LINCOMYCIN

First draft prepared by

Barbara Röstel, Fougères, France
Jan Zmudzki, Pulawy, Poland
James MacNeil, Saskatchewan, Canada

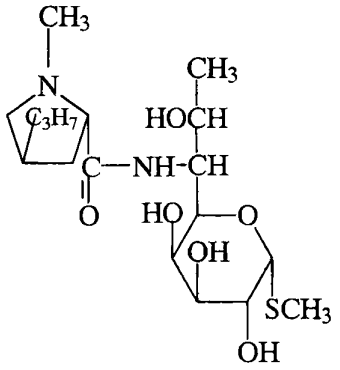
IDENTITY

Chemical name: Methyl 6,8-dideoxy-6-[[(1-methyl-4-propyl-2- pyrrolidinyl)carbonyl]amino]-1-thio- D-erythro-α-D-galacto-octopyranoside, monohydrochloride monohydrate (CAS name); CAS No. 154-21-2 (Lincomycin), 7179-49-9 (Lincomycin hydrochloride monohydrate); 859-18-7 (Lincomycin hydrochloride anhydrous)

Methyl 6,8-dideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrolidone-carboxamido)-1-thio-D-erythro-α-D-galacto-octopyranoside monohydrochloride monohydrate, (IUPAC name)

Synonyms: Lincomycin, Lincomycin hydrochloride, Upjohn: PNU-10149A, Albiotic ®
Non-Proprietary name: Lincocin

Structural formula



Molecular Formula: $C_{18}H_{34}N_2O_6S$

Molecular weight: 406.6

OTHER INFORMATION ON IDENTIY AND PROPERTIES

Appearance: White to tan to brown crystalline powder, color depending on grade of material. The high grade used in injectable products is whiter than the grades used in premixes.

Melting point: 145 – 147 ° C

Solubility: Lincomycin hydrochloride: Soluble in water, (1:1) in ethanol, (1:40) in dimethylformamide, (1:20), in methanol. Very slightly in acetone and practically insoluble in chloroform and ether

Optical rotation: Licomycin hydrochloride (1% in water) $[\alpha]^{25}_{D} = + 142°$

Ultraviolet maxima: none

Stability: Lincomycin is a very stable molecule and does not degrade under normal environmental conditions.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Lincomycin is an antibiotic produced by *Strepromyces linconensis*. It belongs to the class of lincosamides, which are derivatives of an amino acid and a sulfur containing galactoside. Other substances belonging to the lincosamide group are clindamycin and pirlimycin.

Lincomycin is used as oral preparation in feed and water in poultry for treatment of bacterial enteric infections and as a performance enhancer; in pig it is used for treatment and control of bacterial enteric, mycoplasmal respiratory infections, in infectious arthritis and as a performance enhancer. Mono preparations are also used as parental formulations in pig for treatment of bacterial enteric, mycoplasmal respiratory infections and in infectious arthritis.

Combination-preparations with spectinomycin are used in poultry for oral application (water) for treatment and control of respiratory disease and increase in weight gain. In pig oral preparations (water, feed) are intended for treatment and control of enteric and respiratory disease, treatment of infectious arthritis and increase in weight gain. Parental preparations (i.m.) exist for treatment of bacterial enteric and respiratory disease in pig and calves and for treatment of arthritis in pig and contagious foot-rot in sheep. The combination-preparation with sulfadiazine is used orally (feed) in pig for treatment of atrophic rhinitis and enzootic pneumonia. Combination preparations with neomycin are used as intramammary applications in lactating dairy cattle for treatment of acute mastitis.

Dosage:

Table 1. Maximum daily Lincomycin doses per animal species and route of administration (mg/kg body weight or mg/mammary quarter).

Species	Oral	Parental	Intramammary
Poultry	50 mg/kg bw		
Pig	13 mg/kg bw	10 mg/kg bw	
Calves		5 mg/kg bw	
Sheep		5 mg/kg bw	
Dairy Cows			600 mg/quarter

PHARMACOKINETICS AND METABOLISM

Laboratory Animals

Rat

Adult male rats received one single i.m. lincomycin dose of 0 and 100 mg/kg body weight (Davis, Balcolm 1969). Groups of 10 rats were killed at 15, 30 minutes, 1, 1.5, 3, 6 and 8 hours after treatment at which time blood and bone samples were collected. Lincomycin is rapidly absorbed and plasma levels reach mean peak concentrations of 20.9 mg/kg and declined biphasically to concentrations of 2.5 mg/kg at 8 hours.

Dog

One dog (female, 11.5 kg) received one single dose of tritium labelled lincomycin hydrochloride of 500 mg equivalents once i.m. and once orally (Eberts et al. 1963). After oral application peak plasma concentrations of 4.5 mg/kg were reached at 4 hours. The plasma half-life was 4.1 hours. Of the administered dose, 14% was excreted in urine and 77% in feces. Total recovery of the dose was 92%. The amount of oral absorption was calculated as 35%. The half life curves of urinary excretion was biphasic with values of 4.3 and 27.4 hours. After i.m. application, peak plasma concentrations of 25.2 mg/kg were reached within 10 minutes, with a half-life of 4 hours. Of the 87% of radioactivity recovered, 49% were excreted in urine and 38% in feces. The biphasic half-life curve of urinary excretion had values of 3.8 and 20.4 hours. Urinary excretion for both routes of application was essentially complete in less than 24 hours and in fecal excretion, within 48 hours.

Food Animals

Pig

Serum time curves were determined in 3 pigs after a single i.m. dose of 11 mg lincomycin hydrochloride/kg bw (Russel 1979). Blood samples were taken at 0.5, 1, 1.5, 2, 2.5, 4, 6 and 8 hours and analysed by the microbiological assay.

Plasma concentrations peaked between 0.5 and 1 hour after treatment with maximum level ranging between 5.33 and 10.92 mg/kg and gradually declining over 8 hours.

In a GLP conforming study, five groups of two pigs received oral doses of ^{14}C lincomycin hydrochloride of 440 mg per animal per day by capsule (Hornish, Gosline 1981). Capsules were given every 6 hours. One group received 6 doses, one 14 doses and three groups received 10 doses. The groups treated with 6 and 14 doses were killed 4 hours post treatment, and the groups treated with 10 doses were killed 4, 48 and 96 hours post treatment. Blood and tissue samples were analysed. Total ^{14}C accountability was 96%. Highest concentrations were found in liver and kidney samples of the 10 dose treatment animals with mean levels of 14.0 mg/kg-equivalents in liver and 10.1 mg/kg-equivalents in kidney followed by muscle (0.7 mg/kg-equivalents) and fat (<0.1 mg/kg-equivlents) depleting to 1.01 mg/kg-equivalents in liver and 1.1 mg/kg-equivalents in kidney at 96 hours.

In a GLP study, one group of 7 pigs received a single i.v. dose of 10 mg linocmycin hydrochloride/kg bw. After a one week treatment-free interval, the same pigs received the same dose once orally by capsule (Hornisch, et al., 1985). Blood samples were taken at 0, 0.1, 0.2, 0.3, 0.5, 1, 2, 4, 8, 12 and 16 hours after i.v. treatment and at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 8, 12 and 16 hours after oral treatment and the residues examined by microbiological methods. The oral absorption in pig was 53 ± 19%. Plasma protein binding at blood concentrations of 0.5 to 20 mg/kg was 5-15%. The absorption and bioavailability follows a first order kinetic profile. The distribution and excretion for the oral application represents a one compartment and first order elimination. For the i.v. application, a biphasic, two compartment model with an initial fast elimination through the first 0.5 hours followed by a slower terminal elimination phase was observed. C_{max} was determined as 1.45 mg/kg at the t_{max} of 3.6 hours for the oral dose. The large variation coefficient of 48% for C_{max} was explained by the profound effect of food intake exerted on the absorption. The mean $t_{1/2}$ was calculated as 3.36 ± 1.27 for the oral route and 1.95 ± 0.42 for the parental route.

Groups of 4 pigs received a single i.m. dose of lincomycin hydrochloride of 2, 5 and 10 mg/kgb bw, corresponding to 4.4, 11, 22, mg/kg bw per day (Barbiers, Kleckner 1964). Blood samples were taken at 0, 1, 2, 4, 8, 12, 24, 36 and 48 hours after treatment. Dose dependent maximum concentration were seen at 1 hour declining at 16 hours to the limit of quantification (LOQ = 0.1 mg/kg) of the microbiological method. Additionally, groups of 3 pigs received a single oral dose of lincomycin hydrochloride of 10, 25 and 50 mg/kgb bw corresponding to 22, 55, 110 mg/kg bw. The same sampling schedule was applied. Dose related maximum blood concentrations were found at 4 hours after treatment declining at 24 to 36 hours to the LOD of the method.

