modric, et al. 1998) compared the pharmacokinetics of tilmicosin in cattle and sheep after subcutaneous administration of a dose of 10 mg/kg bw. The pharmacokinetic parameters derived from the time concentration curve (T_{max}, C_{max}, $T_{1/2}$, AUC) were not significantly different between species. Individual animal data were not provided and no information about equivalency of tissue distribution and metabolism could be derived.

A peer-reviewed pharmacokinetic study of tilmicosin in goats studied the bioavailability of tilmicosin after intravenous or subcutaneous administration of 10 mg/kg (Ramadan 1997). Concentrations in plasma and milk of goats were determined by a microbiological assay (LOD =5 ng/ml, LOQ = 10 ng/ml). A small fraction of tilmicosin was absorbed very slowly. C_{max} in plasma was 1.56 µg/ml. Tilmicosin was excreted in milk with a mean concentration peak of 11.6µg/mL and a slow depletion rate maintaining detectable concentrations more than 5 days after administration.

A GLP compliant radiometric study was performed in cows which were approximately two months from calving (Donoho and Thomson 1990). Radio-labeled tilmicosin was administered subcutaneously at a single dose of 10 mg/kg bw. The animals were managed as dry cows until parturition and milk samples collected after this time. In colostrums, tilmicosin represented 89 % of the total radioactive residue, which means that the administered dose remained largely unchanged for a long period since the interval between dosing and calving was around 50 days.

Chickens

Studies using ^{14}C-labelled tilmicosin

In a GLP compliant study (T5C749505, Ehrenfried, et al. 1996a) four week old Hubbard White Mountain Cross chickens were given *ad libitum* access to ^{14}C-tilmicosin (specific activity 0.278 µCi/mg) in medicated drinking water for five consecutive days. Two concentrations in water were tested (25 and 50 mg/L, respectively). Of the animals receiving the higher dose two groups were formed. The animals of the lower dose group and one of the higher dose groups were sacrificed 7 days after the end of treatment. The remaining group was sacrificed 10 days after the end of the treatment. Radioactivity was determined by liquid scintillation counting in liver, kidney, thigh and breast muscle, abdominal and skin fat, and bile. Although dosing was variable it is evident that the concentrations of residues in liver and kidney of individual animals were strictly proportional to the dose the individual animals had received. High concentrations of residues were also found in bile. The concentrations in muscle and fat were very low. Table 2 provides a summary of the results of the study.

Table 2: Summary of the results of study T5C749505.

Concentration in water [mg/L]	Animal	sex	Withdrawal time [days]	Dose [mg/kg]	Concentration in tissues [mg/kg]					
					Liver	Kidney	Breast muscle	Abdominal fat	Skin fat	Bile
25	9732	m	7	32.2	0.42	0.33	0.02	0.01	0.02	0.49
25	9739	m	7	20.7	0.21	0.14	<LOD	<LOD	<LOD	
25	9751	m	7	20.0	0.37	0.12	<LOD	<LOD	0.02	0.3
25	9708	f	7	21.0	0.14	0.14	<LOD	<LOD	<LOD	
25	9711	f	7	21.0	0.85	0.26	0.02	0.02	0.03	0.45
25	9715	f	7	19.0	0.15	0.16	<LOD	<LOD	<LOD	<LOD
50	9729	m	7	50.0	0.69	0.41	<LOD	0.02	0.06	0.91
50	9731	m	7	48.2	0.6	0.33	<LOD	0.02	0.05	0.69
50	9730	m	7	50.7	0.72	0.39	0.03	0.04	0.07	0.79
50	9717	f	7	51.3	0.4	0.31	<LOD	<LOD	0.02	0.25
50	9721	f	7	33.1	0.47	0.44	0.02	0.01	0.03	0.22
50	9709	f	7	43.2	0.85	0.37	0.02	0.02	0.03	
50	9742	m	10	76.9	1.96	0.7	0.03	0.02	0.07	
50	9749	m	10	52.5	1.04	0.47	<LOD	0.02	0.05	0.9
50	9738	m	10	44.5	0.8	0.36	0.04	0.02	0.05	0.59
50	9716	f	10	43.9	0.33	0.21	<LOD	<LOD	0.03	0.16
50	9720	f	10	34.5	0.3	0.18	<LOD	0.01	<LOD	0.12
50	9718	f	10	44.8	0.24	0.16	<LOD	<LOD	0.02	

LODs were given in cpm and based on the lowest count which was significantly above the background.

In another GLP compliant study (T5C749601, Ehrenfried, et al. 1996b) two groups of four weeks old Cornish Cross chicken were treated with ^{14}C-tilmicosin (specific activity 2.87 µCi/mg) for five consecutive days followed by a seven day withdrawal period. In the first group six birds were given ad libitum drinking water containing 100 mg/l of ^{14}C-tilmicosin; in the second group four birds were dosed by oral gavage twice daily at 11 mg/kg bw/day. There was some variability in the dosing via drinking water and females consumed significantly lower amounts of medicated water than males. Following sacrifice radioactivity was determined by liquid scintillation counting in liver, kidney, thigh and breast muscle, abdominal and skin fat. The results are summarised in table 3.

In another GLP compliant study (T5C749504, Ehrenfried, et al. 1997a) three groups of four week old Cornish cross chicken were dosed *ad libitum* with ^{14}C-tilmicosin (specific activity 3.13 µCi/mg) for five consecutive days. The concentrations of tilmicosin in drinking water were 150, 300, and 450 mg/l, respectively. The first and third group were sacrificed six hours after their last exposure to medicated water. Group 2 was sacrificed after 5 days withdrawal time. The entire liver minus the gall bladder, both kidneys, thigh and breast muscle, abdominal fat, skin with attached subcutaneous fat (skin fat), the brain, both lungs, bile and excreta were collected and analysed. The results are summarised in table 4.

Table 3: Results of the tissue analyses of study T5C749601.

Animal	Sex	Average daily dose [mg/kg]	Withdrawal time [days]	Concentrations in tissues [mg/kg]					
				Liver	Kidney	Muscle	Abdominal Fat	Skin Fat	Bile
6564	m	23.2	7	5.24	1.91	0.22	0.08		
6559	m	34	7	5.13	2.01	0.2	0.12		6.6
6565	m	23.5	7	2.8	1.3	0.07	0.05		2.3
6573	f	15.2	7	4.53	1.3	0.18	0.08	0.24	1.9
6576	f	17	7	4.05	1.6	0.13	0.07	0.22	4.6
6574	f	18.2	7	2.41	1.19	0.06	0.05	0.11	1.4
6557	m	22	7	4.37	2.18	0.12	0.12	0.29	7
6561	m	22	7	2.96	2.07	0.08	0.07	0.26	2.8
6572	f	22	7	2.75	1.44	0.08	0.06	0.1	2.6
6571	f	22	7	3.75	1.57	0.1	0.07	0.19	3.1

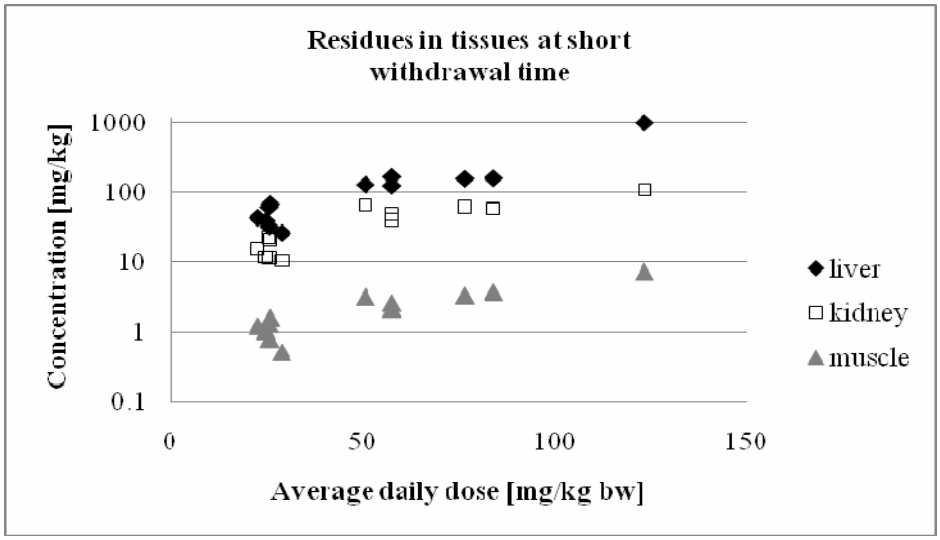
Table 4: Results of the tissue analyses carried out in study T5C749504.

Animal	Sex	Average daily dose [mg/kg bw]	Withdrawal time [days]	Liver	Kidney	Muscle	Abdominal Fat	Skin Fat	Brain	Lung	Bile
				Concentration [mg/kg]							
6531	m	29.0	0.25	25.9	10.6	0.5	0.4	0.6	0.2	2.0	71.4
6538	m	26.0	0.25	68.1	20.9	1.6	1.0	1.5	0.4	5.8	385
6542	m	25.8	0.25	32.0	11.6	0.8	0.6	0.9	0.2	2.6	212
6501	f	25.7	0.25	61.5	22.5	1.3	1.0	1.4	0.3	6.4	216
6504	f	22.8	0.25	43.1	15.6	1.2	0.7	1.3	0.4	5.8	122
6506	f	24.9	0.25	38.8	11.7	1.0	0.7	1.0	0.2	3.2	167
6528	m	50.2	5	73.1	13.4	0.8	0.6	1.4	1.1	9.2	47.6
6543	m	50.7	5	19.6	4.6	0.4	0.3	0.8	0.5	2.7	22.1
6546	m	52.9	5	16.8	6.0	0.3	0.4	0.7	0.3	1.7	13
6505	f	46.0	5	16.6	5	0.4	0.3	0.6	0.4	2.3	14.9
6523	f	51.8	5	5.9	3.3	0.2	0.2	0.3	0.1	1.2	8.4
6527	f	46.4	5	10.6	4.4	0.2	0.2	0.3	0.3	1.2	8.5
6532	m	76.7	0.25	157	62.3	3.4	2.8	3.8	0.7	21.4	1456
6533	m	123.5	0.25	1007	109	7.5	4.7	6.0	1.4	33.0	10650
6548	m	84.2	0.25	160	59.1	3.8	2.6	3.8	0.5	10.3	840
6507	f	50.8	0.25	129	65.5	3.2	3.5	3.0	0.8	15.0	809
6510	f	57.6	0.25	125	40.3	2.6	2.0	2.7	0.6	11.4	802
6518	f	57.6	0.25	168	49.2	2.2	1.2	2.5	0.6	10.1	603

It is evident that the intended high dose could not be achieved in females and the results were highly variable in males. Liver was the edible tissue with the highest concentration of ^{14}C-tilmicosin-equivalents. The concentrations of radioactive residues were very high in samples of bile collected early after the end of the treatment of the animals. At later time points they were in the order of the concentrations found in liver. The concentrations of residues determined in tissues of animals of groups one and three can be directly compared because the withdrawal time was the same (6 hours). These results are plotted in figure 1 as function of the determined average daily dose. The curves for the three tissues are approximately parallel. Reviewing each tissue individually using the most appropriate linear scaling (not shown) there is strict proportionality between the achieved dose and the

concentration of residue – with the exception of one outlying point for liver in the animal that had received the highest dose. Dose linearity is also clearly seen in other studies.

Figure 1: Initial concentrations of total residues in edible tissues as function of dose.



The authors found that approximately 70% of the administered doses were excreted by the end of the treatment period and that excretion had probably reached a steady state at that time. Figure 2 shows the concentrations of radioactive residues in excreta collected on every treatment day. The results seem to confirm this statement despite some variability observed in the highest dose group which might be explained on the basis of the variability of the doses achieved. This is illustrated in figure 3 where the concentrations in excreta in males and females observed on the last treatment day are plotted as function of the average daily dose.

Figure 2: Concentration of radioactive residues (tilmicosin equivalents) in excreta.

