

assay method for the detection of residues in turkeys was adapted from the chicken method. Three days after the end of the treatment, residues were detectable only in liver ( $166 \pm 64 \mu\text{g/kg}$ ). At this time, residues were measured only in one sample of kidney, muscle and fat. After four days post-treatment, all concentrations of residues were below the LOQ or the LOD in all tissues. The results are presented in Table 8.

**Table 8: Mean concentrations of erythromycin A in edible tissues of turkeys treated with 20mg/kg/day for three days.**

Sampling time after treatment	Muscle ( $\mu\text{g/kg}$ )	Fat/skin ( $\mu\text{g/kg}$ )	Liver ( $\mu\text{g/kg}$ )	Kidney ( $\mu\text{g/kg}$ )
3 days	266*	318*	$166 \pm 63.6$	424*
4, 5,6 and 8 days	< LOQ	< LOQ	< LOQ	< LOD

Note: \* only one value; < LOQ: below the quantification limit ( $100 \mu\text{g/kg}$  for all tissues); < LOD: below the detection limit ( $4 \mu\text{g/kg}$  for skin/fat and  $3 \mu\text{g/kg}$  for all other tissues).

## METHODS OF ANALYSIS

### Erythromycin A, B and C and N-methyl-erythromycin A by a LC/MS/MS method

Chicken (MPK/5814 /9812 and validation in MPK/erythromycin/9957).

**Extraction procedure for muscle, liver and kidney:** 1 g thawed tissue was shaken with 1 ml of double distilled water using a vortex mixer. Nine ml of acetonitrile was added and the mixture vortexed for an additional 20 seconds. The mixture was shaken for 10 minutes using a mechanical shaker. After decantation,  $100 \mu\text{l}$  of the supernatant was added to  $300 \mu\text{l}$  of the dilution solution and  $15 \mu\text{l}$  was injected into the chromatographic system.

**Extraction procedure for skin/fat:** 1 g frozen tissue was shaken with 20 ml of acetonitrile for 20 seconds using a vortex mixer followed by 10 minutes using a mechanical linear shaker. After decantation, 1 ml of supernatant and 1 ml of double distilled water and 2 ml of n-hexane were added. The mixture was shaken 10 minutes using a mechanical shaker, centrifuged at 2500 rpm for 5 minutes, n-hexane was decanted off and  $15 \mu\text{l}$  of solution were injected onto the chromatographic system.

**Chromatographic procedure:**  $15 \mu\text{l}$  was injected onto the column using the autosampler. The column used was an RP 18ec  $30 \times 4 \text{ mm}$ ,  $5 \mu\text{m}$ . The elution was done under isocratic conditions with acetonitrile/double distilled water 50:50 v/v with 1% formic acid. The retention times were 2.7 min for erythromycin A, 3.1 min for erythromycin B, 2.0 min for erythromycin C and 2.4 min for N-desmethyl-erythromycin A. Detection was done by mass-mass using a quadripole instrument with an electrospray source in the positive ionization mode. Multiple Reaction Monitoring (MRM) was applied and the following transition ions were monitored  $734.6 \rightarrow 576.6$  for erythromycin A, and  $718.6 \rightarrow 565.2$  for erythromycin B,  $720.6 \rightarrow 576.5$  for erythromycin C, and  $720.5 \rightarrow 562.2$  for N-desmethyl-erythromycin A. No internal standard was used. Quantification of both products was done after interpolation of unknown sample peak areas against the theoretical concentrations of calibration curves. Results were expressed as free erythromycin A and N-desmethyl-erythromycin A ( $\mu\text{g/kg}$ ). The method was validated using the following criteria consistent with internationally recognized guidelines.

**Specificity:** The method was found specific from endogenous compounds, erythromycin B and C, N-desmethyl-erythromycin A, tylosin, tilmicosin and spiramycin.