Two groups of 3 pigs received daily i.m. doses of lincomycin hydroxhloride of 11mg/kg bw, in one group for 3 days, the other group received treatment for 7 days (Barbiers et al 1975). One hour after the last treatment, injection site, muscle, fat, kidney, liver, blood, urine and other tissues and body fluid were collected and analysed by the microbiological method. Lincomycin is absorbed from the injection site and distributed to the various tissues and body fluids. Highest values were found in urine (413 mg/kg in the 3 day treatment group and 261 mg/kg in the 7 day treatment group) confirm very rapid excretion. Plasma concentrations were 6.07 mg/kg (3day treatment group) and 3.80 mg/kg (7day treatment group). In tissues the highest concentrations were seen in injection sites (14.66 mg/kg (3 days) and 93.33 mg/kg (7 days), followed by kidney (21 mg/kg in 3day treatment group and 15 mg/kg in the 7day treatment group), liver (8.43 mg/kg (3days) and 6.40 mg/kg (7days),in muscle, 6.07 mg/kg (3days) and 3.80 mg/kg (7days), and in fat (2.19 mg/kg (3days) and 2.17 mg/kg (7days).

One group of 3 pigs received daily oral doses of 22 mg lincomycin hydrochloride/lb bw for 3 days, a second group of 3 pigs received daily i.m. doses of 22 mg lincomycin hydrochloride/lb bw for 3 days (Barbiers, Kleckner 1964a, 1964b). Blood samples were taken at 0, 1, 2, 4, 8, 12, 24, 36, 48, 60 and 72 hours after treatment. Tissue samples from one pig of each group were taken at 1, 2 and 3 days after treatment. Blood concentrations were comparable to the previous trials with no detectable residues after 24 hours. In tissues, the only residues were found in liver and kidney in the 24 hour samples with higher concentrations found tissues of orally treated animals (liver, 1.1 mg/kg, in kidney 1.6 mg/kg) as measured by a microbiological method.

Four groups of six pigs received lincomycin in feed in concentrations of 0 and 44 mg/kg (DeGeeter, Barbiers 1978). Animals were killed at 0, 1 and 2 days after withdrawal of medication. Blood and tissue samples were analysed by bio-assay. Lincomycin was detected only in liver and kidney samples with values for liver ranging from <0.1 to 0.12 mg/kg and higher values for kidney, from 0.14 to 0.28 mg/kg. No lincomycin was detected in any of the samples at 1 and 2 days withdrawal. The LOQ of the method is 0.1 mg/kg.

Chicken

One group of 8 chickens received twice daily oral doses of 0.47-0.76 mg/kg bw ^{14}C lincomycin hydrochloride/kg bw per capsule for 12 days after having been exposed for 36 day to non labelled lincomycin hydrochloride in feed at a concentration of 10 g/ton (Gosline et al., 1978). Blood, offal, bile and tissues from two chickens were collected for

analysis at 1 hour, 1, 2, and 3 days after final treatment. On-treatment excreta contained 90.2 ± 8.6 % of the administered radioactivity. The half-life in bile and offal were 8.3 and 11.3 hours, respectively. Only liver samples at 1 hour after treatment contained detectable residues (LOD = 0.1 mg/kg), however, the residues were biologically inactive.

Dairy cattle

One first-calf heifer in mid lactation received a single i.v. dose of 11 mg lincomycin/kg bw (Weber et al,. 1981). Blood samples were taken frequently up to 48 hours. Milk samples were taken at 0, 4, 8, 12, 18, 24, 36 and 48 hours. Urine samples were taken in 20 minute intervals up to 4 hours and at 6, 7, 8, 12, 18, 24, 36, 48, 60, and 72 hours. The same animal was used in two trials using an intramammary infusion of 5 mg lincomycin/lb, equally applied to all quarters. In the first trial, blood samples were taken very frequently up to 48 hours, milk samples at 0, 24, 36, 48, 60, and 72 hours. Urine samples were taken in 20 minute intervals up to 3 hours and at 8, 12, 18, 24, 36, 48 and 72 hours. In the second trial, milk samples were taken at one hour intervals through 12 hours and at 16, 20, 36, 48, 50 and 72 hours. Additionally, using the same experimental design, two first-calf heifers in mid lactation received a single i.v. dose of lincomycin of 5.5 and 11 mg/kg bw, respectively. Blood, milk and urine sampling were the same as in the first study.

Lincomycin in cows shows biexponential pharmacokinetics and represent first order kinetics. Intravenous doses of 5.5 and 11 mg/kg bw give linear kinetics. An approximately constant 32% of the dose is excreted in the urine, independent of the route of administration. Only 1.5% of the i.v. dose is excreted in the milk; 85% of an intramammary dose is absorbed into the blood. Changes in the rate of milk production are shown to strongly influence the kinetics of transfer of lincomycin into and out of the udder. Approximately 65% of the dose, regardless of the route of administration is metabolised to bio-inactive metabolites.

Man

Five healthy men received lincomycin as an oral dose of 0.5 and 1.0 g/person (64-104 kg) and as an i.m. dose of 0.6 g/person (Kaplan et al., 1965). The drug was given in a fasting state and after a meal. Blood samples were taken before and 1, 2, 4, 6, 8, 12, and 24 hours after treatment. Urine was collected for 24 hours. After the 0.5 g dose, plasma concentrations were higher in the fasting males and peaked with 1.4 to 1.8 mg/kg at about 4 hours and were maintained at the higher concentrations when compared to non-fasted individuals over a period of 12 hours. Lincomycin levels after eating are lower in plasma with values of 0.6 - 0.7 mg/kg over 2 to 8 hours. About 4 to 7% of the administered dose is excreted in the urine within 24 hours. After i.m. injection, peak plasma concentrations appeared at 1 - 2 hours with values of 6.8 - 11.6 mg/kg decreasing gradually to 1 mg/kg at 24 hours. Approximately 30 to 60% was excreted in urine within 24 hours.

METABOLISM

The metabolism of lincomycin in dog, chicken and pigs has been described. Comparative studies of the metabolism of lincomycin in pigs and rats and in chicken and rats were provided.

Laboratory Animals

Dog

Three beagle dogs received a single i.v. dose of ^{14}C-lincomycin hydrochloride equivalent to 100 mg lincomycin free base per animal 7.7-8.5 mg/kg bw (Daniels and Van Eyk, 1978). Urine and feces were collected continuously for 101 hours. Recovery of radioactivity was complete with 95% of the administered dose accounted for. Biliary excretion plays the predominant role with 55 - 60% of radioactivity excreted in feces and 32 - 40% in urine. Thin layer chromatography (TLC) was used for identification of parent lincomycin, which accounted for 17% of the administered dose excreted in feces and 28.5% in urine. Very small amounts (<3% of the dose) of lincomycin sulfoxide and N-demethyllincomycin were found.

Food Animals

Pig

In pigs, metabolism was rapid and extensive, leading to 26 metabolites in liver. Except for the parent compound, none of the metabolites has been characterized, and each was present at a concentration representing less than 10% of the radiolabelled material (Hornish et al., 1987). In a comparative analysis of microbiological and gas chromatographic-mass spectrometric (GC/MS) methods, lincomycin appeared to account for all of the microbiologically active residues in pig liver and kidney (Nappier et al., 1989, Nappier et al., 1996f).

Comparative metabolism studies were conducted in pigs and rats (Hornisch et al. 1987). Pigs were treated orally (capsules) at doses of 15 mg/kg bw/day, rats at doses of 300 mg/kg bw/day of ^{14}C-lincomycin in drinking water. Of the

total dose administered, 5% was excreted in urine of rats, whereas 14-21% was found in pig urine. Feces and GI tract content contained the major portion of excreted drug in rats (95%) and in pigs (79-86%). All major components separated by HPLC in pig urine were also qualitatively found in rat urine. Analysis of feces demonstrated significant differences between the two species in a quantitative sense but only minor differences on a qualitative basis. Most fecal metabolites found in pig were also found in rats. In liver samples the ratio of the final polar fraction to the chloroform fraction was 2:1 in rats and 5:1 or higher in pigs indicating a relatively higher content of lincomycin in rat liver. Although quantitative differences were found in the different analysed fractions there was greater than 90% qualitative match of pig liver metabolites to rat liver metabolites.