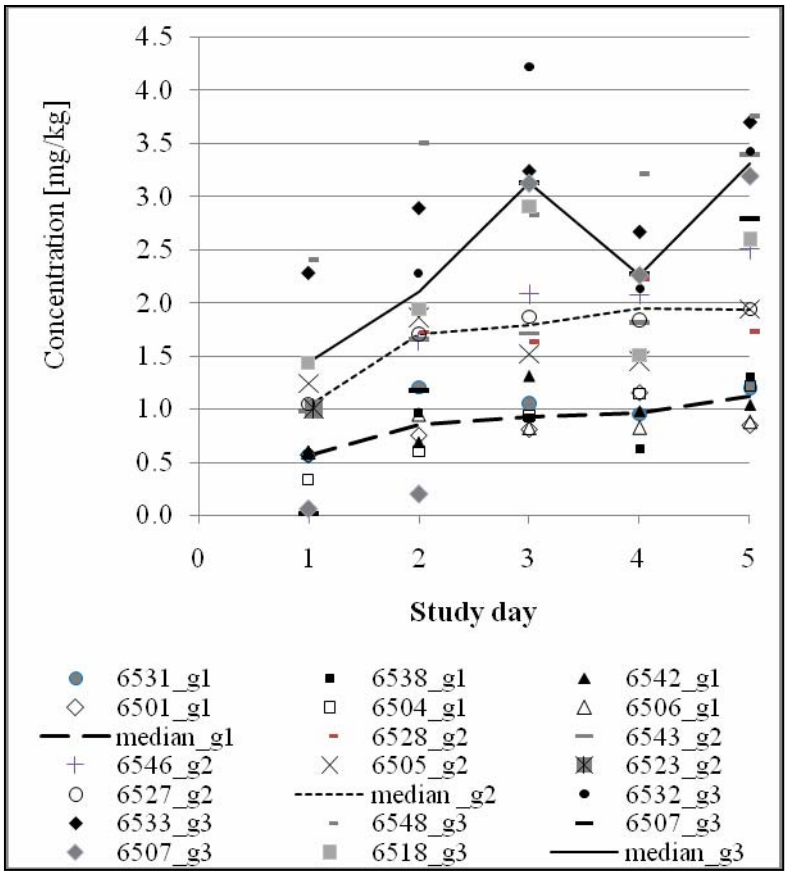
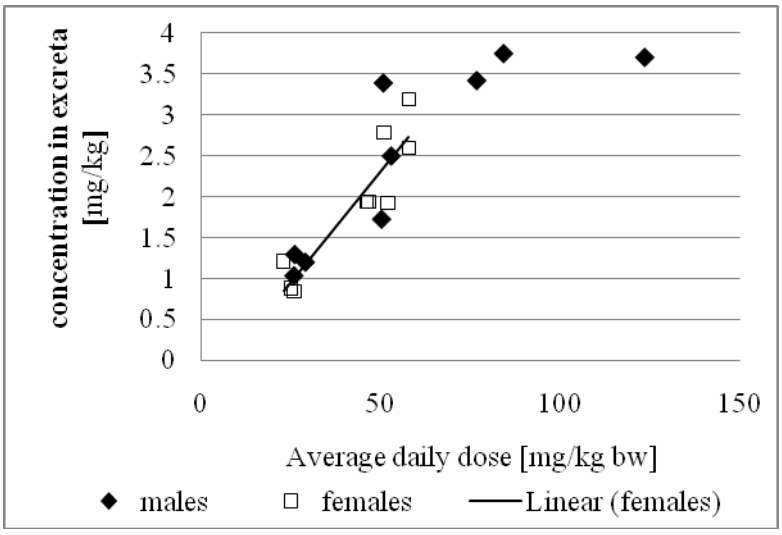


Figure 3: Day 5 concentration of radioactive residues [tilmicosin equivalents] in excreta.



Extracts of liver, kidney, muscle, lung, excreta and bile were prepared and the extracts were subjected to cleanup and complex partitioning schemes. The fractions were analysed by HPLC and radioactivity was determined. The structure of metabolites was determined using ESP-MS. In total, a number of metabolites and parent tilmicosin were found in the extracts. The structures are briefly described in table 5.

Table 5: Main metabolites found in tissues and excreta of chicken in study T5C749504.

Compound	Description
Parent tilmicosin	Including tilmicosin cis-8-epimer
T-1	Tilmicosin desmethylated at the dimethylamine portion of the mycaminose ring
Oxitilmicosin	A form of tilmicosin epoxidised at the macrolide ring
T-3	Replacement of the dimethylamine portion of the mycaminose ring with a hydroxyl group
T-4	Reduced form of tilmicosin, sulphated at the C11 position
T- 6	Tilmicosin devoid of the dimethylamine portion of the mycaminose ring
T- 7	Dehydroxylated form of tilmicosin devoid of the dimethylamine portion of the mycaminose ring
T- 8	Tilmicosin methylated at the mycaminose substituent
T-9	Tilmicosin devoid of its mycinose moiety
T-10	Metabolite T-1 devoid of mycinose moiety

Table 6 summarises the percent of total radioactivity attributable to the parent and major metabolites. All values are expressed in % of total radioactivity. The results suggest that in liver approximately 55% of the total radioactive residue represents parent tilmicosin. The corresponding values for kidney and muscle are approximately 40%.

Table 6: Metabolite profiles of tissues and excreta in chicken of study T5C749504.

Tissues and metabolites	Treatment groups					
	1		2		3	
	females	males	females	males	females	males
Liver	% of total radioactive residue					
Tilmicosin	49.6	55.3	36	50.2	62.3	67.7
T-1	6.6	4.8	5.3	9.1	5.4	4.7
T-2	2	1.7	2.2	4.7	2.2	1.8
Traces T-6, T-7						
Kidney						
Tilmicosin	52.2	36.1	25.2	34	49.1	43.3
T-1	7.1	7	4.9	5.2	9	7.5
T-2	1.7	1.4	1.3	1.2	1.6	1.6
T-9	4.8	2	19.9	12	3.2	2.1
T-10	2.4	1.5	6.7	3.2	1.5	1.1
Muscle						
Tilmicosin	41.8	50.8	25.4	28.8	47.1	37.2
T-1	8.7	6.2	7.4	12.3	12.8	27.5
Lung						
Tilmicosin	37.5	32.5	13	31.4	43.7	53.3
T-1 plus T-3	18.1	21.5	10.3	11.3	14.2	13
Bile						
Tilmicosin	80.9	NA	57	70.7	84.1	83
T-1	3.9	NA	NQ	7.9	3.5	3.9
T-4	2.3	NA	NQ	NQ	2.4	1.3
Oxy-tilmicosin	2.3	NA	NQ	4.8	2.6	3.8
Excreta						
Tilmicosin	31.2	41.9	33.2	36.8	31.5	30.7
T-1	7.4	7.8	5.9	7.2	9.5	8.7
T-4	35.7	26.4	37.7	33	33.1	39.2
Traces T-6, T-7, T-8						

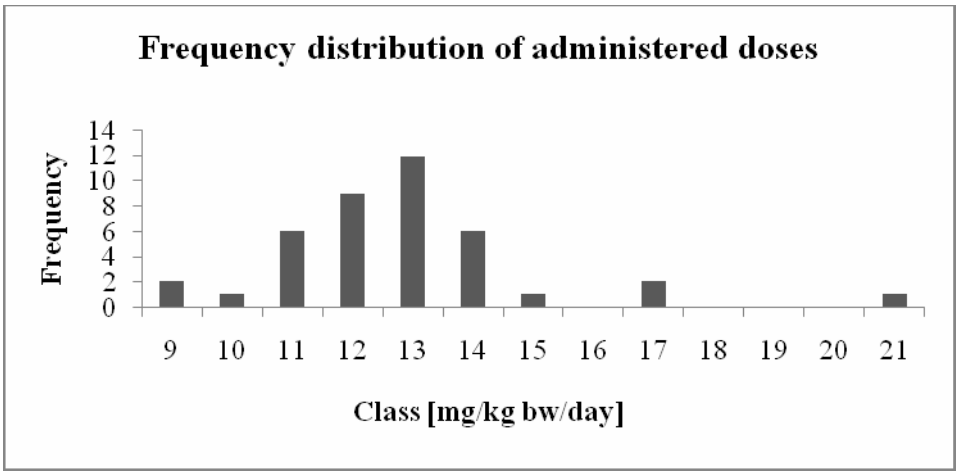
In another GLP-compliant study with ^{14}C-tilmicosin in chicken (T5C749602; Ehrenfried, et al, 1997b), five groups of eight (4 of each sex) 4-week old Cornish Cross chickens received [^{14}C]-tilmicosin (specific activity 2.62 µCi/mg) in medicated drinking water at concentration of 75 mg/L ad libitum for three consecutive days. At withdrawal times of 3, 7, 10, 14, and 21 days one group was sacrificed. The entire liver minus the gall bladder, both kidneys, samples of thigh and breast muscle, abdominal fat, skin fat, bile and excreta were collected from each animal and analysed for total radioactivity by liquid scintillation counting following solubilisation. Four randomly selected samples of liver and kidney from each group and four randomly selected muscle samples from the animals sacrificed 3 and 7 days after the end of treatment were also analysed for parent tilmicosin.

Body weights of the birds were determined twice, the first time before the dosing and the second time after dosing. The authors used the average; this is justified because the weight gains during the dosing period were up to 350g per bird. The achieved doses were variable ranging from 8.5 to 20.4mg/kg bw/day (average 12.3 ± 2.1mg/kg bw/day]. Doses were slightly higher and slightly more variable in males than in females. A frequency distribution of the doses is given below in figure 4.

The highest residue concentrations were observed in liver followed by kidney. Residue concentrations in skin fat, abdominal fat and muscle were very low. The variability of the data was high. A few data points exhibited extreme values. However, no data points were excluded from statistical analysis. A

kinetic analysis based on linear regression was performed. The results are discussed in the below section on tissue residue depletion studies.

Figure 4: Frequency distribution of doses achieved in study T5C749602.



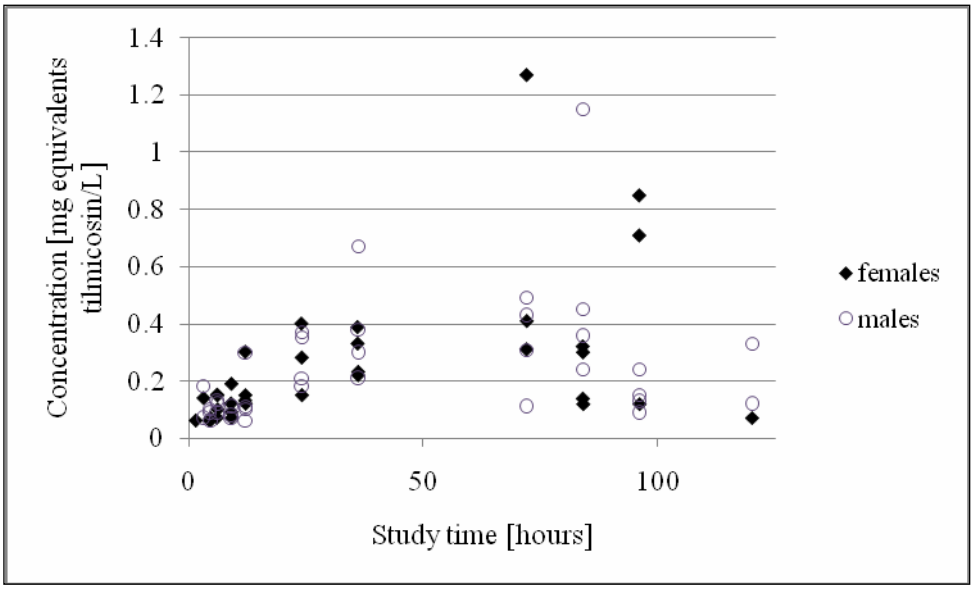
Another GLP compliant study (96 – ELA – 01, Peters, et al., 1997) involved 184 broiler chickens (92 of each sex). At an age of 3 – 4 weeks groups of animals were treated using three concentrations of tilmicosin in drinking water. Water was provided *ad libitum* from hanging drinkers. Body weight ranged from 348 to 758 g per animal on day -2. There were no significant differences between body weights of the groups. Time zero of treatment was staggered between groups in order to allow scheduling of blood samples. Achieved doses were calculated on the basis of group water intakes. Table 7 summarises the results of dosing. The grand average of the administered doses was 12.8, 21.8, and 56.0 mg/kg bw/day for the low dose, middle dose and high dose group respectively.

Table 7: Doses achieved in study 96 – ELA – 01.