**Linearity:** The linearity of detector response was assessed in the four tissues matched with standard solutions using  $100\text{-}5000 \mu\text{g/kg}$ . The correlation coefficients were 0.9996, 0.9993, 0.9998 and 0.9993

for erythromycin A in muscle, liver, kidney and skin/fat respectively and 0.9979, 0.9987, 0.9992 and 0.9976 for N-methyl-erythromycin A in the same tissues, respectively.

**Stability:** Stability was demonstrated at a concentration of 2000 µg/kg after 3 freeze-thaw cycles.

The within-day precision, day-to-day precision, accuracy, recovery rate, LOQ and LOD are presented in the following tables.

**Table 9: Validation parameters for erythromycin A in chicken tissues**

Erythromycin A	Muscle	Kidney	Fat / skin	Liver
Concentration (µg/kg)	100 - 5000	100 - 5000	100 - 5000	100 - 5000
Within-day precision (%)	1.95 - 7.57	2.30 - 7.24	2.51 - 7.80	2.89 - 8.19
Day-to-day precision (%)	2.28 - 7.57	2.30 - 7.24	2.51 - 9.36	4.31 - 13.6
Accuracy (E%)	-1.5 ≤ E% ≤ +2.0	-2.0 ≤ E% ≤ +2.4	-7.0 ≤ E% ≤ +10.1	-3.3 ≤ E% ≤ +3.0
Recovery rate (%)	> 98.9	> 92.1	> 87.4	> 98.1
LOQ (µg/kg)	100	100	100	100
LOD (µg/kg)	3	25	5	30

**Table 10: Validation parameters for N-desmethyl-Erythromycin A in chicken tissues**

N-desmethyl-Erythromycin A	Muscle	Kidney	Fat / skin	Liver
Range (µg/kg)	100 - 5000	100 - 5000	100 - 5000	100 - 5000
Within-day precision (%)	2.56 - 6.37	1.95 - 7.19	2.61 - 9.33	2.48 - 6.85
Day-to-day precision (%)	2.62 - 7.15	1.95 - 7.19	2.61 - 9.33	3.86 - 9.47
Accuracy (E%)	-3.0 ≤ E% ≤ +3.4	-1.2 ≤ E% ≤ +3.8	-4.0 ≤ E% ≤ +10.4	-4.2 ≤ E% ≤ +4.0
Recovery rate (%)	> 99.1	> 96.8	> 81.7	> 97.3
LOQ (µg/kg)	100	100	100	100
LOD (µg/kg)	3	25	24	48

#### Turkey (MPK/5814/0225)

The analytical method was adapted from the chicken method described above. It was validated using the criteria noted above. The method was partially validated as noted below. The retention time was approximately 3.6 min for erythromycin A.

**Specificity:** The method was found specific from endogenous compounds, erythromycin B and C, N-desmethyl erythromycin A, tylosin, tilmicosin and spiramycin.

**Linearity:** The linearity of detector response was assessed in the four tissues matched with standard solutions in the range of 100-5000 µg/kg. The correlation coefficients were 0.9998, 0.9997, 0.9988 and 0.9993 for erythromycin A in muscle, liver, kidney and skin/fat. The within-day precision, accuracy, recovery rate, LOQ and LOD are presented in Table 11.

**Table 11: Validation parameters for erythromycin A in turkey tissues**

Erythromycin A	Muscle	Liver	Kidney	Fat / skin
Range ( $\mu\text{g}/\text{kg}$ )	100 - 5000	100 - 5000	100 - 5000	100 - 5000
Within-day precision (%)	1.1 - 2.3	1.6 - 4.6	1.1 - 2.2	4.9 - 11.2
Accuracy (E%)	-5.0 - 6.5	-8.0 - 8.4	-6.8 - 0.2	-3.3 - 8.8
LOQ ( $\mu\text{g}/\text{kg}$ )	100	100	100	100
LOD ( $\mu\text{g}/\text{kg}$ )	3	3	3	4

Eggs (MPK/ 5814/9908 and validation in MPK/erythromycin/9961)

**Extraction procedure:** 1g frozen mixed egg was shaken with 1 ml of double distilled water for 20 seconds using a vortex mixer. Nine ml of acetonitrile were added and the mixture was shaken for 20 seconds using a vortex mixer and for 10 minutes using a mechanical linear shaker. After decantation, 500  $\mu\text{l}$  of supernatant were added to 500  $\mu\text{l}$  of acetonitrile, 1 ml of double distilled water and 2 ml of n-hexane. The mixture was shaken 10 minutes using a mechanical shaker, centrifuged, the n-hexane was decanted off and 10  $\mu\text{l}$  of solution were injected onto the chromatographic system.