Chicken

Comparative metabolism studies were conducted in chicken and rats (Hornisch et al., 1987). Chickens received doses of 5.5-6.5 mg/kg bw/day of ^{14}C-lincomycin for 7 days. Lincomycin was the major excreta metabolite in chickens with 60-80% on treatment and about 50% 2-4 days after treatment. Small amounts of N-demethyllincomycin and lincomycin sulfoxide were identified only during treatment. An unknown metabolite comprising 10% during treatment and increasing to 50% at 4 days after treatment was considered to be generated by gut microflora of the chicken. In these comparative studies of the metabolism of lincomycin in chicken and rat liver, it was found to be qualitatively similar, although the metabolites were not identified.

Twenty-one female and 21 male broiler chickens (35 days old) were exposed to doses of 128 mg/gal (5.1 to 6.6 mg/kg bw/day) ^{14}C-lincomycin hydrochloride in drinking water for 7 days; six animals (3 male, 3 female) served as control (Hornisch et al. 1984). Total radioactive residue concentrations were measured in liver, kidney, muscle and skin and fat at 0, 0.5, 1, 2, 4, and 7 days after treatment. Liver and kidney contained the highest total residue concentrations, declining in liver from 1.58 (day 0) to 0.02 mg/kg-equivalents (day 7), and in kidney from 1.26 (day 0) to 0.01 (day 7) mg/kg-equivalents. Total residue concentrations declined in muscle from 0.05 (day 0) to <0.05 (day 2) mg/kg-equivalents and in skin/fat from 0.13 (day 0) to <0.05 (day 7) mg/kg-equivalents. At 0 hours 75% of total liver residues were identified as: lincomycin (20%), lincomycin sulfoxide (40%), N-demethyllinocmycin (5%), and N-demethyllincomycin sulfoxide (10%). In muscle, lincomycin (16%) and one unknown, metabolite VI, (37%) accounted for more than 50% of the total residues at 0 hours. In skin and fat, corresponding values were: lincomycin (18%) and unknown metabolite VI (11%); they comprised about 40% of total residues at 0 hours. During treatment excreta contained 60-85% unmetabolised lincomycin declining slightly to 50-55% of total residues at 4 days after treatment. Lincomycin sulfoxide (6-10%), N-methyllincomycin (3-6%) and one unknown (10%) were the remaining on-treatment excreta residues.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled Residue Depletion Studies

The depletion of radiolabelled ^{14}C lincomycin was studied in pigs and chicken.

Pig

In a study conducted prior to GLP, groups of six pigs were fed diets containing ^{14}C-lincomycin at concentrations equivalent to 1.2, 2.0, 6.0-7.0, or 10-12 mg/kg of body weight per day for 3 days and were killed 12 h after the last treatment (Hornish et al., 1987). An additional group of six pigs received the maximum dose for 3 days and were killed 48 h after treatment. The mean concentrations of total residues at 12 h for the four different treatments were 0.40, 0.64, 1.6, and 3.4 mg/kg in liver; 0.22, 0.41, 1.2, and 3.1 mg/kg in kidney; 0.01, 0.02, 0.053, and 0.15 mg/kg in muscle; and 0.021, 0.024, 0.13, and 0.35 mg/kg in fat for each treatment group, respectively. At 48 h, the mean concentrations of total residues were 0.82 mg/kg in liver, 0.64 mg/kg in kidney, 0.091 mg/kg in muscle, and 0.097 mg/kg in fat in animals given lincomycin at 10-12 mg/kg of body weight. At 12 h, the mean concentrations of microbiologically active residues in tissues of animals given 10-12 mg/kg of body weight were 0.096 mg/kg in liver and 0.42 mg/kg in kidney. The samples of liver were reanalysed with an improved microbiological assay and by GC/MS. In liver samples from the group given the highest dose, the ratio of total residues to lincomycin was 17:1 at 12 h and 40:1 at 48 h after treatment.

In a study conducted using GLP conditions, twelve pigs received intramuscular doses of 11 mg/kg of body weight of ^{14}C-lincomycin daily for 3 days, and groups of three pigs were killed at 12 and 24 h and six pigs at 48 h after treatment (Nappier et al., 1988). Of the administered radiolabel, 78-85% was accounted for. The highest mean concentrations of total residue at 12 h were 17 mg/kg in liver and 12 mg/kg in kidney. The concentrations in other tissues were 1.0 mg/kg at the injection site, 0.59 mg/kg in fat, and 0.39 mg/kg in muscle. By 48 h, these concentrations had declined to 3.8 mg/kg in liver, 3.1 mg/kg in kidney, 0.58 mg/kg at the injection site, 0.20 mg/kg in fat, and 0.14 mg/kg in muscle. When the liver and kidney samples were reanalysed by an improved microbiological assay and by GC/MS, the ratio of total residues to lincomycin was 7.2 :1 at 12 h, 31 :1 at 24 h, and 62 :1 at 48 h in liver, and 1.8 :1 at 12 h, 5.1 :1 at 24 h, and 15 :1 at 48 h in kidney. Results are summarized in table 2.

Table 2. Mean ^{14}C-lincomycin total residue concentrations (mg/kg) in pig tissues after 11 mg/kg BW/day intramuscularly for three days.

Hours after treatment	Liver	Kidney	Muscle	Inj. Site	Fat
12	17.54 ± 6.24	12.02 ± 4.25	0.39 ± 0.04	1.05 ± 0.32	0.59 ± 0.36
24	13.61 ± 1.87	5.75 ± 0.24	0.13 ± 0.02	0.86 ± 0.28	0.26 ± 0.14
48	3.84 ± 1.41	3.08 ± 1.09	0.14 ± 0.04	0.58 ± 0.33	0.20 ± 0.07

Chicken

In a study conducted according to GLP, ^{14}C-lincomycin was given to 21 female and 21 male broiler chickens, 35 days old, in drinking-water at doses of 5.1-6.6 mg/kg of body weight per day for 7 days (Hornisch et al., 1984). Total radiolabelled residue was measured in liver, kidney, muscle, and skin with adhering fat immediately after withdrawal of the treated water and 0.5, 1, 2, 4, and 7 days after treatment. Liver and kidney contained the highest concentrations, with residues decreasing between day 0 and day 7 from 1.6 to 0.02 mg/kg in liver and from 1.3 to 0.01 mg/kg in kidney. The concentrations in muscle decreased from 0.052 at 0 h to <0.005 mg/kg at day 2, and in skin/fat from 0.132 at 0 h to <0.005 mg/kg at day 7. Lincomycin represented 20% of the radiolabel in liver at 0 h, 12% at 12 h, 8% at 24 h, 2% at 48 h, and 5% at 96 h. It represented 16% in muscle and 18% in skin with adhering fat immediately after the last treatment.

Table 3. Mean ^{14}C-lincomycin total residue concentrations (mg/kg) in chicken tissues treatment in drinking water for 7 days.

Days after treatment	Liver	Kidney	Muscle	Skin/Fat
0	1.58	1.26	0.052	0.013
0.5	0.503	0.56	0.027	0.051
1	0.224	0.23	0.027	0.065
2	0.107	0.10	<0.005	0.028
4	0.028	0.03	<0.005	0.017
7	0.020	0.01	<0.005	<0.005

In a study conducted according to GLP, eighteen laying hens were given capsules containing of ^{14}C-lincomycin, equivalent to 0.5 mg/kg of body weight per day, twice daily for 12 days (Gosline, Hornish 1980). Eggs were collected on days 1-3 of treatment, and tissues were collected from six animals 4, 28, and 76 h after treatment. The mean concentrations of total residue in eggs rose from 0.002 mg/kg at day 1 to 0.008 mg/kg at day 10 during treatment and had decreased to 0.005 mg/kg by day 2 after treatment. The mean concentrations of total residues were highest in kidney (0.15 mg/kg) and liver (0.14 mg/kg) and were lower in muscle (0.02 mg/kg) and skin with fat (0.02 mg/kg) 4 h after treatment. The residues in all tissues were depleted to < 0.01 mg/kg within 76 h. Results are summarized in table 4, while table 5 describes residues in eggs.

Table 4. Mean ^{14}C-lincomycin total residue concentrations (µg/kg) in laying hens after treatment for 12 days.

Hours after treatment	Liver	Kidney	Muscle	Skin & fat
4	141 ± 60.2	152 ± 94.6	19.7 ± 12.4	18.9 ± 4.4
28	24.3 ± 11.6	20.6 ± 5.5	12.7 ± 11.5	14.0± 7.3
76	5.7 ± 64	6.0 ± 6.8	9.8 ± 3.8	2.9 ± 7.2

Table 5. ^{14}C-Lincomycin total residue concentrations (μg/kg) in chicken eggs after treatment for 12 days.