Study day	Targeted concentrations in drinking water [mg/L]	Measured concentrations in drinking water [mg/L]	Achieved doses [mg/kg bw/day]		
			Minimum	Maximum	Average
0	37.5	34	10.3	13.3	11.7
	75	67	19.6	23.6	21.6
	150	138	40.3	54.4	46
1	37.5	36	10.5	19.1	13
	75	68	20.1	23.1	21.6
	150	210	61.2	75.2	68.8
2	37.5	34	10.4	23.7	13.8
	75	66	19.6	24.9	22.3
	150	137	45.6	65.9	53.2

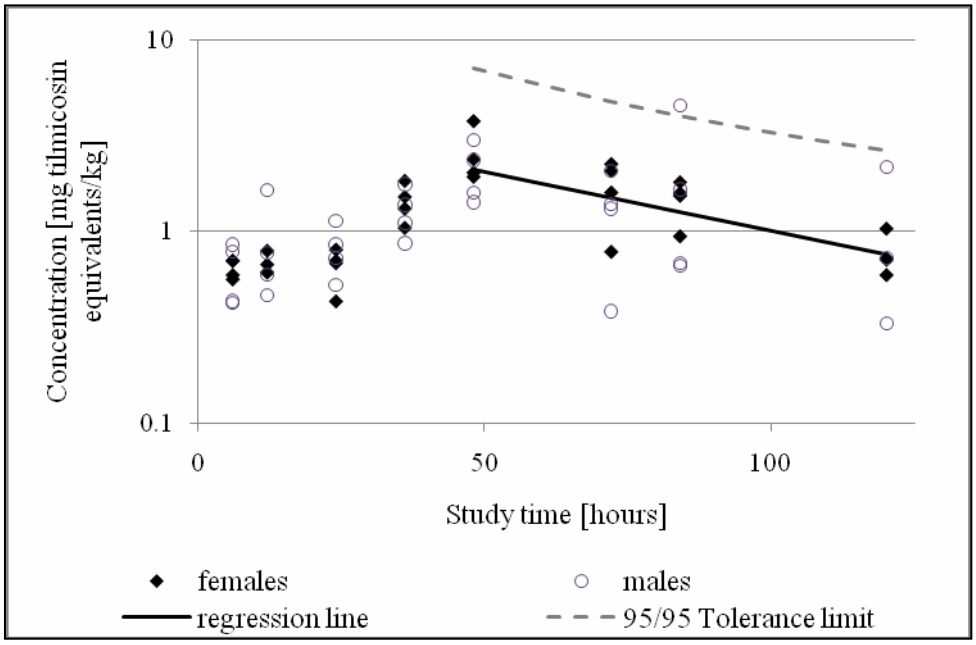
Serial blood samples were taken from the wing veins of eight birds of each group from time 0 to 120 hours after treatment. If both wing veins collapsed of developed hematomas spare chickens were used. Some haemolysed samples could not be used for the analyses. In addition, samples with a volume below 0.8 ml could not be analysed. Analyses were performed in plasma using a validated HPLC method and UV detection. Only for the high dose group there were sufficient measured values to produce a graph. The results are shown in figure 5. Results were variable and there were no significant differences observed between males and females. T_{max} cannot precisely determined because there was a data gap between 36 and 72 hours and the results obtained at 72, 84, and 96 hours were highly variable.

Figure 5: Radioactive residues in plasma samples obtained in study 96 – ELA – 01.



Four male and four female birds of the middle dose group were slaughtered at several time points from 6 to 120 hours after begin of treatment and lungs and airsac tissues were analysed using a validated HPLC method with UV detection. Since only small amounts of airsac tissue could be obtained from the animals all tissues sampled at a given time point were pooled and analysed as one sample. The total radioactive residue increased from the beginning of treatment until approximately hour 48 of the study. The depletion of the residues could be described by linear regression on a semi-logarithmic scale. Compared to plasma the residues accumulated in lungs. The results are given in figure 6.

Figure 6: Kinetics of formation and depletion of total radioactive residues in lung tissues obtained in study 96 – ELA – 01.



High concentrations of total residue also accumulated in airsac tissues. The analytical results obtained with pooled tissues of animals treated with the middle dose are shown in table 8.

Table 8: Residues in airsac tissues.

Study time [hours]	Concentration [mg equivalents tilmicosin/kg]
6	0.3
12	0.52
24	0.89
36	1.79
48	3.29
72	2.38
84	3.1
120	2.86

Turkeys

A study with unlabeled tilmicosin was performed to identify metabolites in turkey liver using HPLC-ESP-MS (Study 870 566, Ehrenfried et al. 1998). Parent drug was the main component of the extract, supporting it as marker residue for turkey.

Laying hens/eggs

Eight laying hens received by gavage, two times by day, a dose close to 10 mg/kg bw of ^{14}C-tilmicosin, during three days (SBL 004-00780, Beauchemin, et al. 2007a). Total radioactivity was determined in egg white and yolk during 24 days after the beginning of treatment. Pools of egg whites and egg yolks were extracted and analysed by HPLC-MS/MS to determine the metabolites. The ratio of tilmicosin to total residue was calculated and a value of 0.7 was estimated from the data base provided.

TISSUE RESIDUE DEPLETION STUDIES

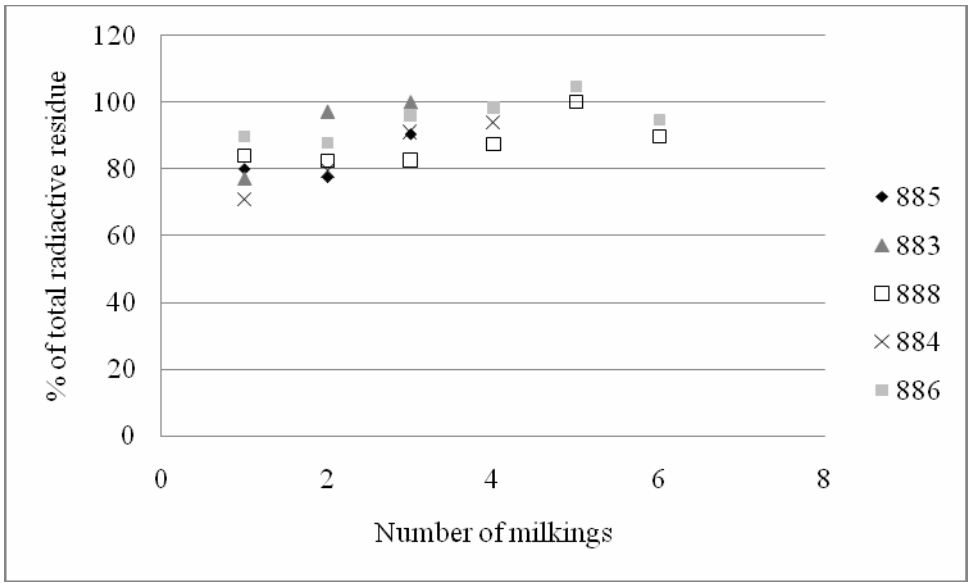
Studies in milk producing animals

Cattle

Study with ^{14}C-labelled tilmicosin

Five Holstein cows which were approximately two months from calving were injected subcutaneously with ^{14}C-tilmicosin of a specific activity of 1.28 μCi/mg at a single dose of 10 mg/kg bw (Study ABC-0447, Donoho and Thomson 1990). The study was GLP compliant. The animals were managed as dry cows until parturition. Milk samples were collected twice daily after this time and assayed for total radioactivity by liquid scintillation counting. The milkings analysed were numbers 11, 27, 14, 15, and 19, respectively for the five animals in the study. The first three to six milkings were also analysed for tilmicosin following extraction and fractionation on an HPLC column. The first milkings are usually considered colostrum unfit for human consumption. The seventh milking represents about the first which could be marketed for human consumption. With one exception no milking suitable for human consumption was analyzed. In this exceptional case it was the 15th milking obtained from one cow and the concentration of residues was below the limit of detection. In the other colostrum samples the parent drug tilmicosin represented 88.9 ± 8.8% of the total radioactive residue. This is an important finding since the interval between dosing and calving was 52, 50, 44, 59, and 49 days respectively. During this long time the administered dose remained largely unchanged in the bodies of the animals and there was no significant time trend observable over the first six milkings (see figure 7).

Figure 7: Percent of parent drug tilmicosin in the total radioactive residue in cow's milk.

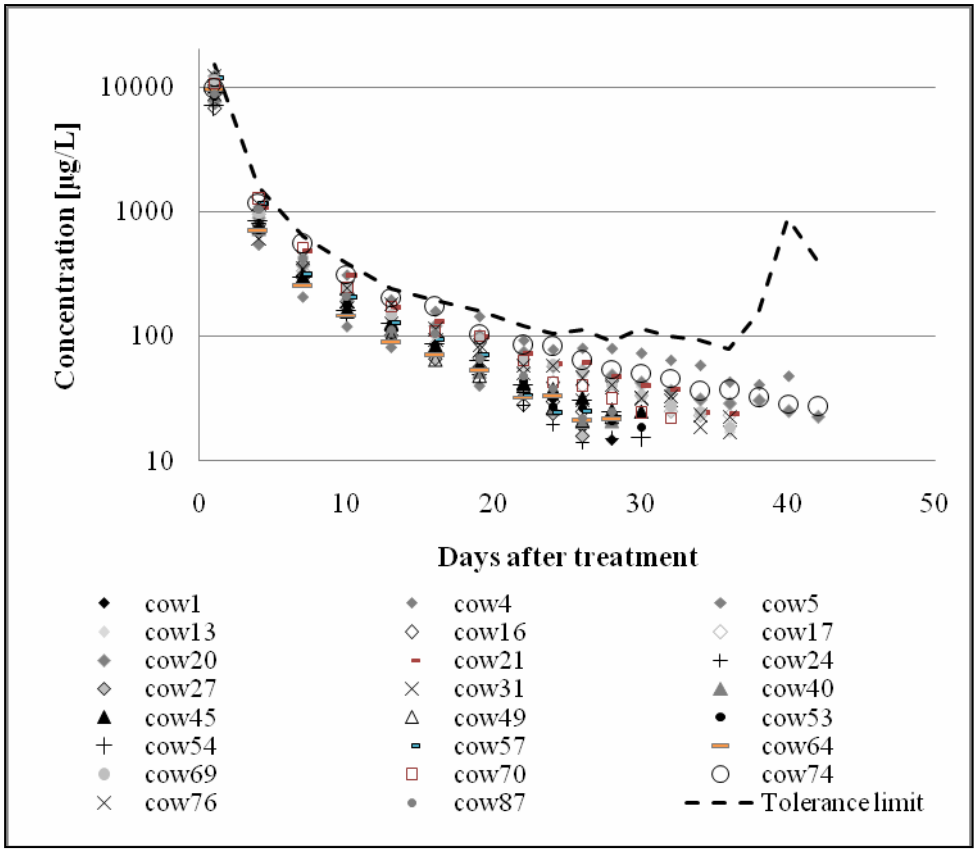


Studies with unlabelled tilmicosin

The depletion of tilmicosin was also investigated in a GLP compliant study with Holstein dairy cows (A03586/T5CCFF0301, Lacoste 2003). 25 animals received a subcutaneous injection of Micotil 300® corresponding to 10 mg/kg (range from 10.1 to 10.4 mg/kg). The labels of registered products provided by the sponsors warn that tilmicosin should not be used in cows producing milk for human consumption.

Animals in early, mid, and late lactation were used. Milk samples were taken before treatment and every evening on days 1, 4, 7, 10, 13, 16, 19, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, and 42. The samples were analyzed using HPLC. Method validation details are not given. When two consecutive concentration values fell below 50 µg/kg, subsequent samples were not analysed. Therefore, the upper one sided confidence limit over the 95th percentile increases again after 36 days (see figure 8). If one would consider recommending an MRL on the basis of a 36 day milk discard time the value would be approximately 80 µg/kg, a concentration still causing inhibitory activity in the Delvotest.

Figure 8: Depletion of tilmicosin in cow's milk.

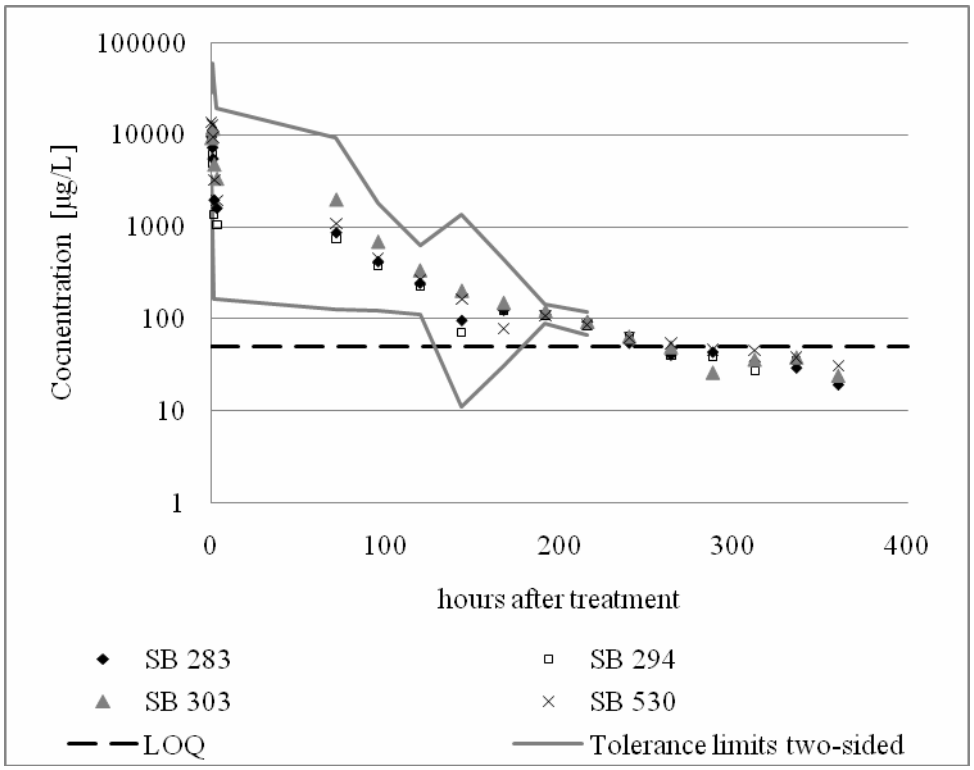


Sheep

A single subcutaneous dose of 10 mg/kg bw was given to 4 lactating Suffolk X ewes (CVLS3/92, Parker et al. 1992). They were all about 52 days after lambing and the lambs had been weaned seven days before the beginning of the study. Milk samples were taken from all four animals until day 28 after treatment. Milk was analysed for parent tilmicosin using an HPLC method. It is stated in the report that the method had been validated and that the limit of quantification was 50 µg/l. The milk was also subjected to a Delvotest and full inhibition was found for the first 6 to 7 days. No inhibition in any sample was found after day 12. The range of concentrations of parent tilmicosin was from 26 to 46 µg/kg on this day. A number of samples contained residues at concentrations below the LOQ. In order to identify those samples a line corresponding to the LOQ is drawn parallel to the x-axe in figure 9.