**Chromatographic procedure:** 15  $\mu\text{l}$  were injected onto a RP 18ec 30 x 4 mm, 5  $\mu\text{m}$  column. The elution was obtained under isocratic conditions with acetonitrile/double distilled water 50:50 v/v with 1% formic acid. The retention times were 3 min for erythromycin A and 2.5 min for N-desmethyl-erythromycin A. Detection was done by mass-mass using a quadripole instrument with a turbo ionspray source in the positive ionization mode. MRM was applied and the following transition ions were monitored 734.6 $\rightarrow$ 158.0 for erythromycin A and 720.5  $\rightarrow$ 144.2 for N-desmethyl-erythromycin A. No internal standard was used. Quantification of both products was done after interpolation of unknown sample peak areas against the theoretical concentrations of calibration curves. Results were expressed as free erythromycin A and N-desmethyl-erythromycin A.

**Specificity:** The method was found specific from endogenous compounds, erythromycin B and C, N-desmethyl erythromycin A, tylosin, tilmicosin and spiramycin.

**Linearity:** The linearity of detector response was assessed matched with standard solutions in the range of 50-5000  $\mu\text{g}/\text{kg}$ . The correlation coefficients were 0.9993 and 0.9997 for erythromycin A and N-methyl-erythromycin A respectively.

**Stability:** The stability of erythromycin A and N-desmethyl-erythromycin A was evaluated at the concentration level of 2000  $\mu\text{g}/\text{kg}$  after 3 freeze-thaw cycles. The mean concentration was approximately 104% of the mean reference value. Long term stability was evaluated in both compounds in a mixed frozen egg stored at -80  $^{\circ}\text{C}$  for 1 month at the concentration level of 2000  $\mu\text{g}/\text{kg}$ . The mean concentration of the pool was 102% and 104% of the nominal value of erythromycin A and N-methyl-erythromycin A, respectively.

The within-day precision, day-to-day precision, accuracy, recovery rate, LOQ and LOD are presented in Table 12.

**Table 12: Validation parameters for erythromycin A and N-desmethyl-erythromycin A in eggs**

	Erythromycin A	N-desmethyl-Erythromycin A
Range (µg/kg)	50 - 5000	50 - 5000
Within-day precision (%)	1.76 - 9.87	1.10 - 7.79
Day-to-day precision (%)	1.76 - 11.87	1.11 - 7.79
Accuracy (E%)	-5.0 ≤ E% ≤ +7.8	-1.3 ≤ E% ≤ +2.0
Recovery rate (%)	> 82.6	> 71.9
LOQ (µg/kg)	50	50
LOD (µg/kg)	0.92	2.2

**Microbiological assay method for erythromycin in chicken tissues and eggs**

A microbiological method was used for the assay of erythromycin A and its related metabolites with potential microbiological activity in edible tissues (muscle, kidney, liver and fat/skin) of chicken (MPK/erythromycin/9957) and eggs (MPK/Erythromycin/9961).

**Extraction procedure:** 1 g of test sample was vortexed with 4 ml of acetonitrile, placed in an ultrasonic bath for five minutes and on a liner shaker for ten minutes. The extract was centrifuged at 7500g for 10 minutes and the supernatant transferred into a disposable tube. Two wells of each Petri dish were filled with this mixture.