	Days on treatment										Days after treatment		
Animal No	1	2	3	4	5	6	7	8	10	12	1	2	3
101	2.9	2.9	–	5.2	5.7	–	6.2	8.0	*	–	7.8	5.9	K-3
102	1.7	2.5	–	3.4	5	5.7	5.7	7.1	–	–	K-1		
103	1.3	NS	2.1	–	3.1	4.9	5.3	6.2	6.9	6.2	6.1	5.2	K-3
104	1.9	NS	2.9	3.2	3.7	4.4	5.0	5.5	5.9	5.1	–	K-2	
105	1.4	4.2	4.9	6.1	7.0	*	*	*	8.0	–	K-1		
106	2.3	3.2	–	6.9	7.4	8.0	–	9.0	12	–	9.8	K-2	
107	1.4	NS	–	2.4	3.4	4.1	4.3	–	6.9	4.7	–	4.1	K-3
108	1.4	NS	2.6	3.4	4.0	4.4	5.2	–	9.1	–	K-1		
109	–	–	3.3	4.3	4.9	6.0	5.9	6.5	7.3	6.7	5.9	K-2	
110	2.0	2.8	3.6	4.5	5	6.0	5.8	6.7	9.3	–	7.6	K-2	
111	1.2	NS	2.8	3.8	4.7	4.2	4.4	4.8	6.4	–	5.3	4.3	4.0, K-3
112	1.6	2.4	3.0	3.8	4.9	5.3	5.3	6.5	7.6	6.8	K-1		
113	1.7	3.5	–	4.9	6.5	–	7.8	–	–	10	–	K-2	
114	1.5	2.8	4.5	–	–	7.0	–	6.7	9.6	–	K-1		
115	2.0	NS	–	2.6	*	5.2	4.8	7.7	7.7	–	9.0	K-2	
116	1.4	2.6	2.4	2.9	–	4.1	4.8	–	6.6	5.0	5.1	4	K-3
117	–	3.2	4.5	5.7	8.6	7.5	7.9	–	11.9	*	9.2	–	K-3
118	2	*	3.6	–	5.0	6.3	*	7.9	–	*	K-1		
n	16	10	12	15	15	15	14	12	14	7	9	5	1
Mean	**1.7**	**3.0**	**3.4**	**4.2**	**5.3**	**5.5**	**5.6**	**6.9**	**8.2**	**6.4**	**7.4**	**4.7**	**4.0**
SD	0.4	0.5	0.9	1.3	1.5	1.3	1.1	1.2	1.9	1.8	1.7	0.8	–

Note: NS means not sampled.

Residue Depletion Studies with Unlabelled Drug

Lactating cows

In a study conducted prior to GLP, five lactating cows received three consecutive doses of 200 mg of lincomycin on one quarter of the udder at 12-h intervals (Barbiers et al. 1971). Milk samples were taken during treatment and at 12-h intervals for the following 10 milkings and analysed by microbiological assay (LOQ, 0.2 mg/kg). The mean concentrations of residue in milk decreased from 115 mg/kg at 12 h to 18 mg/kg at 24 h and 1.4 mg/kg at 36 h to below the LOQ at 48 h.

Three studies conducted according to GLP were reported of intramammary application of lincomycin.

The study in which the highest recommended dose was used involved 24 cows that received three consecutive intramammary infusions of 330 mg of lincomycin per quarter into each of the four quarters of the udder at 12-h intervals (Deluyker et al., 1996a. 1996b). Pooled milk samples were taken at 12-h intervals at eight milkings after the last application and analysed by GC/MS. The mean concentrations of lincomycin were 53 mg/kg at 12 h, 7.0 mg/kg at 24 h, <LOQ-4.0 mg/kg at 36 h, 0.02-1.5 mg/kg at 48 h, <LOQ-0.20 mg/kg at 60 h, and <LOQ (0.015 mg/kg) at all other times. Individual animal results are indicated in table 6.

Table 6. Lincomycin residue concentrations (mg/kg) in milk after 3 intramammary infusions of 330 mg/quarter/12 hour interval.

	Hours after treatment							
Cow No.	12	24	36	48	60	72	84	96
4	22.8	8.23	0.20	0.06	0.06	0.01	<LOQ	<LOQ
9	30.3	8.10	0.68	0.28	0.04	0.01	<LOQ	<LOQ
11	65.0	11.4	0.76	0.19	0.02	<LOQ	<LOQ	<LOQ
16	26.3	2.49	0.74	0.03	0.03	<LOQ	<LOQ	<LOQ
18	70.1	5.89	0.10	0.06	0.01	<LOQ	<LOQ	<LOQ
22	72.6	9.20	0.26	0.12	0.01	<LOQ	<LOQ	<LOQ
27	37.0	2.67	<LOQ	0.08	<LOQ	<LOQ	<LOQ	<LOQ
28	59.1	3.44	0.33	0.04	<LOQ	<LOQ	<LOQ	<LOQ
31	71.4	9.40	0.22	0.18	0.04	NA	NA	NA
37	43.0	5.10	0.72	0.14	0.06	<LOQ	<LOQ	<LOQ
40	34.4	1.95	0.55	0.06	<LOQ	<LOQ	<LOQ	<LOQ
41	58.0	6.75	0.22	0.08	0.01	<LOQ	<LOQ	<LOQ
46	53.8	5.00	0.35	0.14	0.02	NA	NA	NA
47	68.7	13.8	4.01	1.47	0.20	0.09	0.016	<LOQ
48	25.5	1.83	1.65	0.02	<LOQ	<LOQ	<LOQ	<LOQ
50	46.2	6.37	0.08	0.12	0.01	<LOQ	<LOQ	<LOQ
53	64.5	4.53	0.38	0.07	<LOQ	<LOQ	<LOQ	NA
57	61.7	9.60	0.31	0.24	0.02	<LOQ	<LOQ	<LOQ
60	73.2	10.4	0.84	0.24	0.05	<LOQ	<LOQ	<LOQ
61	57.4	7.67	0.91	0.26	0.07	<LOQ	<LOQ	<LOQ
63	77.2	8.25	0.88	0.24	0.04	<LOQ	<LOQ	<LOQ
65	36.5	6.14	0.91	0.24	0.03	<LOQ	<LOQ	<LOQ
71	70.7	14.0	0.75	0.38	0.16	<LOQ	<LOQ	<LOQ
73	49.5	5.23	0.46	0.07	0.03	<LOQ	NA	NA
Mean*	**53.1**	**6.98**	**0.68**	**0.20**	**0.04**			
SD*	17.4	3.44	0.80	0.29	0.05			
Min.	22.8	1.83	<LOQ	0.02	<LOQ			
Max.	77.2	14.0	4.01	1.47	0.20			

Note: NA is not analysed

Ten healthy cows received 3 consecutive doses of 197 mg lincomycin/quarter in two heterolateral quarters (right front and left rear) in 12 hour intervals (Nouws et al., 1994). Quarter and pooled milk samples were taken during treatment and at the 12 milkings following the last treatment and analysed using a bioassay (LOQ = 0.013 mg/kg). During treatment lincomycin concentrations in treated quarters were 36.1, 27.8 and 52.8 mg/kg declining to or below 0.07 mg/kg at 58 hrs and below 0.025 mg/kg at 72 hours.

Twelve healthy cows received 3 consecutive doses of 200 mg lincomycin/quarter (in two heterolateral quarters (n = 6), and right hind quarter, (n = 3), and left front quarter (n = 3) in 12 hour intervals (Deluyker et al, 1996b). Quarter and pooled milk samples were taken during treatment and at the 4 milkings following the last treatment and analysed using a bioassay (LOQ = 0.025 mg/kg). Mean residue concentrations during treatment were 36.7, 41.4 and 42.0 mg/kg in the three treatment groups, respectively, declining to 2.17 mg/kg at 24 h, 0.60 mg/kg at 36 h and 0.13 mg/kg at 48 hours.

In a study conducted according to GLP, sixteen cows were given three consecutive intramammary infusions of 330 mg of lincomycin into each of the four quarters of the udder at 12-h intervals (DeGraeve et al, 1997). Tissue samples from four cows sacrificed at 1, 7, 14, and 21 days after treatment were analysed by GC/MS. The mean concentrations of lincomycin in liver were 0.23 mg/kg on day 1 and 0.058 mg/kg on day 7, and the ranges of concentrations of residues in liver were 0.017-0.040 mg/kg on day 14 and 0.007-0.051 mg/kg on day 21. Residues were found in muscle and kidney only at day 1, and no residues were found in fat.