The data base of this study is very limited. Figure 9 visualises the extreme distances (n=4) between the measured values and the calculated (here a 2-sided for better visualisation) tolerance limits. These limits cannot be derived from linear regression like in the case of edible tissues of poultry and slaughter animals because the data points on the depletion curves are obtained from the same four animals every day. The weaknesses of the study cannot be compensated by recommending high MRLs. Consumption of milk obtained within the first 144 hours after treatment likely leads to intakes exceeding the ADI.

Figure 9: Depletion of tilmicosin in sheep's milk.



To consider recommending MRLs on the basis of longer milk discard times calculations like those shown in table 9 could be used. The MRL is derived from upper one-sided tolerance limits calculated in a conservative manner using the logarithms of the concentrations and calculating the antilog of the mean of the logarithms plus 6.37 standard deviations (for n=4).

Table 9: Example of the way of calculating MRLs for milk.

Withdrawal time [hours]	Mean (logarithms)	s.d. (logarithms)	k	One-sided Tolerance limit (antilog) [µg/l]	Intake equivalent to tolerance limit [µg/person/day]
168	2.06354	0.117100	6.37	645	1075
192	2.04976	0.022098	6.37	155	258
216	1.94515	0.025737	6.37	129	214

Another important consideration is that concentrations above 50 µg/l will most likely result in antimicrobial activity of the milk if tested in the Delvotest. While it seems possible to find MRLs in a way that the human gut flora is not affected, it would not be so to derive an MRL and a corresponding milk discard time from the available data base that provides insurance that the milk has no inhibitory properties. An MRL of 50 µg/l would require a milk discard time > 360 hour hours.

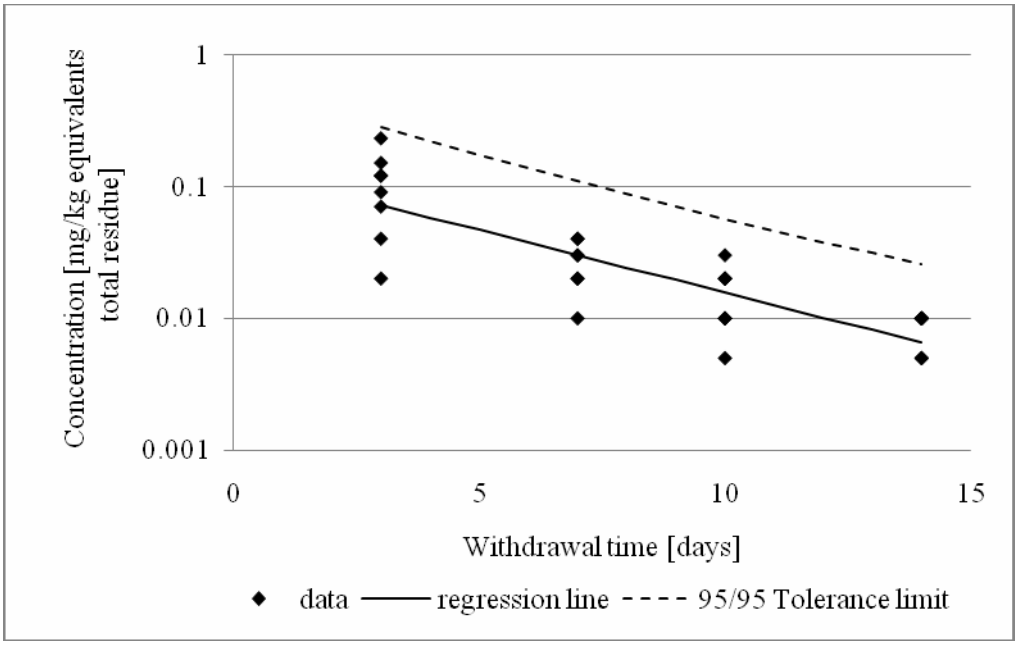
Chickens

Study with ^{14}C-labelled tilmicosin

A kinetic analysis based on linear regression was performed using the data of the above mentioned study T5C749602 (Ehrenfried, et al, 1997b). For liver and kidney all data points were used as given.

For abdominal and skin fat the analysis was limited to 3-14 days withdrawal time because at later time points too many concentrations were below the limit of detection. For non-detects occurring before or at 14 days, 0.005 mg/kg was substituted. For muscle only the data obtained for days 3-10 were suitable for statistical analysis. Non-detects were replaced by 0.01 mg/kg. Figure 10 gives an example of such analyses.

Figure 10: Example of statistical analysis of depletion data for skin fat.



The results of the statistical analysis are presented below in table 10. The authors have calculated averages of the results obtained on a given day for all animals. Since the data are not normally distributed and on day 3 there was one animal with extreme concentrations of residues in its tissues, such calculations can be misleading and suggest much higher residues than were encountered. The values predicted from the regression line and the calculated tolerance limits provide much more reliable estimates of the trends and the variability of the residue concentrations. Therefore it was preferred to perform such analysis even in cases where the data were only marginally suitable for this type of analysis. The results of this study are best suited to calculate the estimated daily intake (EDI) for total residues for the first ten days after treatment. For this time period results for all tissues are available. Skin fat was used in the food diet because of its higher concentrations of residues.

Table 10: Results of the statistical evaluation of kinetic residue data obtained in study T5C749602.

day	Predicted from regression line	Tolerance limit	Predicted from regression line	Tolerance limit	Predicted from regression line	Tolerance limit	Predicted from regression line	Tolerance limit	Predicted from regression line	Tolerance limit
	Liver		Kidney		Muscle		Skin fat		Abdominal fat	
	Concentration of total residue [mg/kg tilmicosin equivalents]									
3	2.94	20.18	0.88	3.03	0.11	0.71	0.14	0.45	0.07	0.28
7	1.56	9.93	0.66	2.18	0.04	0.24	0.07	0.20	0.03	0.11
10	0.97	6.01	0.54	1.74	0.02	0.12	0.04	0.11	0.02	0.06

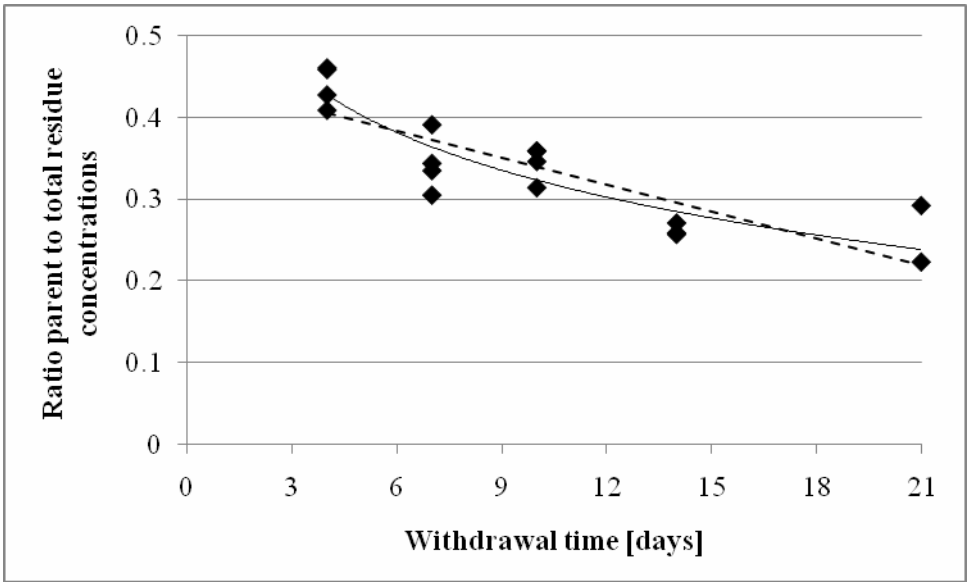
These values may need to be adjusted depending on the dose resulting from authorised treatments according to the label instructions. The information on the Irish label suggests a range of daily doses from 10 to 25 mg/kg of body weight. The French and Swiss labels assume a range of 15 to 20 mg/kg bw per day. In the "cold residue study" discussed below average daily doses ranged were 15. 9 – 20.9 mg/kg bw in females and 16.5 – 21.7 mg/kg bw in males. The average used in the study was 17.5 ± 2.2 mg/kg bw. The data given in table 11 are based on the unchanged results of the study. The ADI is 2400 µg/60 kg person/day.

Table 11: Calculation of the EDI of total tilmicosin related residue using data of study T5C749602.

day	Liver	Kidney	Muscle	Skin fat	Abdominal fat	All tissues	% of ADI
	EDI [µg/60 kg person/tissue/day]						
3	294	44	33	7.0	3.6	378	15.7
4	251	41	26	5.8	2.9	323	13.5
5	214	38	20	4.8	2.3	277	11.6
6	183	36	16	4.0	1.9	238	9.9
7	156	33	12	3.3	1.5	205	8.5
8	133	31	10	2.7	1.2	177	7.4
9	114	29	7	2.3	1.0	152	6.4
10	97	27	6	1.9	0.8	132	5.5

For a number of animals the concentration of parent drug was determined separately. For liver it was possible to establish a time trend which is given graphically in figure 11. The graph shows the data points and two possible trend lines (linear and logarithmic interpolation of the data). Similar time trends could not be established for other tissues. The ratio in kidney on day three was 0.3. The ratio in muscle did not change between days three and seven and was approximately 0.66.

Figure 11: Ratio of marker to total residue concentrations in liver.



Some representative tissue samples were extracted and metabolite profiles were determined. Table 12 shows percent of parent drug in tissue extracts. These values overestimate the parent to total ratio because the reference is the radioactivity in the extract and not the total radioactivity. They are used here to demonstrate that for kidney the ratio decreases over time. Because of the uncertainties in the determination of ratios it might be more appropriate to derive MRLs from a marker residue study and

calculate in parallel the corresponding intakes for each time point directly from the study discussed here, rather than to use highly uncertain conversion factors. The EDI would then represent a conservative "worst case" estimate.

Table 12: Percent of total extracted residue representing parent drug.

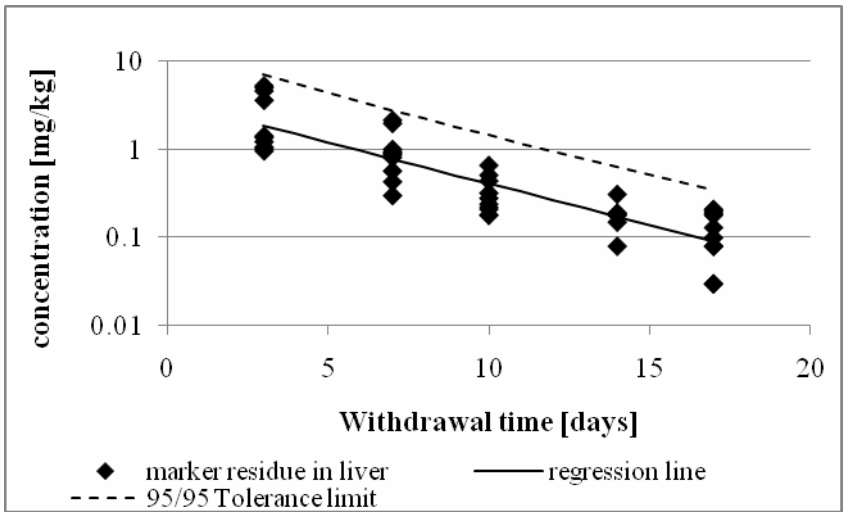
day	% parent tilmicosin in extract	
	Liver	Kidney
3	49.5	19.2
7	46.5	6.3
10	37.6	11.1
14	26.2	2.7
21	18.9	7.2

Studies with unlabelled tilmicosin

Chickens were dosed with tilmicosin in drinking water (75 mg/l) for three consecutive days in a GLP compliant study (T5C619610, Readnour, et al., 1997). Access to water was *ad libitum*. Five male and five female chickens were sacrificed on days 3, 7, 10, 17, and 21 after the end of treatment. Four males and three females were sacrificed 14 days after treatment. Three animals of this group were lost due to death or injury. Liver, kidney, breast and leg muscle, skin fat and abdominal fat were analysed. The limit of quantification was 0.06 mg/kg for liver and kidney (0.3 for day 17 and day 21 tissues) and 0.025 for muscle and fat.