**Petri dishes:** Plates were prepared (diameter 100mm) using agar medium and *Micrococcus Luteus ATCC9341* as test organism. For each point, two plates with two replicates per plates were used. The diameter of bacterial growth inhibition was the measured response. Amounts of erythromycin were calculated by interpolating the inhibition diameter into the standard curve. The method has been validated according to the following criteria.

**Specificity:** The method shows specificity between erythromycin and endogenous component of the tissues. Three different matrixes are tested under experimental conditions for each tissue. No inhibition zone was observed for any tissue.

**Linearity in chicken tissues:** The linearity of detector response was assessed in the four tissues matched with standard solutions in the range from 100 to 2000µg/kg. The correlation coefficients were 0.9994, 0.9513, 0.9571 and 0.9969 for erythromycin A in muscle, liver, kidney and skin/fat, respectively. Other validation parameters are presented in the following tables for each tissue.

**Linearity in eggs:** The linearity of detector response was assessed matched with standard solutions in the range from 100 to 2000µg/kg. The correlation coefficient was 0.9567 as average of 9 standard curves. Other validation parameters are presented in the following tables.

**Table 13: Validation parameters of the microbiological method for erythromycin in chicken**

**Table 13-A**

Concentrations (µg/kg).	Validation parameters for kidney						Overall
	100	200	500	1000	1500	2000	
Recovery rate (%) n=9	85.5	86.5	88.2	85.1	84.8	86.1	86.0
Repeatability, Cv,% n=3 per day	5.9	9.4	7.8	10.8	5.7	6.0	-
Intermediate precision, CV, %/ 3 days	12.9	12.8	13.5	10.8	5.7	10.5	-
Accuracy: difference (% value)	-5.11	3.78	7.07	4.02	-1.90	-4.27	0.60
Limit of Detection (LOD)				50 µg/kg			
Limit of Quantification (LOQ)				100 µg/kg			
- = not calculated							

**Table 13-B**

Validation parameters for fat + skin							
Concentrations ( $\mu\text{g}/\text{kg}$ ).	100	200	500	1000	1500	2000	Overall
Recovery rate (%) n=9	101.5	102.9	100.0	97.5	97.1	96.6	99.2
Repeatability $C_v$ ,% n=3 per day	3.7	5.6	4.6	7.5	6.9	6.7	-
Intermediate precision, $C_v$ , %/ 3 days	3.7	5.6	5.4	7.5	6.9	6.7	-
Accuracy: difference (% value)	0.22	-0.22	-1.13	4.99	-3.44	0.67	0.18
Limit of Detection (LOD)	50 $\mu\text{g}/\text{kg}$						
Limit of Quantification (LOQ)	100 $\mu\text{g}/\text{kg}$						
- = not calculated							

**Table 13-C**

Validation parameters for muscle							
Concentrations ( $\mu\text{g}/\text{kg}$ ).	100	200	500	1000	1500	2000	Overall
Recovery rate (%) n=9	94.1	94.0	93.6	92.6	93.1	95.9	93.9
Repeatability $C_v$ ,% n=3 per day	9.4	13.3	7.9	4.6	7.3	4.5	-
Intermediate precision, $C_v$ , %/ 3 days	9.8	13.3	7.9	4.6	7.3	6.2	-
Accuracy: difference (% value)	-3.22	5.22	2.53	-2.00	-1.45	1.06	0.36
Limit of Detection (LOD)	50 $\mu\text{g}/\text{kg}$						
Limit of Quantification (LOQ)	100 $\mu\text{g}/\text{kg}$						
- = not calculated							

**Table 13-D**

Validation parameters for liver							
Concentrations ( $\mu\text{g}/\text{kg}$ ).	100	200	500	1000	1500	2000	Overall
Recovery rate (%) n=9	86.6	83.7	88.5	87.8	87.8	88.8	87.2
Repeatability, $C_v$ ,% n=3 per day	5.2	7.6	7.0	8.8	6.1	6.3	-
Intermediate precision, $C_v$ , %/ 3 days	9.2	7.9	13.3	8.8	6.2	6.3	-
Accuracy: difference (% value)	0.22	-5.72	9.47	4.04	1.26	-6.51	0.46
Limit of Detection (LOD)	50 $\mu\text{g}/\text{kg}$						
Limit of Quantification (LOQ)	100 $\mu\text{g}/\text{kg}$						
- = not calculated							