Veal calves

In a study conducted according to GLP, four groups of five veal calves weighing 60-80 kg received daily intramuscular injections at different sites on both sites of the neck of 5 mg of lincomycin for 5 days. Two doses were given on the first day (Hoffman et al., 1996). The animals were killed and tissue samples were taken 8 h and days 7, 14, and 21 after treatment and analysed by GC/MS. At 8 h, the highest mean concentrations of residues were found in kidney (3.3 mg/kg) and at the last injection site (2.4 mg/kg). The mean concentration of residues in muscle was 0.72 mg/kg. The

concentrations were <LOQ-0.14 mg/kg in liver and <LOQ-0.26 mg/kg in fat at 8 h. The only other sample in which residues were detected was one of liver at day 14 (0.07 mg/kg).

Pig

Several residue depletion studies involving oral or intramuscular application of lincomycin to pigs were submitted.

The most relevant study conducted according to GLP, in which the maximal intramuscular dose was applied, involved two groups of 24 pigs which were given intramuscular doses of 11 mg/kg of body weight of two different formulations of licomycin for 3 days (Nappier et al, 1996d). The animals were killed 0, 3, 6, 12, 24, 48, and 144 h after treatment, and samples of liver, kidney, muscle, fat ,and injection site tissue were taken from four pigs in each group and analysed by GC/MS. The mean residue concentrations animals treated with the two formulations decreased rapidly between 3 and 48 h, from 6.4 and 4.7 mg/kg to 0.059 and 0.065 mg/kg in liver and from 29 and 21 mg/kg to 0.17 and 0.24 mg/kg in kidney. In both tissues, the concentrations were <LOQ at 144 h. In muscle, the mean concentrations were 3.6 and 2.6 mg/kg at 3 h, 0.061 and 0.085 mg/kg at 24 h, and <LOQ at all other times. In fat, the mean concentrations were 0.47 and 0.47 mg/kg at 3 h, 0.024 and 0.033 mg/kg at 24 h, and <LOQ at all other times. At the injection site, the mean concentrations were 115 and 250 mg/kg at 3 h, 0.022 and 0.025 mg/kg at 48 h, and <LOQ at 144 h. The results are summarised in table 7.

Table 7. Mean lincomycin residue concentrations (mg/kg) in pigs after intramuscular treatment for 3 days.

Preparation	Hours after treatment	Liver (n = 4)	Kidney (n = 4)	Muscle (n = 4)	Fat (n = 4)	Injection Site (n = 4)
Lincomix ® 100	3	6.37 ± 1.58	29.3 ± 5.67	3.60 ± 0.41	0.47 ± 0.30	115 ± 75
Lincomix ® 300	3	4.70 ± 0.61	21.0 ± 5.96	2.46 ± 0.42	0.47 ± 0.26	250 ± 68
Lincomix ® 100	6	4.36 ± 1.12	16.0 ± 1.77	2.31 ± 0.09	0.67 ± 0.20	15.1 ± 11.6
Lincomix ® 300	6	4.86 ± 1.33	18.4 ± 0.99	1.84 ± 0.53	0.46 ± 0.26	47.6 ± 44.1
Lincomix ® 100	12	2.42 ± 0.73	5.83 ± 1.45	0.69 ± 0.24	0.15 ± 0.14	2.55 ± 0.73
Lincomix ® 300	12	2.46 ± 0.65	7.47 ± 2.28	0.64 ± 0.10	0.20 ± 0.12	7.12 ± 4.20
Lincomix ® 100	24	0.32 ± 0.14	0.91 ± 0.15	0.06 ± 0.02	0.02 ± 0.01 1 <LOQ	0.23 ± 0.14
Lincomix ® 300	24	0.55 ± 0.32	1.36 ± 0.45	0.08 ± 0.02	0.04 ± 0.03 1 <LOQ	1.27 ± 0.50
Lincomix ® 100	48	0.06 ± 0.02	0.17 ± 0.09	2 of 4 <LOD	4 <LOQ 1 <LOD	0.02 ± 0.01 1 <LOQ
Lincomix ® 300	48	0.06 ± 0.02	0.24 ± 0.17	0.01 ± 0.00	4 <LOQ 1 < LOD	0.02 ± 0.01 1 <LOQ
Lincomix ® 100	144	<LOQ	<LOQ	3 <LOD	4 <LOD	0.003 ± 0.00
Lincomix ® 300	144	<LOQ	<LOQ	3 <LOD	4 <LOD 3 <LOD	0.031 ± 0.05 3 <LOQ

Sheep

In a study conducted according to GLP, four groups of five sheep received daily intramuscular doses of lincomycin at 5 mg/kg of body weight (formulated with 10 mg/kg spectinomycin) for 3 days (Brown et al, 1996a, 1996b). The animals were killed 8 h and days 7, 14, and 21 after treatment, and liver, kidney, muscle, and injection site tissue were analysed by GC/MS. At 8 h after the last treatment, the mean concentrations of lincomycin were highest in kidney (9.0 mg/kg), liver (4.3 mg/kg), and at the site of injection (14 mg/kg); a lower mean concentration (0.95 mg/kg) was found in muscle. By day 7, only two of five samples of liver contained concentrations of residues that were above the LOQ of the method.

Chicken

Chicken broilers were treated orally via the drinking water with lincomycin /spectinomycin of doses of 1 g + 2 g/gallon for 7 days (Barbiers, Smith 1968). Tissue samples were taken at 0 (n = 2), 6, 12, 18 (n = 4 each) for liver, muscle and fat/skin, (n = 2) for kidney at 24 h and at 48 h (n = 4 each) for liver, muscle and fat/skin, (n = 2) for kidney. Samples were analysed by bio-assay. Lincomycin was detected at 0 h in one liver sample at 0.98 mg/kg and at 6 h in one kidney sample at 0.85 mg/kg. Respective serum samples showed only positive results at 0 h (0.18) and at 12 h (0.34) mg/kg.

EFFECT OF LINCOMYCIN ON STARTER CULTURES IN MILK PROCESSING

The effect of lincomycin on bacterial starter cultures was investigated in cultures used for the production of Italian cheese, yoghurt, butter milk and sour cream. For each starter culture, the four- parameter Weibull growth curve was used to model the pH as a function of time. At concentrations up to 0.16 mg/kg no significant effects were observed.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUE AND MILK

Various methods have been used to determine the concentrations of residues of lincomycin in animal-derived foods. The methods include microbiological assay, thin-layer chromatography-bioautography, GC with an alkaline flame detector, and GC/MS.

Screening test for milk

In AOAC Official Method 988.08 (AOAC International, 1995), lincomycin is detected in milk samples based on a binding reaction between incurred residue and binding sites on a substrate. The method uses a commercially available test (Charm Test) validated at 0.40 mg/kg for the detection of lincomycin residues in milk.

Microbiological Assays

In one of the early reports of analytical methods for lincomycin residues in milk, *Sarcina lutea* ATCC 9341 was used as the test organism, seeded on a test plate containing Antibiotic Medium 11 (Barbiers and Gosline, 1973). The pH of the milk was adjusted to 8.5 with NaOH (3.5 and 1.0 M) and a sample was added to a steel sample cylinder in contact with the test medium. Following incubation for 18 hrs at 32°C, the size of inhibition zone was measured and compared with standards. The limit of detection (LOD) was claimed to be 0.1 mg/kg. Subsequently, the same organism was used in a microbiological plate assay was used for screening for lincomycin residues in tissue samples, as well as in TLC-bioautography for confirmation (Barbiers and Neff, 1976). The samples were cleaned up using a XAD resin, then chromatographed on silica gel TLC plates. The developed plate was dried, then brought into contact with growth medium for 30 min, after which the TLC plate was removed. Following overnight incubation, lincomycin was identified by the presence of a zone of inhibition at a location corresponding to the R_f of lincomycin on the TLC plate. The method was tested for liver, kidney, muscle and skin/fat of poultry, calf, lamb and pig, with a LOD of 0.1 mg/kg.

More recently, lincomycin was extracted from milk using a C-18 SPE cartridge and quantified using *M. luteus* as a test organism for agar diffusion test plates at pH 8 (Deluyker et al, 1996a). Recovery was 70%, with a limit of quantitation (LOQ) of 0.025mg/kg, based on 6 replicate spiked samples at this level, and a LOD of 0.013 mg/kg. The analytical range tested was 0.025-0.80 mg/kg.