The range of body weights of the animals was 890 – 1256g (mean 1065g) for males and 853 – 1170g (mean 976g) for females before the animals were treated. The report of the study does not provide individual animal based dosing information. The dose calculation was based on mean pen weight of the animals (for each animal the average of the body weights before and after treatment was used) and on total pen water intake. Even under these conditions of calculation average daily doses ranged from 15.9 – 20.9 mg/kg bw in females to 16.5 – 21.7 mg/kg bw in males. The average used in the study was 17.5 ± 2.2 mg/kg bw.

Figure 12: Depletion of marker residue in chicken liver.



The results of the determination of residues were subjected to statistical data treatment in this monograph. For liver it was possible to use all data points from 3 – 17 days withdrawal time. In kidney, too many results were below the LOQ after 10 days. For skin fat and muscle only the data for days 3 and 7 could be used. Results marked as below the limit of quantification were replaced by half the LOQ. Figure 12 shows as an example the depletion of marker residue in liver. Table 13

summarises all results obtained by using statistical methods. Despite the limited number of data for some kinetics the statistical approach was considered the most appropriate to obtain quantitative information on both trends and variability.

Table 13: Results of the statistical evaluation of the chicken marker residue study.

day	Liver		Kidney		Muscle		Skin Fat	
	predicted from regression	Tolerance limit	predicted from regression	Tolerance limit	predicted from regression	Tolerance limit	predicted from regression	Tolerance limit
	Concentration [mg/kg of marker residue]							
3	1.83	7.12	0.54	2.54	0.08	0.49	0.10	0.47
7	0.77	2.82	0.14	0.61	0.03	0.16	0.05	0.24
10	0.40	1.45	0.05	0.24				
14	0.17	0.63						
17	0.09	0.35						

A rational approach to setting MRLs would be to interpolate the tolerance limits values for a withdrawal time between 3 and 7 days on the basis of a complete data set for all tissues. The official withdrawal times for the products registered in the four countries 1 were 10 (1 country) to 12 (3 countries) days. To base the MRLs on withdrawal times > 7 days is difficult because valid quantitative data for the marker residue in muscle and skin/fat are not available.

It seems possible to determine the ratio of marker to total residue concentrations by an alternative approach, namely by dividing the values of the two regression lines (the present marker residue study T5C619610 and the total residue study T5C749602 for all given time points for which they are valid. However, in this case the results of the total residue study have to be adjusted taking into account the 1.43 fold higher dose in the marker residue study. The following ratios – given in table 14 - are then obtained:

Table 14: Alternative to estimate the chicken marker to total residue concentrations.

day	Liver	Kidney	Muscle	Skin fat
	Ratio of the values of the depletion curves for marker and total residues			
3	0.67	0.62	0.91	0.53
7	0.53	0.22	1.25	0.45
10	0.45	0.10		
14	0.35			
17	0.30			

The results are in reasonable agreement with the results of study T5C749602 for liver if one takes into account all uncertainties. For the other tissues the values given in table 14 are possibly the more reliable estimates and can be used for the intake assessment with turkey tissues for which no total residue study is available. However, for EDI estimates with chicken tissues it seems to be most appropriate to directly use the total residue study after adjustment of the values as described above.

If only the EDI < ADI criterion is examined, then MRLs could be based on the tolerance limits observed on day 3 after treatment or later. Using the above mentioned adjustment factor of 1.43 the EDI values calculated in table 11 would change as given in table 15.

Table 15: Chicken EDI estimates adjusted to the dose range of the marker residue study.

day	Liver	Kidney	Muscle	Skin fat	All tissues	% of ADI
	EDI [µg/person/day]					
3	419	62	47	10	538	22.4
7	223	47	17	4	291	12.1
10	139	38	38	2	187	7.8

The ADI is numerically also the microbiological ADI for this substance. It is therefore desirable to ensure that occasional high intakes to be expected due to the high variability of the data also remain below the ADI with reasonable statistical certainty.

A computer modelling exercise was carried out in which on the basis of normally distributed random numbers and the kinetic parameters obtained from regression analysis of the logarithms of the residue concentrations 29220 “food packages” were generated. This number corresponds to 80 years of human life. From the results which are summarised in table 16 the recommended MRLs should not be based on three days withdrawal time because in this case approximately up to 2.5 % of calculated intakes would exceed the ADI. By using the data of day 7 this frequency could be reduced to < 0.3 %. Statistically based MRLs cannot be set for kidney, muscle and skin/fat for withdrawal periods beyond 7 days. Table 16 also shows that for this study the results for the median intake of the computer modelling and the calculated EDI are within 0.6 % identical.

Table 16: Comparison of the results of computer modelling of intakes and of the chicken EDI calculation.

Withdrawal time [days]		3	4	5	6	7	7	7	7	7	7	7	7	7	7
Upper class limit expressed as:						Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10
% ADI	µg/ day	**Cumulative frequency [%]**													
10	240	6.1	10.8	16.8	25.0	33.6	33.6	33.1	33.6	34.0	34.3	33.5	33.8	33.9	34.0
20	480	38.8	48.6	58.6	66.8	74.4	74.0	74.7	74.2	74.4	74.4	74.7	74.6	74.8	74.6
30	720	64.0	71.5	79.1	84.2	88.9	88.4	88.7	88.5	89.0	89.1	89.0	89.0	89.0	89.0
40	960	78.1	83.2	88.5	91.7	94.6	94.4	94.4	94.4	94.6	94.6	94.7	94.7	94.5	94.5
50	1200	85.7	89.8	93.1	95.2	97.1	97.0	97.1	97.1	97.1	97.2	97.2	97.2	97.1	97.0
60	1440	90.1	93.4	95.7	97.1	98.4	98.3	98.4	98.3	98.4	98.4	98.4	98.4	98.3	98.4
70	1680	93.6	95.7	97.3	98.3	991	99.0	99.1	99.1	99.1	99.1	99.1	99.1	99.0	99.1
80	1920	95.6	97.0	98.2	98.8	99.4	99.3	99.5	99.5	99.4	99.5	99.4	99.4	99.4	99.4
90	2160	96.8	97.9	98.8	99.2	99.6	99.6	99.6	99.6	99.6	99.6	99.7	99.6	99.6	99.6
95	2280	97.2	98.3	99.0	99.4	99.7	99.7	997	99.7	99.7	99.7	99.7	99.8	99.7	99.7
100	2400	97.6	98.5	99.1	99.5	99.8	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7
200	4800	99.8	99.9	100.	100.	100.	100.	100.	100.	100.	100.	100.	100.	100.	100
300	7200	100.	100.	100	100	100	100	100	100.	100.	100.	100.	100	100.	100
Lowest intake [µg]:		71	71	73	58	45	39	42	47	47	44	38	49	44	40
Median intake [µg]:		571	492	417	357	312	311	310	309	308	308	311	310	308	308
Highest intake [µg]:		14192	7717	7508	7623	5290	5857	7272	6394	5021	9844	7147	5523	6293	4259
EDI	Liver	419.0				222.7									
	Kidney	62.4				47.2									
	Muscle	47.2				17.5									
	Skin/fat	10.5				4.2									
	Basket	539				292									

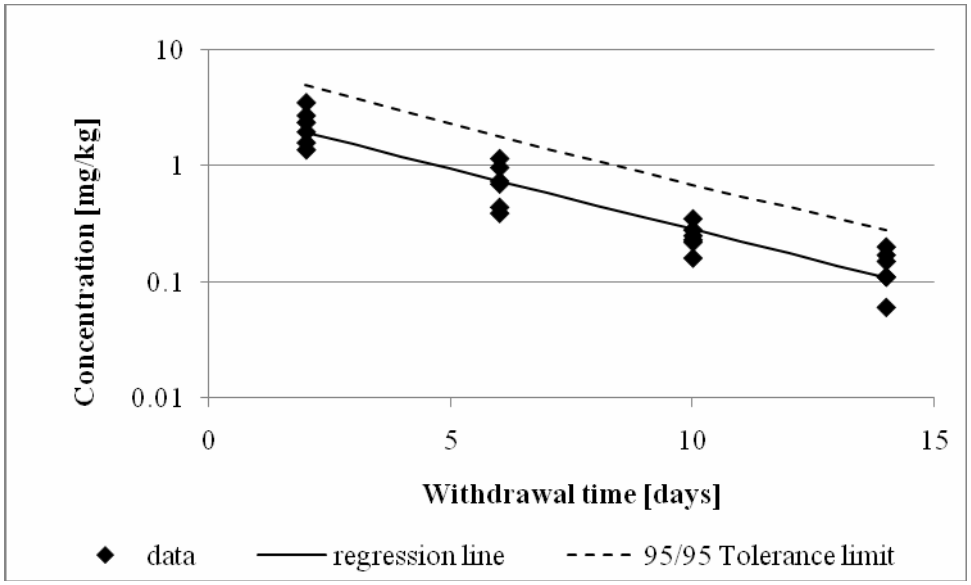
Turkey

In a GLP compliant study (TUR – 99 – 10, Warren, 2000) grower turkeys, 7-8 weeks of age were given continuous access *ad libitum* over 72 hours to medicated drinking water containing 75 mg/L of tilmicosin. Water consumption was given only on a pen basis. The average body weight of the animals on day 0 was 3.42 kg with a range from 2.58 to 4.56 kg. An average daily dose of 9.9 mg/kg bw was calculated (range from 9.6 to 10.5 mg/kg bw/day). According to the label, the Irish authority expects a dose range of 6 – 30 and the French authority a dose range of 10 – 27 mg/kg bw/day resulting from the recommended treatment. Thus the study ranges are at the lower end of the expected dose range.

Three male and three female birds were sacrificed 2, 6, 10, 14, and 18 days after cessation of treatment. Residue data were provided for liver, kidney, skin/fat and muscle. A validated HPLC method was used for the determination of tilmicosin. In liver quantifiable concentrations of residues

were observed from day 2 to 14. In kidney, samples of two female animals were below the limit of quantification. For statistical evaluations half the limit of quantification was used for these samples. The situation was similar for skin/fat. In muscle quantifiable results were only obtained in samples of days 2 and 6. Compared with the chicken marker residue study doses were less variable and also the variability of the residue data was much smaller. An example of the results of statistical treatment of the data is given in figure 13 below.

Figure 13: Statistical evaluation of residue data for liver of turkey.



When linear regression analysis was performed in a semi-logarithmic system (logarithms to the base 10), the following parameters (table 17) were obtained (where "a" is the log of the extrapolated concentration at zero withdrawal time, "b" is a measure of the depletion rate constant and $s_{y.x}$ is the residual variance. Analysis shows that in liver of turkey the initial concentrations were slightly higher compared to chicken liver. In muscle the two concentrations were similar and in fat and muscle concentrations were lower in turkey compared to chicken. However, the rate of depletion was higher in chicken with the exception of liver in which the depletion rate in turkey was higher. The residual variance in chicken was significantly higher, possibly due to the high variability in the doses found in chicken studies.

Table 17: Comparison of statistical parameters for chicken and turkey tissues.

Turkey

Parameter	Liver	Kidney	Skin/Fat	Muscle
a:	0.49379	0.25866	0.60122	0.75071
b:	-0.10411	0.10022	0.06573	0.08942
$s_{y.x}$:	0.16467	0.21830	0.17113	0.12490
n	24	24	24	12

Chicken

a:	0.54487	0.16678	0.77698	0.74695
b:	-0.09394	0.14458	0.07329	0.11919
$s_{y.x}$:	0.26726	0.29110	0.26670	0.31530
n	47	30	20	20

These results do not support the same MRLs in turkey and chicken tissues. The figures, 14a and 14b, support this observation by visualizing the regression lines obtained for the two species and the four tissues. MRLs should be recommended on the basis of seven day withdrawal time. The practical

withdrawal time to comply with these limits could be longer if the dose range observed in practice is in fact higher than the one used in the study TUR – 99 – 10. For this time point the following values for the median value and the tolerance limits have been obtained by statistical data analysis:

Figure 14: Comparison of a) regression lines and b) tolerance limits for chicken and turkey tissues.