**Table 14: Validation parameters of a microbiological assay method for erythromycin in eggs**

Validation parameters							
Concentrations ( $\mu\text{g}/\text{kg}$ ).	100	200	500	1000	1500	2000	Overall
Recovery rate (%) n=9	87.5	93.5	94.2	93.4	92.4	92.8	92.3
Repeatability, $C_v$ ,% n=3 per day	2.9	2.6	4.7	3.9	4.3	4.6	-
Intermediate precision, $C_v$ , %/ 3 days	5.0	4.5	5.1	5.7	4.5	4.6	-
Accuracy : difference (% value)	-4.89	3.11	3.87	5.77	-2.21	-4.59	0.18
Limit of Detection (LOD) :	50 $\mu\text{g}/\text{kg}$						
Limit of Quantification (LOQ) :	100 $\mu\text{g}/\text{kg}$						
- = not calculated							

Wang, et al. (2005) published a method for determination of five macrolide antibiotic residues in eggs (spiramycin, tilmicosin, oleandomycin, erythromycin and tylosin) using liquid chromatography/electrospray ionization tandem mass spectrometry. Data acquisition under MS/MS was achieved by multiple reaction monitoring of two or three fragment ion transitions for both quantification and confirmation. A full experimental design was used to study the measurement uncertainty arising from intermediate precision and trueness or proportional bias. The overall recoveries of spiramycin, tilmicosin, oleandomycin, erythromycin and tylosin at fortified levels of 60, 100, 200 and 300 µg/kg were 96.8, 98.2, 98.3, 98.8 and 95.4, respectively. The method detection limits (S/N ≥ 3:1) of five macrolides were <1.0 µg/kg).

### APPRAISAL

Erythromycin is an old drug. It was first reviewed by the Committee in 1968. No ADI was established but acceptable levels of residues were defined in milk (0-0.04 mg/ml) and meat (0-0.3 mg/kg). Erythromycin is a mixture of three compounds produced during fermentation. The main product is erythromycin A with small portions of B (≤ 5%) and C (≤ 5%). In veterinary medicine, erythromycin is used for the treatment of clinical and subclinical mastitis in lactating cows, for the treatment of infectious diseases due to erythromycin sensitive bacteria (cattle, sheep, swine, and poultry) and for the treatment of chronic diseases due to mycoplasma in poultry. The maximum recommended therapeutic dose in veterinary use is 20/mg/kg/day as erythromycin base.

Data from pharmacokinetic and metabolic studies in experimental and target animals and humans were submitted for evaluation by the Committee together with two earlier residue studies in calves and chickens. Three new non radiolabeled residue depletion studies in chickens, laying hens and turkeys treated with erythromycin and the description and validation of the analytical procedures employed were provided.

Erythromycin is rather slowly absorbed in humans, rats, cattle and chicken with some differences related to the mode of administration (IM, IV and oral), the salt form and the coating of the administered compound. Protein binding is variable ranging from 90% in man to 38-45% in cattle. The major site of absorption is rats, dogs and humans is the small intestine. Erythromycin is only slightly absorbed in the stomach. The tissue concentrations are higher than in serum and persist longer. Erythromycin is mainly excreted in the faeces through the bile, 37 to 43 % of the dose was recovered in the intestinal tract plus faeces of rats. Urinary excretion ranged from 10 to 36 % in different species (human, rats and dogs).

Erythromycin is rapidly metabolized in the liver, mainly through an N-demethylation process in a variety of species of rodents, ruminants and humans. N-desmethyl-erythromycin was the major metabolite and the only microbiologically active metabolite of erythromycin. However, its antimicrobial activity is low and the only form of erythromycin known to be active *in vivo* is erythromycin free base.