Chemical Methods

A method was reported in which regulatory samples of bovine and porcine kidney were analyzed by gas chromatography with nitrogen specific detection after an extensive clean-up which included an initial clean-up using a solid phase extraction cartridge, collection of a fraction from a high performance liquid chromatography separation, followed by solid phase extraction of the collected fraction (Farrington et al, 1987). The final extract was derivatized with a silylating reagent and analyzed by gas chromatography with an alkali flame detector operated in the nitrogen-specific mode. Recovery from bovine and porcine kidneys spiked at 0.1 mg/kg ranged between 31-51% and 38-64%, respectively. Four batches of each tissue were run on separate days, with 3 replicates per run. The LOD was estimated to be 0.02 mg/kg, with a linear analytical range from 0.02 to 0.20 mg/kg. The method was applied in a pilot survey to 54 samples. However, the length and complexity of the clean-up procedure would make this method rather difficult to apply on an on-going routine basis.

The method used by the sponsor in various residue depletion trials and proposed for regulatory use is based on a GC/MS procedure originally developed for lincomycin residues in human serum (Fourtillan et al, 1987). Following clean up by solid phase extraction and derivatization with acetic anhydride, lincomycin was detected as the m/z 126 fragment ion using GC/MS in the electron impact (EI) mode. The method was later validated for the analysis including pig fat, liver, kidney and muscle, chicken fat, liver, kidney, muscle and skin, and calf liver (Mignot et al, 1991).

The method was also adapted and applied to the analysis of pig liver, with the introduction of an acidic extraction step (Nappier and Hoffman, 1989). A correlation of >0.98 was obtained when the results of the GC/MS and microbiological assays were compared. In a later study, it was shown that the original method for lincomycin in serum could also be successfully applied to pig serum using an ion trap mass spectrometer, rather than a conventional quadrupole mass spectrometer (Hoffmann et al, 1990). Additional work demonstrated that the GC/MS and microbiological assays for lincomycin in pig plasma gave comparable results (Davis and Hamlow, 1990).

Subsequently, it was discovered during sample storage stability studies with freeze-thaw cycles that the recovery of incurred lincomycin residues from pig liver increased by over 2-fold when the sample was incubated at room temperature overnight (Nappier and Rizzo, 1995; Nappier et al, 1996b). Further study demonstrated that the analysis of pig kidney and muscle, as well as sheep liver and kidney, did not require the 18 h incubation required for pig liver to release residues (Nappier et al, 1996c; DeYoung et al, 1996a). Results obtained with and without the incubation step for these tissues agreed within 15%.

The original tissue version of the method was modified to require a smaller sample size, to include the incubation step for pig liver and to provide requirements for confirmatory analysis (Nappier et al, 1995). After an extensive sample clean-up, a 1µl aliquot is injected into the GC/MS at an oven temperature of 175°C, held for 0.5 min., then ramped at 30 °C/min to 325 °C and held there for 5 min. Total run time is 10 min, with injection port and transfer line temperatures at 300 °C and helium flow rate of 1 mL/min. A 30m x 0.25mm id containing a cross-linked methyl silicone coating, 0.33 µm film thickness, was used for the analysis, in combination with a 5m x 0.25mm id pre-column. Assay precision was similar for analysis of duplicates of both fortified and incurred samples (11.2-12.1% from 0.078 to 0.321mg/kg for liver and 9.6-11.9% from 0.115 to 0.616 mg/kg for kidney). The confirmatory procedure for residues in pig liver uses the molecular ion fragment m/z 575 and three fragments at m/z 126, 515 and 527 in chemical ionization mode, using methane as the reactant gas. Confirmation required a retention time within 2% of the mean standard retention times, relative ion intensities within 10% of the mean standard relative ion intensities and a signal-to-noise ratio of >3 for at least 3 ions.

In a method comparison, 19 pig kidney and 17 pig liver samples previously assayed by GC/MS were also analysed by the cylinder plate assay method, with results from the two methods correlated at 0.95 for both liver and kidney analyses (Nappier et al, 1996f).

The determinative method was also validated for sheep liver (Gammill et al, 1995), sheep kidney (DeYoung et al, 1995), sheep muscle (DeYoung et al, 1996b), chicken liver (DeYoung et al, 1997a), chicken kidney (DeYoung et al, 1997b), chicken gizzards (DeYoung et al, 1997c) and chicken muscle (DeYoung et al, 1997d). In these more recent studies, the capillary column used in the GC/MS analysis is a 12.5m x 0.20mm id column coated with an 0.33 µm film thickness of cross-linked methyl silicone.

The method was modified for the analysis of fat by including an initial extraction of the 3 g analytical sample with chloroform: hexane (DeYoung et al, 1996c,d) and also applied to the analysis of chicken fat (DeYoung et al, 1997e). The modification was also shown to be necessary for the analysis of bovine fat, but bovine liver, kidney, muscle and milk could be analyzed without the inclusion of the initial organic extraction (Nappier et al, 1997a). A method has also been tested for the analysis of lincomycin in eggs, but a full validation report was not available (Nappier to Thomas, 1998). The results of the validation tests for various matrices have been summarised in Table 8 (Nappier, 1998).

Table 8. Summary of validation study results for analysis of lincomycin residues by electron impact gas chromatography – mass spectrometry (GC/MS-EI) in various edible tissues and milk.

Species	Edible Tissue or Milk	Limit of Detection (mg/kg)	Limit of Quantitation (mg/kg)	Mean Recovery (%)	Repeatability (%)	Reproducibility (%)
Bovine	Milk	0.008	0.015	102	2.9	9.6
	Muscle	0.015	0.015	90	5.0	11.2
	Liver	0.005	0.015	93	8.0	9.9
	Kidney	0.007	0.015	96	5.5	12.7
	Fat	0.005	0.015	97	5.9	6.9
Pig	Muscle	0.002	0.017	96	3.8	4.8
	Liver	0.03	0.06	97	5.7*	11.7
	Kidney	0.03	0.06	91	5.2	12.0
	Fat	0.005	0.017	85	7.1*	10.0
Chicken	Muscle	0.003	0.017	85	5.8*	8.2
	Liver	0.003	0.017	92	5.6*	9.1
	Kidney	0.003	0.017	85	5.5*	9.4
	Fat	0.002	0.017	93	7.3*	9.9
Sheep	Muscle	0.002	0.017	93	3.1*	4.1
	Liver	0.003	0.017	84	5.2*	6.7
	Kidney	0.002	0.017	85	2.8*	5.0

*Based on analysis of variance techniques applied to data from referenced technical reports.

MAXIMUM RESIDUE LIMITS

In recommending MRLs, the Committee took into account the following:

- An ADI of 0-0.03 μg/kg of body weight was recommended by the Committee on the basis of a microbiological end-point, which results in a maximum daily intake of 1.8 mg for a 60-kg person.
- In pig tissues, lincomycin is the major component with significant microbiological activity.
- In milk, lincomycin accounts for 90% of the total residues.
- There was insufficient evidence that lincomycin is the major component with significant microbiological activity in tissues of cattle, sheep, and chicken and in chicken eggs.
- Lincomycin is the marker residue.
- Kidney and liver contain the highest concentrations of residues.
- A validated GC/MS method is available which could be used routinely in many laboratories and has a LOQ of 0.02-0.06 mg/kg in tissues of pigs, cattle, calves, sheep, and chicken.
- 0.16 mg/kg is the concentration below which lincomycin has no effect on bacterial starter cultures in the production of milk products.
- Lincomycin was considered as a drug with a long history of use.

On the basis of the above considerations, the Committee recommended the following MRLs for lincomycin in edible tissues of pigs and in cows' milk, expressed as parent drug : muscle,0.1 mg/kg, liver,0.5 mg/kg, kidney.1.5 mg/kg, fat,0.1 mg/kg and milk of dairy cattle, 0.15 mg/kg. Considering its old drug policy, the Committee extended the same MRLs as temporary for muscle, liver, kidney and fat in cattle, calves, sheep and chicken. The Committee was unable to recommend MRLs for chicken eggs.

Using the conservative consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, and 50 g of fat and 1.5 kg milk, the theoretical maximum intake of lincomycin residues would be 0.385 mg.

Before reviewing the compound again, the Committee would wish to receive the following information by 2002:

1. Comparable data as have been provided for tissues of pigs, which show that lincomycin is the major component with significant microbiological activity in tissues of cattle, calves, sheep, chicken and in chicken eggs.
2. Residue depletion study in chicken eggs using the GC/MS method.

REFERENCES

AOAC International. (1995) AOAC International Official Method 988.08: Antimicrobial Drugs in Milk. Microbial Receptor Assay. AOAC Official Methods of Analysis, 16th Edition, AOAC International, Gaithersburg, MD, Chapter 33, pp. 42-43.