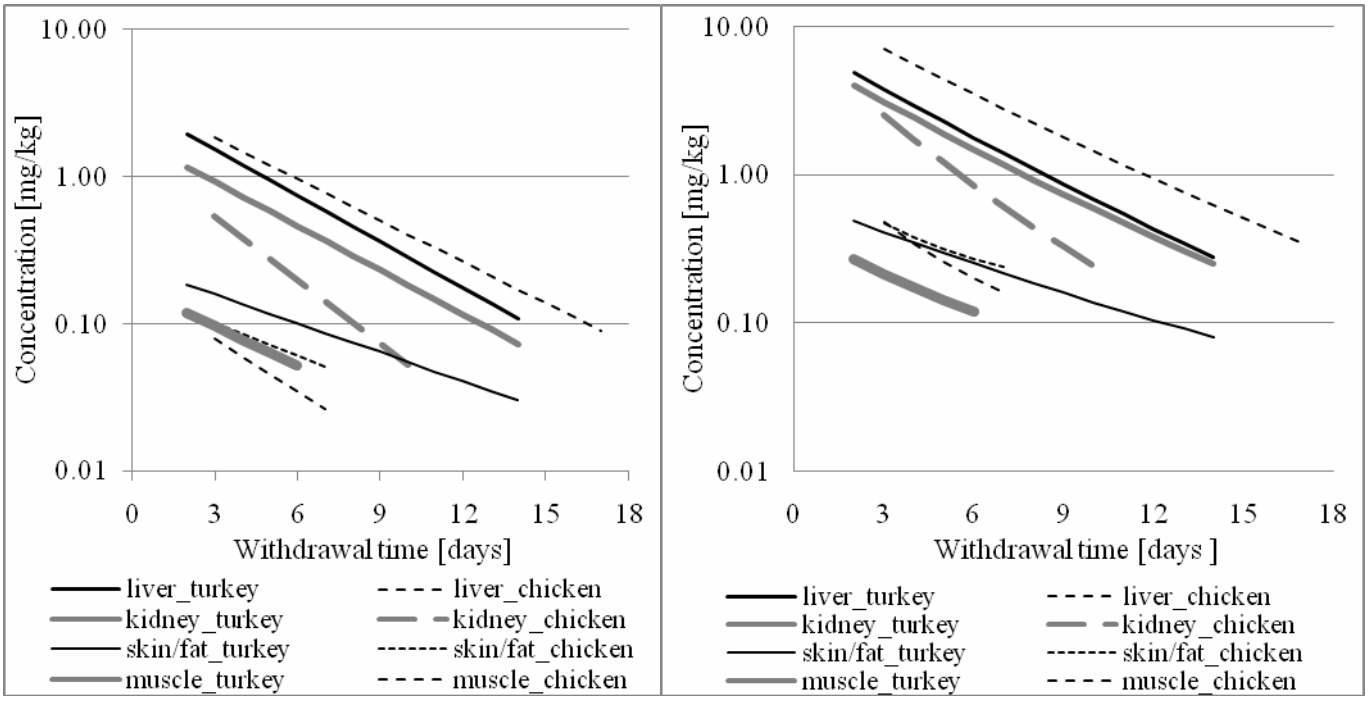


Table 18: Basis for recommending MRLs in turkeys.

day	Liver		Kidney		Skin/fat		Muscle	
	median	Tol.-limit	median	Tol.-limit	median	Tol.-limit	median	Tol.-limit
7	0.582	1.400	0.361	1.154	0.087	0.216	0.042	0.101

The following factors could be used for the conversion of marker to total residue concentrations: liver 0.5, kidney 0.25, skin fat 0.45, and muscle 1.0.

Chicken eggs

Study with ^{14}C-labelled tilmicosin

In the study 004-00780 mentioned previously the hens received daily for three consecutive days oral doses via gavage of 19.1 ± 0.1 mg/kg bw of ^{14}C-tilmicosin as two divided doses in the morning and in the evening. The initial body weights of the hens (day -1) ranged from 1.166 to 1.463 kg. The total number of eggs produced per animal and within the study days 0-23 ranged from 20-24. Table 19 summarizes the animal data. Animal V19 produced the lowest number of eggs including one soft-shelled egg, and the lowest amount of egg material during the 24 days observation period. Concentrations of residues were highest in egg white and egg yolk of this animal on every day.

Table 19: Egg and animal data.

Animal ID	bw [kg]	Daily Dose [mg/kg bw]		Weight [g]			Amount of residues [µg]		
			Number of eggs	Egg white	Egg yolk	Total egg content	In egg white	In egg yolk	In total egg content
V10	1.25	20.0	23	768	322	1089	735	249	984
V12	1.37	20.0	24	714	339	1053	580	228	808
V14	1.39	20.0	23	759	320	1078	712	278	990
V17	1.39	20.0	24	853	354	1207	501	219	720
V19	1.26	19.9	20	631	285	916	2445	999	3444
V22	1.17	19.8	22	605	284	890	505	305	811
V24	1.37	19.8	21	825	386	1211	539	218	758
V25	1.46	19.8	24	936	382	1317	832	233	1065

Figure 15 shows the kinetics of depletion of total radioactive residues in total egg content. The concentrations in egg white and in egg yolk were in the same order of magnitude. The ratio of the concentrations in egg white and in egg yolk was 1.24 ± 0.41. The median concentration in total egg content reached a peak of 4.2 mg/kg on day 3. The maximum of 13.7 mg/kg was observed in an egg of animal V19 on day 4. The concentrations of residues are not normally distributed. If one assumes a log-normal distribution, the values obtained with animal V19 fall within 3 standard deviations of the geometric mean and should not be excluded from calculations.

Marker residue and ratio marker to total residue

Pools of all egg whites and egg yolks (except from animal V19) from days 3, 7, 11, and 18 were formed. The report states that equivalent masses were taken from each egg. The corresponding samples from animal V19 were analysed separately. Samples were twice extracted with acetonitrile. The remaining pellet is called “nonextracted” in table 20 below and expressed in percent. The extract was further cleaned and analysed by HPLC-MS/MS. The authors provide the concentrations of tilmicosin and T-12 on the basis of the initial sample mass. However, the percent of total radioactivity is calculated on the basis of the radioactivity in a given peak and total radioactivity injected onto the column. This approach overestimates the ratio. It is better to base the ratio of marker to total residue concentrations on the basis of the mass of the samples in order to take account of the residues remaining in the pellet. This approach slightly underestimates the ratio since the unknown recoveries cannot be taken into account; however, it seems appropriate to follow the more conservative approach. The results of the calculations are given in table 20. The values obtained for the pools established from eggs collected on day 18 are outside the range of all other values. It is proposed not to use these results, in particular since intake estimates for such late time points of the depletion kinetics will not be made. For the early time point a value of 0.7 for the ratio of marker to total residue concentrations is sufficiently conservative.

Figure 15: Depletion of residues of ^{14}C-tilmicosin in eggs.

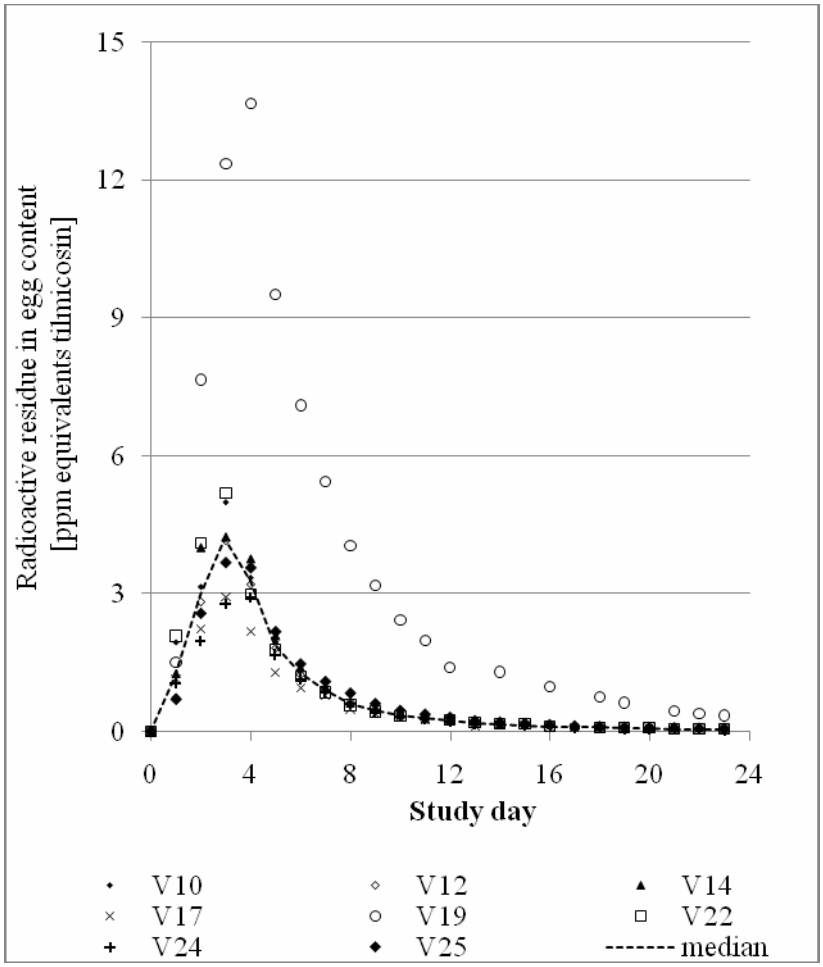


Table 20: Ratio of marker to total residue concentrations.

day	Calculated total residue [mg/kg]	% Not extracted	Tilmicosin [mg/kg]	T-12 [mg/kg]	Ratio	Measured total residue[mg/kg]	% not extracted	Tilmicosin[mg/kg]	T-12[mg/kg]	Ratio
3	4.10	4.9	3.04	0.10	0.74	12.92	5.2	8.34	1.12	0.65
7	0.90	4.0	0.58	0.04	0.65	5.51	4.4	3.46	0.52	0.63
11	0.32	4.1	0.21	0.03	0.66	2.03	4.8	1.26	0.23	0.62
18	0.10	5.6	0.15	0.02	1.42	0.85	5.4	0.51	0.12	0.60
3	3.76	6.5	2.84	0.03	0.76	11.09	6.9	7.74	0.23	0.70
7	0.97	7.4	0.64	0.01	0.65	5.29	6.7	3.48	0.15	0.66
11	0.26	9.2	0.18	0.01	0.68	1.89	8.3	1.34	0.07	0.71
18	0.09	9.3	0.10	0.00	1.16	0.59	12.5	0.36	0.02	0.62

Study with unlabelled tilmicosin

In a GLP compliant study (004-00781, Beauchemin, et al. 2007b), fifteen hens of an approximate age of 41 weeks and a body weights of 1.59 to 2.15 kg were dosed for three days via drinking water. Dose amounts were calculated based on study day (-1) body weights. The targeted dose was 15 to 20 mg/kg bw/day. The average calculated dose was 17 mg/kg bw/day. The individual doses per animal and day are not given. Information on registered doses is not available since all label copies provided by the sponsor warn that tilmicosin should not be used in birds producing eggs for human consumption. The light/dark cycle was set to 17 hours of light and 7 hours of dark. Two animals did not drink much of the treated water and had decreased egg production. One of these animals was treated as an outlier and excluded from data analysis. The data were used from the other animal (ID 270).

Eggs were collected from day (-1) to day 23. Some animals produced two eggs on a day (animal 293/day 0; animal 265/day 3; animal 270/day 11). In these cases the two eggs were combined into one sample. Weights of the egg contents were not given. Egg contents were analysed only for the odd days of the study. Therefore, for some animals the highest observed concentration may not represent the peak concentration. HPLC-MS/MS was used for analysis. Table 21 summarises animal-related data. Figure 16 shows the quantified results above the LOQ on a double linear scale.

The residue concentrations found are not normally distributed. Several alternative quantitative evaluations of the data are discussed. In the first two alternatives, the logarithms of the concentrations are used. A mean, a standard deviation, and an upper 95% confidence limit of the 95th percentile is calculated for each time point on the basis of the logarithms. The calculation was performed once including the data of animal 270, and once excluding the data. Since the sample size is very small and the variability of the results is extreme, the tolerance limits are very high. The results are given in table 22. The last column shows the results obtained if the data of animal 270 were not used.

Table 21: Animal body weights and egg production.

Animal ID	Body weight on day [-1) [kg]	Number of eggs produced from day 0-23
252	1.80	21
253	1.70	20
254	2.15	22
255	2.05	21
257	2.11	22
264	1.75	24
265	1.87	24
267	1.73	21
268	1.86	24
270	1.72	21
272	1.89	23
277	1.68	19
289	1.87	22
293	1.63	23

In figure 16, the data of the hens producing eggs with the lowest (270) and the highest (267) concentrations of residues are connected by a dotted line.