Two studies were performed in 1988 in calves and poultry using erythromycin thiocyanate. In poultry, the residues of erythromycin were determined in chicken tissues after administration by the oral route in drinking water for 3 consecutive days. In a similar study the residues of erythromycin in whole eggs were determined following medication administered to laying hens for 7 consecutive days in drinking water. The concentration of the erythromycin in chicken tissues declined to values below the limit of detection three days after the end of treatment and six days after the end of treatment in whole eggs.

There was no radiolabelled study reported, however, four new residue depletion studies with unlabelled erythromycin were performed in poultry. The route of administration, the dose and the species are those intended for therapeutic use.

The residues with microbiological activity were measured in chickens and laying hens by a microbiological plate assay using agar medium and *Micrococcus luteus* ATCC9341 as test organism

(LOD: 50 µg/kg for all chicken tissues and eggs; LOQ: 100 µg/kg for all chicken tissues and eggs). The concentration of erythromycin A and its metabolites were simultaneously assayed using a LC/MS/MS method. For chickens, erythromycin A, B and C, the LOD are as follows: 25 µg/kg for kidney, 30 µg/kg for liver, 3 µg/kg for muscle and 5 µg/kg for skin + fat and the LOQ is 100 µg/kg in all tissues. For N-desmethyl-erythromycin A, the LOD are as follows: 25 µg/kg for kidney, 48 µg/kg for liver, 5 µg/kg for muscle and 24 µg/kg for skin + fat and the LOQ is 100µg/kg in all tissues. For eggs, the erythromycin LOQ is 50 µg/kg and the LOD is 0.92 µg/kg. For turkey, the erythromycin LOQ is 100 µg/kg for all tissues and the LOD is 4 µg/kg for skin/fat and 3 µg/kg for all other tissues.

In the chicken studies, all tissue residue depletion results showed that from day 1 to day 3 after the end of the treatment period, low concentrations of erythromycin A and N-desmethyl-erythromycin A were measured in only a few liver samples. Prolonged treatment for up to eight days resulted in the same tissue residue concentration characteristics. In eggs, during the three days of treatment, mean concentrations of erythromycin A ranged from 109 µg/kg (day 1) to 83 µg/kg (day 3) measured by the LC/MS/MS method. Taking into account the standard deviation, there are no significant differences in residue concentration between days. The egg residues of erythromycin after the end of the treatment were 57±6 µg/kg, 71± 6 µg/kg and 54± nc µg/kg for days 1, 2 and 3, respectively.

Results show that erythromycin A and its metabolite N-desmethyl-erythromycin A were the major compounds observed (the ratio erythromycin/total active microbiological metabolites was 0.33 and the ratio of N-desmethyl-erythromycin/total active microbiological metabolites was 0.75). Erythromycin could only be quantified in 25% of eggs at day 1 and 12.5% of the eggs at day 2. Concentrations of erythromycin after the end of the treatment measured by LC/MS/MS were near the LOQ (50 µg/kg). N-desmethyl-erythromycin was present at higher concentrations but had very low antimicrobial activity. Both compounds were below the LOQ at 6 days after the end of treatment. Erythromycin A was identified as the marker residue for eggs.

Tissue residue depletion studies in turkeys yielded similar results. The LC/MS/MS assay method was adapted from the chicken method. At three days after the end of the treatment, residues were detected only in liver (166±64 µg/kg). At this time, residues were measured only in one sample of kidney, muscle and fat. Four days post-treatment, all concentrations of residues were below the LOQ or the LOD.

The 66<sup>th</sup> meeting of the Committee agreed to apply a new approach to estimate chronic (long term) exposure using the median residue concentration of residues, using the standard food basket, in addition to the historically used theoretical maximum daily intake calculation based on the MRL. For numerical values reported below the LOD and the LOQ, one-half the analytical limit was applied to each, respectively, for estimating daily exposure concentrations. For erythromycin, the summary of the median residue concentrations in chickens, turkeys and eggs are presented in the following tables, derived from using individual values in all studies using an Excel spreadsheet.