Barbiers, A.R. and Gosline, R.E (1973). An assay for fincomycin in milk sensitive to 0. 1 ppm. Upjohn Interoffice Memo to AW Neff, 2 August 1973

Barbiers, A.R. and Kleckner, M.D. (1964) Lincomycin serum levels in pigs following intramuscular and oral administration of 2 and 5 mg/kgb. The Upjohn Company Interoffice Memo. 13 July 1964.

Barbiers, A.R. and Kleckner, M.D. (1964) Serum levels and tissue residues of lincomycin in pigs following treatment for three consecutive days, intramuscularly and orally. The Upjohn Company Interoffice Memo, 17 September 1964.

Barbiers, A.R., Krzeminski, L.F., Perrel, P.N., Schiltz, R.A., Bell, R.L., Lallinger, A.J., Cox, B.L., Smith, L.J. and Subacz, C.J. (1971) Persistence study in lactating dairy heifers following intramammary infusion with Lincocin Forte®. Upjohn Technical Report 712-9760-9, 9 December 1971.

Barbiers, A.R. and Neff, A.W. (1976) Screening and confirmatory methods for determining lincomycin residues in animal tissues. J. of the AOAC 1976; 59; 849-54.

Barbiers, A.R. and Smith, L.J. (1968) Lincomycin broiler tissue residues following oral spectinomycin/lincomycin via drinking water. Upjohn Technical Report 723-9760-13, 21 November 1968.

Barbiers, A.R., Smith, L.J., Swenson, G.H. and Lallinger, A.J. (1975) Distribution of lincomycin in tissues and body fluids in pig following intramuscular administration. Upjohn Technical Report 003-9760-8. 30 January 1975.

Brown, S.A., Johnson, R.A., Craigmill, A.L. and Wetzlich, S. (1996a) LINCO-SPECTIN™ Sterile Solution in Sheep. Part IV. Method validation and residue decline of lincomycin in sheep liver after multiple intramuscular administration at a dose of 15 mg/kg (5 mg lincomycin and 10 mg spectinomycin/kg). Upjohn Technical Report 803-7926-95-003, 12 January 1996.

Brown, S.A., Johnson, R.A., Craigmill, A.L. and Wetzlich, S. (1996b) LINCO-SPECTIN™ Sterile Solution in Sheep. Part V. Method validation and residue decline of lincomycin in sheep liver after multiple intramuscular administration at a dose of 15 mg/kg (5 mg lincomycin and 10 mg spectinomycin/kg). Upjohn Technical Report 803-7926-96-001, 14 February 1996.

Daniels, E.G. and VanEyk, R.L. (1978) Metabolism of lincomycin hydrochloride in dogs. Excretion of radioactivity and characterisation of urinary and faecal metabolites. Upjohn Technical Report 7261-78-7261-008. 1 November 1978.

Davis, W.M. and Balcolm III, J.H. (1969). Lincomycin studies on drug absorption and efficacy. Oral surgery,1969. 27 (5): 688 -96.

Davis, R.A. and Hamlow, P.J. (1990). Comparison of microbiological and instrumental assays for the determination of lincomycin and spectinomycin in pig plasma. Research Report 803-9760-90-002, Sponsor Submitted.

DeGeeter, M.J. and Barbiers, A.R. (1978) Lincomycin residues in tissue and serum from pig fed diets fortified with O or 44 mg lincomycin per kilogram. Upjohn Technical Report 524-78-9660-016. 19 October 1978.

DeGraeve, J., Van Heugen, I-C, Nappier, J.L. and Deluyker, R.A. (1997) Tissue residue depletion study of UNCOCIN® FORTE Sterile following intramammary infusions to dairy cows. Part II - lincomycin assay validation, and lincomycin residues. Pharmacia & Upjohn Technical Report 804-7926-97-00 1, 11 February 1997.

Deluyker, H.A., Nouws, J.F.M., Van Oye, S. and Gilberston, T.J. (1996a) Concentrations of neomycin and lincomycin in milk during and following three successive intramammary infusions of Lincocin Forte ® in normal lactating cows. Upjohn Technical Report 804-7926-95-001, 23. January 1996.

Deluyker, H.A., VanHeugen, J.C., DeGraeve, J. and Nappier, J.L. (1996b) Tolerance, kinetics, and milk residue depletion study of LINCOCIN FORTE™ Sterile following intramammary infusions to dairy cows - Part II Pharmacia & Upjohn Technical Report 804-7926-96005, 18 December 1996.

DeYoung, L.A., Grostic, M.F., and Johnson, R.A. (1995). Method Validation Report: GC/MS assay for lincomycin in sheep kidney tissue. Research Report 95-550, Sponsor Submitted.

DeYoung, L.A., Johnson, R.A., Nappier, J.L., and Brown, S.A. (1996a). Method validation report addendum: tissue incubation and parallelism results. Research Report 803-7926-96-003, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A., Nappier, J.L. (1996b). Method validation report: GC/MS-EI assay for lincomycin in sheep muscle tissue. Research Report 768-7926-96-002, Sponsor Submitted.

DeYoung, L.A., Gammill, J.C., Johnson, R.A., Johnson, K.M., and Grostic, M.F. (1996c). GC/MS assay for lincomycin free base in pig fat tissue. Research Report 96-670, Sponsor Submitted.

DeYoung, L.A., Johnson, R.A., Nappier, J.L. and Rizzo, V.L. (1996d). Method validation report: GC/MS-EI assay for lincomycin in pig fat tissue. Research Report 768-7926-96-009, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997a). Method validation report: GC/MS-EI assay for lincomycin in chicken liver tissue. Research Report 768-7926-97-002, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997b). Method validation report: GC/MS-EI assay for lincomycin in chicken kidney tissue. Research Report 768-7926-97-003, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997c). Method validation report: GC/MS-EI assay for lincomycin in chicken gizzard tissue. Research Report 768-7926-97-004, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997d). Method validation report: GC/MS-EI assay for lincomycin in chicken muscle tissue. Research Report 768-7926-97-005, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997e). Method validation report: GC/MS-EI assay for lincomycin in chicken fat tissue. Research Report 768-7926-97-008, Sponsor Submitted.

Eberts, F.S., Meeks, R.C. and Vliek, R.W. (1963) Status report: Metabolism of ^{3}H-Lincomycin in the dog. The Upjohn Company Interoffice Memo, 17. 26 September 1963.

Farrington, W.H.H., Cass, S.D., Patey, A.L. and Shearer, G. (1987) A method for the analysis of lincomycin in porcine and bovine kidney. Food Add. and Contam. 1: 67-76.

Fourtillan, J.B., Mignot, A., Lefebvre, M.A., Girault, J., Parsy, P., Senon, J., and Ingrand, I. (1987) Assay of lincomycin-A in plasma samples by combined gas chromatography – electron impact mass spectrometry. Research Report CE572H, Sponsor Submitted.

Gammill, J.C., DeYoung, L.A., Grostic, M.F. and Johnson, R.A. (1995) Method Validation Report: GC/MS assay for lincomycin in sheep liver tissue. Research Report 95-520, Sponsor Submitted.

Gosline, R.E., Barbiers, A.R., Subacz, C.J. and Smith, L.J. (1978) ^{14}C-Lincomycin (U-10, 149A) fate study in chickens. Upjohn Technical Report 768-78-9760-001, 26 April 1978.

Gosline, R.E. and Hornish, R.E. (1980). The disposition of ^{14}C-Lincomycin HCl (U-10,149A) in laying hens. Upjohn Technical Report 536-9760-80-001, 29 April 1980.

Hoffman, G.A., Nappier, J.L., Hanson, B.J., and Gilbertson, T.J. (1990). Assay validation of a gas chromatography/mass spectrometry – ion trap detection procedure for the determination of lincomycin (U-10,149A) as the anhydrous free base in the plasma of pig. Research Report 768-9760-90-002, Sponsor Submitted.

Hoffman, G.A., Delahaut, P., DeGraeve, J., Brown, S.A., Gilbertson, T.J. and Lens, S.T. (1996) Lincomycin residues in the tissues of calves at various times after multiple injections of LINCO-SPECTIN™ Sterile Solution at a dose rate of 15 mg per kg body weight (5 mg lincomycin + 10 mg spectinomycin/kg). Upjohn Technical Report X803-7926-95004, 29 January 1996.