Figure 16: Depletion curves of marker residue in total egg content.

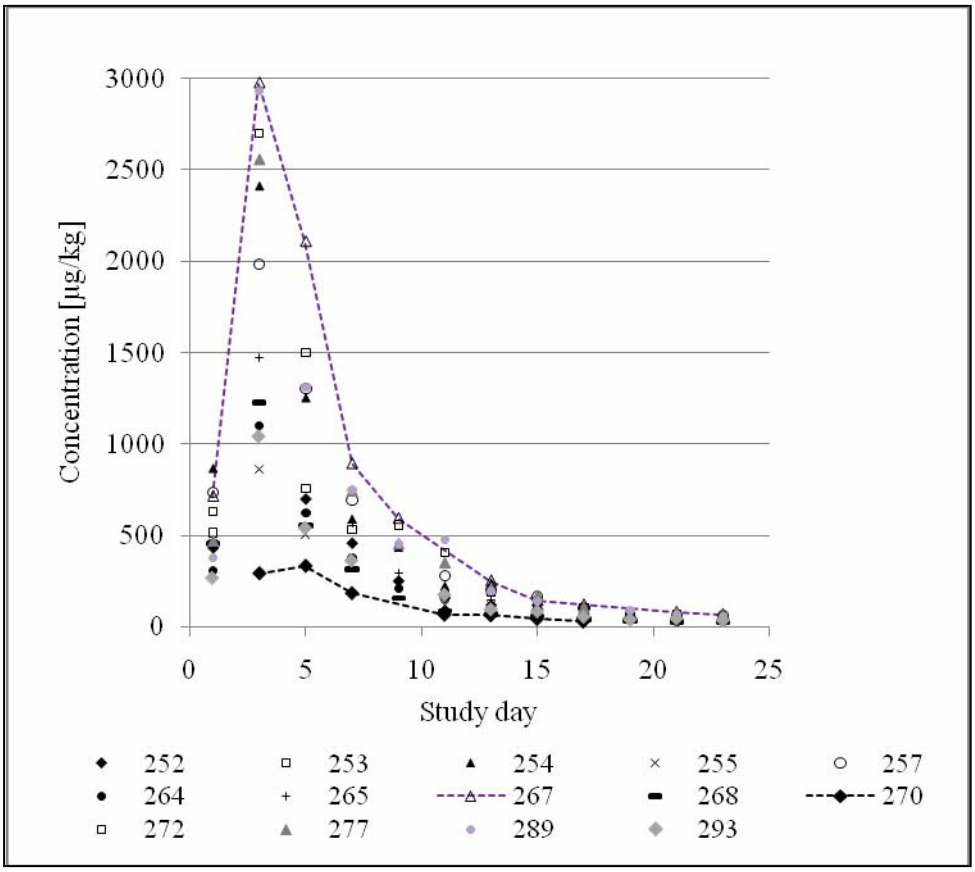


Table 22: Statistical evaluation of the laying hen eggs data.

Study day	n	mean (log scale)	s.d. (log scale)	k	Tolerance limit (Mean + k x s.d.)	Antilog mean	Antilog Tolerance limit	Antilog Tolerance limit excluding animal 270
		[dimension-less]				[µg/kg]		
1	13	2.56999	0.46595	3.081	4.00559	372	10130	10130
3	12	3.18108	0.29633	3.162	4.11807	1517	13124	7980
5	12	2.92075	0.23969	3.162	3.67864	833	4771	4504
7	12	2.69558	0.19536	3.162	3.31330	496	2057	1633
9	10	2.52326	0.19621	3.379	3.18626	334	1536	1536
11	13	2.25325	0.26589	3.081	3.07244	179	1182	1138
13	13	2.14317	0.20244	3.081	2.76688	139	585	558
15	13	2.02723	0.18442	3.081	2.59543	106	394	338
17	13	1.84576	0.18193	3.081	2.40628	70	255	221
19	10	1.66248	0.23934	3.379	2.47120	46	296	168
21	13	1.67755	0.22138	3.081	2.35963	48	229	150
23	13	1.62244	0.19941	3.081	2.23681	42	173	173

A plot of the same data on a semi-logarithmic scale system would show that the results obtained within days 3 and 15 follow roughly a linear pattern. The sponsor proposes to carry out a statistical analysis on this basis using linear regression. This approach is not appropriate since the eggs are obtained every day from the same hens. If the product would be registered for use in laying hens, the tolerance limits calculated in a table of the type of table 22 would form the basis for the calculation of MRLs. However, in the present case an MRL cannot be proposed because the number of animals used in the study is too small to adequately assess the great variability of the residue concentrations. The amount of data was further reduced because the only eggs of every second day were analysed and – on the present limited data base - one cannot exclude that the egg discard times required to ensure an acceptable distribution of daily intakes are not practicable. Furthermore it cannot be judged whether the dose regimen was adequate because the product is not registered for use in laying hens.

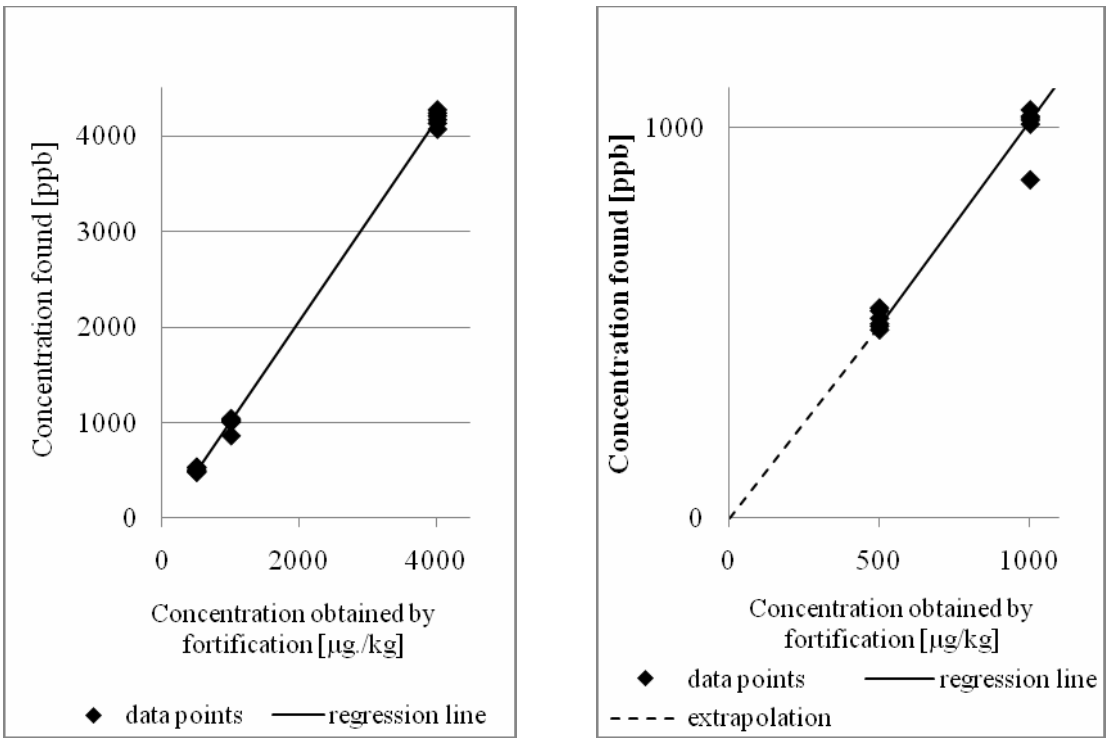
Rabbits

A tilmicosin tissue residue study (RTC study 6483, Luperi and Brightwell, 1999a) was conducted in the rabbit. Test animals were New Zealand White Rabbits of a body weight range of 1890 – 2150g for the males and 1924 – 2200g for the females. Animals received a single subcutaneous injection of tilmicosin calculated to result in a 10 mg/kg bw dose. The only example of a registered use of tilmicosin in rabbits recommends oral administration in the feed on the basis of a granulate and the doses vary depending on the indication between 5 – 6 and 10 – 12 mg/kg bw.

In the present study five animals (at least two of each sex) were sacrificed after various withdrawal times (7, 14, 21, 28 and 35 days) and the contents of parent drug tilmicosin were determined in liver, kidney, abdominal fat and muscle (tissue from the semimembranosus and semitendinosus muscle). Injection sites were excised in a portion of tissue of the trapezius and longissimus thoraci muscle of approximately 36 – 54g and were analysed for tilmicosin. The method used involved HPLC separation and UV detection at 280 nm. The method was only partially validated (Luperi and Brightwell, 1999b) using the following concentrations of tilmicosin obtained by fortifying blank tissues: muscle, 125.5, 251 and 1004; liver and kidney, 502, 1004 and 4016; fat, 25.1, 50.2 and 200.8 µg/kg, respectively.

The concentrations of all incurred tissues except 4 kidney samples and two fat samples were outside the range of concentrations for which the method was validated. At all the above given concentrations the method did fulfil the required accuracy and precision criteria. The authors declared the lowest concentration used in the validation study the limit of quantification though this is not often the case. When it happened that the incurred concentrations were lower, the analytical curve was extrapolated down to the origin of the coordinate system though this is not a good practice. The concentrations determined by this way were reported quantitatively – if they were above the limit of detection, but were labelled with an asterisk if they were below. The following figure 17 describes the approach on the example of liver. The left part shows the analytical curve obtained in the validation study. The right part explains at higher magnification the extrapolation procedure.

Figure 17: Use of analytical curves in the residue study in rabbits.



Normally, this approach would not be acceptable. However, it could not be excluded that the reported residue concentrations represented valid data and only the LOQ had been inadequately estimated. The problem was discussed with the sponsor in order to explore the possibility of a solution, but the sponsor confirmed that they only supported the use of the data above the limit of quantification.

The limit of detection was determined from the average plus three standard deviations obtained from the analyses of 21 blank tissues. It is not reported whether these were 21 independent tissues or 21 replicate determinations of one tissue. The sponsor could not answer this question, but was assuming that the 21 samples were replicate of the same composite sample composited from different animals.

Residues above the limit of detection of 3.5 µg/kg were not found in any sample of muscle. Residues at injection sites were below the limit of detection of 3.5 µg/kg in all samples collected at and after 14 days. Residues in fat were above the limit of detection of 3.2 µg/kg in all samples collected on day 7 and in about 50% of the samples obtained on days 14 and 21. In liver, residues above the limit of detection were found in all samples until 14 days after treatment and in three of five samples collected on day 21. Kidney was the organ with the highest concentrations found. All samples obtained until day 28 and one sample of an animal sacrificed on day 35 contained tilmicosin in concentrations above the limit of detection of 0.78 µg/kg. Figures 18 and 19 demonstrate the problems of the data base in view of the method validation data of the study.

Figure 18: Relationship of the measurements in liver of rabbits to the LOQ.