**Table 15a: Median residues in chicken and turkey tissues (µg/kg)**

Tissue & Time	Chicken			Turkey			
	12 hours	1 day	2 days	1 day	2 days	3 days	4 days
Muscle	1.5	1.5	1.5	50	50	1.5	1.5
Liver	15	15	15	50	50	15	15
Kidney	12.5	12.5	12.5	50	12.5	12.5	12.5
Fat+Skin	50	2.5	2.5	50	25.8	2.5	2.5

**Table 15b: Median residues in chicken eggs ( $\mu\text{g}/\text{kg}$ )**

Time (days)											
0 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days
0.45	25	25	25	25	25	25	41	25	25	25	25

The LC/MS/MS analytical methods provided are applicable for the determination of residues of erythromycin in chicken and turkey tissues (muscle, fat, kidney and liver) and eggs. The method was validated.

#### MAXIMUM RESIDUE LIMITS

In recommending MRLs for erythromycin, the Committee considered the following factors:

- The marker residue is erythromycin A. Metabolites exhibited little or no microbiological activity.
- Only MRLs in poultry tissues and eggs were considered.
- Residue studies in bovine and ovine tissues suggest mean ratios of marker residue to total residues for tissues to be 0.7 for muscle and kidney, 0.5 for liver and 0.85 for fat. The ratio was not always available for all species and when it was, it was only available based on few individual data. From submitted studies, the marker residue to total residues for eggs was estimated to be 0.33.
- Residue depletion studies generated very limited numbers of residue concentrations above the limit of quantification for all studies in chickens, turkeys and eggs.
- A validated LC/MS/MS method is available with a limit of quantification of 100  $\mu\text{g}/\text{kg}$  for all tissues and 50  $\mu\text{g}/\text{kg}$  for eggs. The limit of quantification of the microbiological method is 100  $\mu\text{g}/\text{kg}$  for all tissues and eggs.
- For residue concentrations reported below the LOD and the LOQ, one-half the analytical limit was applied to each, respectively, for estimating daily exposure concentrations.
- The ADI for erythromycin A was 0 – 0.7  $\mu\text{g}/\text{kg}$  body weight, equivalent to 42  $\mu\text{g}$  per person per day.

Noting the factors noted above, the Committee recommended MRLs of 100  $\mu\text{g}/\text{kg}$  for chicken and turkey muscle, liver, kidney and fat/skin and 50  $\mu\text{g}/\text{kg}$  for eggs at the limit of quantification of the LC/MS/MS method, measured as erythromycin A.

Applying the MRLs and the standard food basket, the theoretical maximum daily intake is 55  $\mu\text{g}$ , equivalent to approximately 130% of the ADI. The 66<sup>th</sup> meeting of the Committee agreed to apply the principle of using median residue concentrations to better estimate long-term (chronic) exposures to residues. Estimated daily intake (EDI) values were determined using median residue values for each tissue from each food-producing species for which data were available. Median residue values were determined using an Excel<sup>®</sup> spreadsheet program. Where residue values were below the LOD or LOQ of the validated method, values of  $\frac{1}{2}$  the LOD and  $\frac{1}{2}$  the LOQ, respectively, were used in the calculations. Applying the highest estimated median residue concentrations in turkeys (50  $\mu\text{g}/\text{kg}$  in all tissues) – the median residue concentrations in turkey tissues were higher than the corresponding median residue concentrations in chicken tissues - and eggs (41  $\mu\text{g}/\text{kg}$ ), the estimated daily intake is 29.1  $\mu\text{g}/\text{kg}$ , equivalent to approximately 69% of the ADI. Results are shown in Table 16.



**Table 16: Estimated daily intake**

Tissue	Median intake ( $\mu\text{g}/\text{kg}$ )	Standard Food Basket (kg)	Daily intake ( $\mu\text{g}$ )
Muscle	50	0.3	15
Liver	50	0.1	5
Kidney	50	0.05	2.5
Fat	50	0.05	2.5
Eggs	41	0.1	4.1
Total			29.1

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