Hornish, R.E., Arnold, T.S., Cox, T.D., Hubbard, V.I., Nappier, J.M., Pierce, P.A., Reeves, D.R., Stahl, G.I., Subacz, C.J. and Zaya, M.J. (1987) Lincomycin residues in the tissues of pig treated with ^{14}C-lincomycin hydrochloride by feed additive at 20,40, 100, and 200 grams per ton. Upjohn Technical Report 768-9760-87-001, 6 August 1987.

Hornish, R.E. and Gosline, R.E. (1981) Metabolism of ^{14}C-lincomycin fed at 200 g/ton in pig. Part I - Accountability, distribution and depletion of ^{14}C in tissue. Upjohn Technical Report 768-9760-81-001. 27 January 1981.

Hornish, R.E., Gosline, R.E., Nappier, J.M., Butine, T.J. and Subacz, C.J. (1984) The metabolism and depletion of 14C-Lincomycin (U-10,149A) in broiler chickens exposed at 128 mg/gal in the drinking water. Part I. Upjohn Technical Report 783-9760-84-001, 14 February 1984.

Hornish, R.E., Gosline, R.E., Arnold, T.S., Butine, T.J., Smith, L.J. and Subacz, C.J. (1985) Pharmacokinetics and oral absorption of lincomycin (U-10, 149) in pig. Upjohn Technical Report 002-9760-85-002. 25 September 1985.

Hornish, R.E., Gosline, R.E. and Nappier, J.M. (1987) Comparative metabolism of lincomycin in the Pig, Chicken and Rat. Drug Metabolism Reviews, 1987. 18 (2&3): 177-214.

Kaplan K, Chew, W.H. and Weinstein, L. (1965) Microbiological pharmacological and clinical studies of lincomycin. Am. J. of Med. Sci., 1965, 250:137-146.

Mignot, A., LeFebvre, M.A., Parsy, P., Renoux, A. and Gilbertson, T.J. (1991). Validation of a GC/MS assay for lincomycin with limit of detection of 0.04 mg/kg. Research Report 768-7926-91-001, Sponsor Submitted.

Nappier, J.L. and Hoffman, G.A. (1989) The determination of lincomycin in the liver of intramuscularly and orally treated pig by GC/MS analysis. Research Report 768-9760-89-001, Sponsor Submitted.

Nappier, J.L., Hoffman, G.A., Arnold, T.S., Cox, T.D., Reeves, D.R., Flook, T.F. and Paulissen, J.B. (1988). Total residues of ^{14}C-lincomycin in the tissues of intramuscularly treated pig. Upjohn Technical Report 768-9760-88-001, 8 September 1988.

Nappier, J.L., Hoffman, G.A., Yein, F.S., Zaya, M.J. and Paulissen, J.B. (1989) The determination of lincomycin in the liver of intramuscularly and orally treated pig by a modified GC/MS procedure: Validation, correlation and analysis. Pharmacia and Upjohn Technical Report 768-9760-89-002, 23 June 1989.

Nappier, J.L. and Rizzo, V.L. (1995) Determination of the appropriate conditions for the improved extraction of lincomycin from treated swine liver. Research Report 768-9760-89-001. Sponsor submitted.

Nappier, J.L., Rizzo, V.L., Arnold, T.S., Janose, R.l., Cox, T.D., Flook, T.F., Lewis, V.R., and Hanson, B.J. (1995). Validation of two methods of analysis of lincomycin hydrochloride residue in pig by GC/MS. A determinative method in pig liver and kidney and a confirmatory method in pig liver. Research Report 768-7926-95-001, Sponsor Submitted.

Nappier, J.L., Hanson, B.J., DeYoung, L.A. and Johnson, R.A. (1996a) The determination of the residue decline of lincomycin in the muscle tissue of pig treated with an intramuscular injection of lincomycin hydrochloride (U-10149A) at 11 mg of lincomycin free base equivalents per kg of body weight. Pharmacia & Upjohn Technical Report 768-7926--96-004, 23 October 1996.

Nappier, J.L., Rizzo, V.L., Fate, G.D., and Benner, C.P. (1996b). The discovery and resolution of the incomplete extraction of antibiotic residues from the liver of treated pig. Proceedings of the Euroresidue III Conference, Veldhoven, the Netherlands, pp. 740-744.

Nappier, J.L., Rizzo, V.L., Staskiewicz, S.G., DeYoung, L.A., and Johnson, R.A. (1996c). Evaluation of the need for incubation of pig and sheep tissues in the residue analysis of lincomycin. Research Report 768-7926-96-006, Sponsor Submitted.

Nappier, J.L., Rizzo, V.L., Staskiewicz, S.G., Stafford, L.K., Arnold, T.S., Janose, R.L., Lewis, V.R., Cox, T.D., Flook, T.F. and Hanson, B.J. (1996d) The determination of the residue decline of lincomycin in the liver and kidney tissues of pig treated with an intramuscular injection of lincomycin hydrochloride (U-10,149A) at 11 mg of lincomycin free base equivalents per kg of body weight. Upjohn Technical Report 768-7926-95-004, 13 February 1996.

Nappier, J.L., Rizzo, V.L., Staskiewicz, S.G., Stafford, L.K., Arnold, T.S., Janose, R.L., Lewis, V.R., Cox, T.D., Flook, T.F. and Hanson, B.J. (1996e) The determination of the residue decline of lincomycin in the liver and kidney tissues of pig treated with lincomycin hydrochloride (U-10,149A) at 44, 110, or 220 grams of lincomycin free base equivalents per 1000 kg of feed. Upjohn Technical Report 768-7926-95-003, 13 February 1996.

Nappier, J.L., Stafford, L.K.E., Rizzo, V.L., Staskiewicz, S.G., DeYoung, L.A., Johnson, R.A. and Fate. G.D. (1996f) Correlation of the lincomycin liver and kidney tissue residue assays: GC/MS assay vs microbiological assay. Pharmacia & Upjohn Technical Report 768-7926-96-007, 3 September 1996.

Nappier, J.L., DeGraeve, J, Van Heugen, J.C. and Deluyker, H.A. (1997a). Lincomycin bovine feasibility study: selection of an appropriate analytical assay for the determination of lincomycin residues in milk and animal tissue. Research Report 804-7926-97-002, Sponsor Submitted.

Nappier, J.L., Hanson, B.J., DeYoung, L.A. and Johnson, R.A. (1997b). The determination of the residue decline of lincomycin in the injection site tissues of pig treated with an intramuscular injection of lincomycin hydrochloride (U- 10 149A) at 11 mg of lincomycin free base equivalents per kg of body weight. Pharmacia & Upjohn Technical Report 768-7926-96-008, 12 May 1997.

Nappier, J.L., Hanson, B.J., DeYoung, L.A. and Johnson, R.A. (1997c) The determination of the residue decline of lincomycin in the fat tissue of pig treated with an intramuscular injection of lincomycin hydrochloride (U- 10149A) at I I mg of lincomycin free base equivalents per kg of body weight. Pharmacia & Upjohn Technical Report 768-7926-96-011, 13 May 1997.

Nappier, J.L. (1998). Additional clarification of lincomycin MRL residue file. Pharmacia & Upjohn Interoffice Memo, 15 June 1998.

Nappier, J.L. (1998). A summary of the GC/MS assay procedures for the analysis of lincomycin in biological matrices. Research Report 768-7926-97-001, Sponsor Submitted.

Nappier, J.L. to Thomas, R.W. (1998) Lincomycin GC/MS egg assay. Interoffice memo, August 25 1998, Sponsor Submitted.

Nappier, J.L., Brown, S.A., Craigmill, A.L., Wetlich, S. (1998) LINCO-SPECTIN Sterile Solution in sheep. Part VI. Residue decline in sheep muscle and injection site after multiple intramuscular administrations at a dose of 15 mg/kg (5 mg lincomycin and 10 mg spectinomycin/kg). Pharmacia & Upjohn Study Report aOO 18987, 5 October 1998.

Nouws, J.F.M., Deluyker, H.A., Gilbertson, T.J. and Chester, S.T. (1994) Concentration of neomycin and lincomycin during and following three successive intramammary infusions of Lincocin Forte ® in normal lactating dairy cows. Upjohn Technical Report 804-7926-94-001, 3 August 1994.

Russel, C.A. (1979) Production of serum time curve in the pig following a single intramuscular injection of Lincocin ® Sterile Solution (100 mg/mL). Upjohn Technical Report 777-0008-79-001. April 1979.

Weber, D.J., Barbiers, A.R. and Lallinger, A.J. (1981) Pharmacokinetics of lincomycin in bovine following intravenous and intramammary doses of Lincocin ®. Upjohn Technical Report 7256-81-7256-001. 24 June 1981.