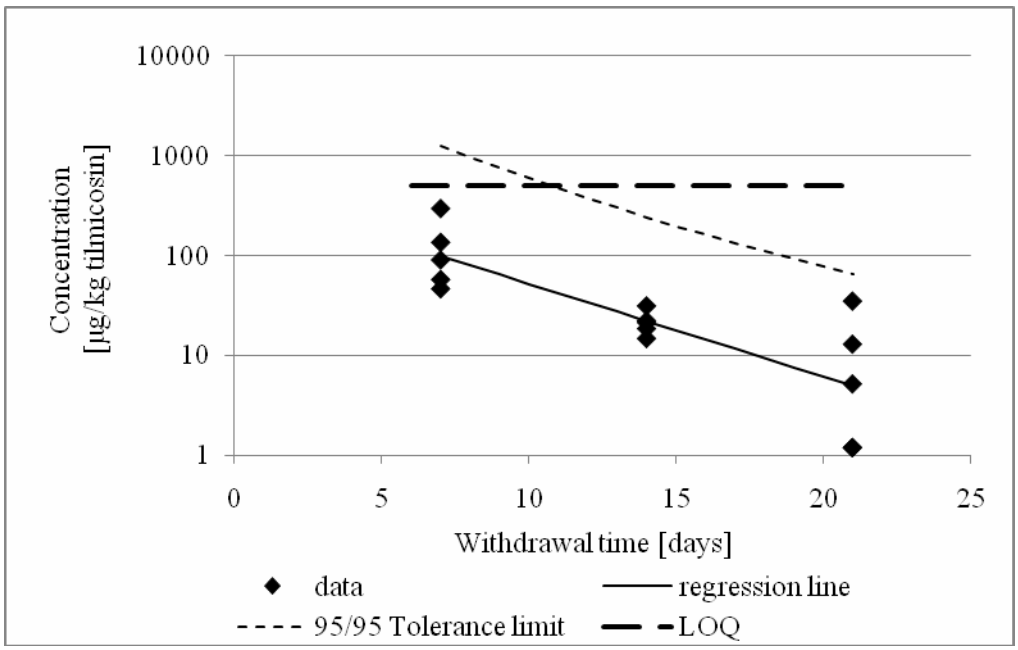
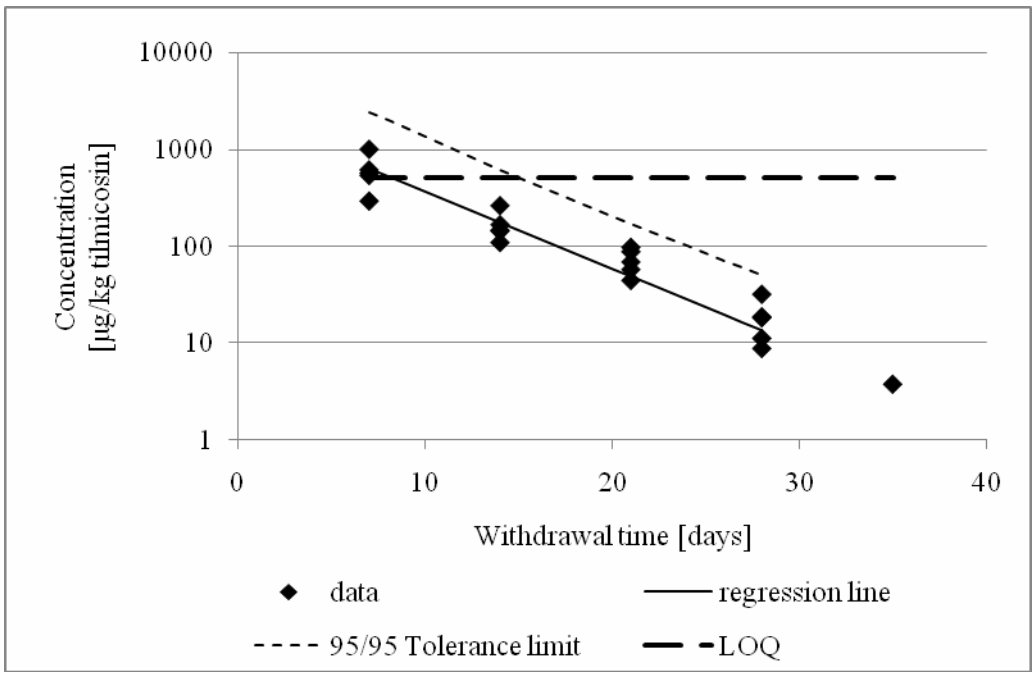


Figure 19: Relationship of the measurements in kidney of rabbits to the LOQ.



The ratio of marker to total residue is not known for rabbit tissues. The basic pattern of metabolites found in other species has been qualitatively confirmed by Montesissa, et al. (2004) using primary hepatocyte cultures and liver microsomes from rabbits and LC-MS methods for the identification of the metabolites. The data base provided by the sponsor is not suitable for recommending MRLs.

ESTIMATION OF DAILY INTAKE

All intake estimates were based on the information obtained from kinetic residue depletion studies. Three approaches were followed.

- For residues of tilmicosin in chicken the EDI was calculated directly from the total residue study at the same time point (7 days) on which the estimation of MRLs was based. The results are summarised in table 23.
- In a second approach, a computer modelling exercise was carried out in which on the basis of normally distributed random numbers and the kinetic parameters obtained from regression analysis of the logarithms of the residue concentrations 29220 "food packages" were generated. This number corresponds to 80 years of human life. The results showed that at 7 days withdrawal time the frequency of occurrence of above ADI "food packages" was below 0.3%. The modelling also showed that for this study the results for the median intake of the computer modelling and the conventionally calculated EDI were within 0.6 % identical. The results are presented in table 16.
- The third approach was applied to estimate intakes resulting from the consumption of turkey tissues. It was the conventional approach involving median marker residue concentrations and factors to adjust for the ratio of marker to total residue concentrations. The factors obtained for chicken were used for turkey tissues. The results are summarised in table 24.

Table 23: Estimate of chronic intake derived from total residue study in chickens on day 7.

	Liver	Kidney	Muscle	Fat/skin	All tissues
Predicted median concentration of total residue equivalents [µg/kg] on day 7 after treatment	2227	943.8	58.3	83.1	
Daily amount consumed [kg]	0.1	0.05	0.3	0.05	0.5
Daily intake of total residue equivalents	223	47	18	4	292
% of upper limit of ADI	9.3	2.0	0.7	0.2	**12**

Table 24: Estimate of chronic intake derived from marker residue study in turkeys on day 7.

	Liver	Kidney	Muscle	Fat/skin	All tissues
Predicted median concentration of marker residue concentration [µg/kg] on day 7 after treatment	582	361	42	87	
Daily amount consumed [kg]	0.1	0.05	0.3	0.05	0.5
Daily intake of marker residue [µg/kg]	58	18.0	13	4	
Conversion factor marker to total	1/0.5	1/0.25	1	1/0.45	
Daily intake of total residue equivalents [µg/kg]	116	72	13	10	211
% of upper limit of ADI	4.9	3.0	0.5	0.4	**8.8**

METHODS OF ANALYSIS

A validated HPLC method was provided to analyse tilmicosin in edible tissues of several species including chicken and turkey tissue (Lilly Method B04228 rev 7). It is based on a solid-phase extraction, gradient elution and UV detection. It was validated for chicken tissues as to linearity, precision, accuracy, specificity, ruggedness, and stability of tilmicosin. The modification for turkey tissues was validated for the same criteria in an additional study (Hawthorne, 1999). The LOQ is 60µg/kg for liver and kidney and 25µg/kg for muscle and fat.

An LC/MS-MS method was provided to analyse tilmicosin in whole egg with a LOQ of 25 µg/kg (MPI Method V0003516). It was validated according to U.S. FDA guidelines (McCracken, 2007).

A validated HPLC method, based on a solid-phase extraction, gradient elution and UV detection is available to analyse tilmicosin in cow and sheep milk with a LOQ of 10µg/kg (Method B05704, Revision 3). A validation document for this method was also provided. Tilmicosin residues can be detected in milk using commercial bacterial growth inhibition test.

APPRAISAL

The forty-seventh meeting of the Committee established an ADI of 0-40 µg/kg body weight (0-2400µg per day for a 60 kg person) and MRLs (µg/kg) for cattle, sheep and pigs were recommended in muscle, liver, kidney and fat tissues. A temporary MRL was recommended for sheep milk. The temporary MRL of 50 µg/kg for milk of sheep was not extended by the Committee at the fifty-fourth meeting because results of a study with radioactively labeled drug in lactating sheep to determine the relationship between total residues and parent drug in milk was not available. The present Committee addressed both new and relevant previously submitted data.

The sponsor requested the Committee to recommend MRLs for tilmicosin in chicken, turkey and rabbit tissues, chicken eggs and an MRL for milk of sheep. In this submission the sponsor explained the reasons for not having provided a total residue study in sheep milk using ^{14}C-tilmicosin as requested by the 47th JECFA. The sponsor proposed MRLs and provided deliberations about dietary intakes resulting from all uses of the products under conditions of compliance with the proposed MRLs.

In chickens, using radiolabel studies, the structure of metabolites was determined using ESP-MS. In total, a number of metabolites and parent tilmicosin were found in the extracts. The structures are briefly described in table 5. Studies suggest that in liver approximately 55% of the total radioactive residue represents parent tilmicosin. The corresponding values for kidney and muscle are approximately 40%. The highest residue concentrations were observed in liver followed by kidney. Residue concentrations in skin fat, abdominal fat and muscle were very low. No similar study was provided for turkeys.

Although tilmicosin is not recommended for production of eggs for human consumption, the sponsor provided data on residues in eggs using radiolabel studies. The ratio of tilmicosin to total residue was calculated and a value of 0.7 was estimated from the data base provided

Studies were also provided on milk from lactating dairy cows. Residues may persist for more than 50 days and tilmicosin represented up to 89 percent of the total radioactive residue in one study. The labels of registered products provided by the sponsors warn that tilmicosin should not be used in cows producing milk for human consumption.

The sponsor had been requested to provide a radiolabel study for consideration of an MRL in sheep milk but none was provided. Only limited residue studies were provided. Milk was analysed for parent tilmicosin using an HPLC method with a limit of quantification of 50 µg/l. The milk was also subjected to a Delvotest and full inhibition was found for the first 6 to 7 days. No inhibition in any sample was found after day 12. The data base of this study was very limited. The weaknesses of the study cannot be compensated by recommending high MRLs. Consumption of milk obtained within the first 144 hours after treatment likely leads to intakes exceeding the ADI.

A rational approach to recommending MRLs in chickens would be to interpolate the tolerance limits values for a withdrawal time between 3 and 7 days on the basis of a complete data set for all tissues. The registered withdrawal times based on provided labels for the products registered in the four countries were 10 (1 country) to 12 (3 countries) days. To base the MRLs on withdrawal times > 7 days is difficult because valid quantitative data for the marker residue in muscle and skin/fat are not available.

The sponsor proposed to carry out a statistical analysis on the egg studies using linear regression to recommend an MRL. This approach is not appropriate since the eggs are obtained every day from the same hens. However, in the present case an MRL cannot be proposed because the number of animals used is too small to adequately assess the great variability of the residue concentrations. Furthermore it cannot be judged whether the dose regimen was adequate because the product is not registered for use in laying hens.

In the rabbit studies, the concentrations of all incurred tissues except four kidney samples and two fat samples were outside the range of concentrations for which the analytical method was validated. The authors declared the lowest concentration used in the validation study as the limit of quantification though this is not often the case. When it happened that the incurred concentrations were lower, the analytical curve was extrapolated down to the origin of the coordinate system, though this is generally not a good practice.

MAXIMUM RESIDUE LIMITS

The Committee considered data for recommending MRLs in chicken, turkeys, eggs, rabbit and sheep milk. The sponsor provided information on registered uses, which showed that there is at present no registered use for laying birds. The residue concentrations in eggs were very high and could result in long withdrawal times.

In the rabbit, the residue depletion study was performed using subcutaneous administration. However, the registered oral use administration route was not covered by an adequate residue depletion study.

The argument of the sponsor that a radiolabelled residue in sheep milk was not necessary, as new data were provided to bridge between cattle and sheep, was accepted in principle. The only residue study in lactating ewes contained an insufficient number of animals to allow MRLs to be recommended and showed that long milk withdrawal times of approximately 15 days may be required.

For chickens, a satisfactory data set was available to derive MRLs. For turkeys, the available residue did not include a total residue study, but the data could be bridged by using ratios of marker to total residue concentrations derived from the study in chickens.

When recommending MRLs the Committee considered the following points:

- The ADI for tilmicosin was 0-40 µg/kg bw/day corresponding to an upper bound of acceptable intakes of 2400 µg per day for a person with a body weight of 60 kg.
- The time point on which the MRLs were set was based on an EDI < ADI approach *and* on modelling of possible intakes resulting from the consumption of the four standard edible tissues showing that > 99.7 % of all intakes in 80 years life time would be below the ADI.
- The residue depletion kinetics in turkeys were different from those found in chickens.
- The most suitable time point for the calculation of MRLs was 7 days after the end of treatment in chickens and turkeys.
- The studies provided clear evidence of dose-linearity of the residues in tissues of chicken.
- The range of therapeutic doses was covered by the studies performed with chickens. The dose used in the depletion study with turkeys was at the lower end of the registered dose regimes; however, the residue data from turkeys showed less than did the data from chickens.
- A total residue study in chicken could be directly used for the intake estimates following adjustment to account for the slightly higher range of therapeutic doses.
- The data from the marker residue study enabled statistical MRL calculations for chickens and for turkeys. MRLs were calculated on the basis of upper one-sided 95% confidence limits over the 95th percentile of residue concentrations.
- The ratio of marker to total residue concentrations was determined for chicken tissues and was applied for the estimated intakes of residues from turkey tissues.

- Data submitted to support MRLs for rabbit tissues, chicken eggs and sheep milk were not suitable to derive MRLs compatible with the registered conditions of use for tilmicosin.
- A validated method of analysis was available for chicken and turkey tissues.

The Committee recommended MRLs, determined as tilmicosin, as follows:

	MRLs [µg/kg]			
	Liver	Kidney	Muscle	Skin/Fat
Chicken	2400	600	150	250
Turkey	1400	1200	100	250

The Committee was not able to recommend an MRL for sheep milk.

Before a re-evaluation of tilmicosin with the aim to recommend MRLs in tissues of rabbits, the Committtte would require adequately designed residue studies with doses and routes of administration under authorized conditions of use and using a validated method suitable for the purpose.

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