

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

LINCOMYCIN

SUMMARY REPORT (1)

1. Lincomycin is an antibiotic derived from *Streptomyces lincolnensis*. It belongs to the lincosamide group which also includes pirlimycin and clindamycin. In veterinary medicine, it is used in mono-preparations and in combination product with other antibiotics such as spectinomycin, sulfadimidine and gentamicin. It is administered to poultry orally, sometimes in the feed or drinking water, at doses equivalent to up to 50 mg/kg bw/day for up to 7 days. It is administered to swine orally, at doses of up to 10 mg/kg bw/day for up to 21 days, or intramuscularly at a dose of 15 mg/kg bw/day for up to 7 days. In calves and sheep, it is administered intramuscularly at doses of up to 15 mg/kg bw/day for up to 4 days. There is also a preparation containing lincomycin, neomycin and methylprednisolone for intramammary administration to dairy cattle; 1 tube (200 mg lincomycin) is administered per teat. Most of the safety studies used either "premix" grade lincomycin or lincomycin complying with the specification in the US Pharmacopoeia. The grades differed in their lincomycin factor B content, up to 5% in the case of the USP grade and up to 10% in the "premix" grade. It was considered that the differences in specification would not result in significant differences in toxicity.

In humans, the usual dosage is 500 mg to 2 g lincomycin, orally per day for 7 to 10 days. It may also be administered by intramuscular injection, at doses of 600 to 1800 mg/person/day. Lincomycin has been largely replaced by clindamycin in human therapy.

2. Lincomycin is active mainly against Gram-positive bacteria. Lincomycin exerts its antibacterial action by inhibiting RNA-dependent protein synthesis by acting on the 50S subunit of the ribosome. No classical pharmacological studies in laboratory animals were provided. It was considered that such studies were not needed for lincomycin.
3. Peak serum concentrations of 0.6 to 0.7 mg/ml bw were achieved after oral administration of 0.5 g to humans; the dose was given after a meal. Higher concentrations (1.8 to 1.4 mg/ml) were achieved in fasted individuals. About 4 to 7% of the dose was excreted as unmetabolised lincomycin, in the urine, within 24 hours. Around 40% of the administered dose was recovered from faeces. The oral bioavailability was estimated to be 25 to 50% in humans. A peak plasma concentration of 4.5 mg/ml was achieved 4 hours after oral administration of ³H-lincomycin to a Beagle dog; 14% was excreted in the urine and 77% in the faeces with over 80% excretion occurring within 48 hours. In humans and laboratory animals, excretion was predominantly via the faeces. The main component of the urine following oral and intramuscular administration to humans and dogs and intravenous administration to rats was unchanged lincomycin. When rats were dosed orally in the drinking water, the primary urinary metabolite was lincomycin sulphoxide. Compounds in the faeces of rats dosed intravenously (dose not stated) consisted of 40% lincomycin and 60% unidentified metabolites. In the target species, metabolism was primarily by S oxidation to the sulphoxide or de-methylation to the N-desmethyl derivative followed by conversion of both metabolites to N-desmethyl-lincomycin-sulphoxide.

¹ Correction introduced in July 2008 in paragraph 22 concerning sacrifice times, replacing days by hours.

4. Lincomycin was shown to be of low acute toxicity. In rats and mice, the acute oral LD₅₀ of different pharmacopoeial and premix grades of lincomycin hydrochloride was always greater than 5000 mg/kg bw. Signs of toxicity observed at doses of 8000 mg/kg bw and above included prostration, depression, diarrhoea and convulsions. The substance was more toxic when administered parenterally with acute intravenous LD₅₀ values of 214 and 342 mg/kg bw in mice and rats, respectively. The acute subcutaneous LD₅₀ was considerably lower in newborn rats compared with adult rats (783 mg/kg bw versus 9778 mg/kg bw).
5. Several repeated-dose toxicity studies were carried out in rats during the 1960s. There was no chemical analysis of the dosing suspensions and no monitoring of clinical chemistry values in any of these studies. Groups of Wistar rats were given daily oral doses of 0, 30, 100 or 300 mg/kg bw/day by gavage for 3 months. There were no treatment-related effects on body weight gain, food conversion ratios, haematology values (measured only at termination) or pathological findings. The NOEL for this study was therefore greater than 300 mg/kg bw. A second 3-month study used oral doses of 0, 600 and 1000 mg/kg bw/day. The weight of the intestines was significantly increased in both treated groups in comparison with the controls but there were no corresponding pathological findings. In a one-year study, groups of Wistar rats were given daily oral doses of 0, 30, 100 or 300 mg/kg bw/day. Treated rats gained more weight than the controls. There were no changes in haematology values or pathological findings attributable to treatment. The NOEL for this study was therefore greater than 300 mg/kg bw/day.

Groups of B6C3F1 mice were fed diets containing the equivalent of 0, 10, 30, 100, 300 or 3000 mg/kg bw/day for 90 days. At 3000 mg/kg bw, there was a significant reduction in body weight gain, an increase in food consumption and in females, an increase in serum corticosterone concentrations. Serum glucose concentrations were significantly reduced in both sexes given 300 and 3000 mg/kg bw. There was a trend towards increased intestinal weight. In the 300 and 3000 mg/kg bw groups, there was an increase in luminal distension and dilatation of the small and large intestines. The complete data set were not provided for this study. The NOEL was 100 mg/kg bw/day.

Several repeated-dose toxicity studies which carried out in Beagle dogs during the 1960s were poorly conducted and reported. Groups of 2/sex/dose dogs were given daily oral doses of 0, 400 or 800 mg/kg bw/day for 3 months. Transient increases were observed in some transaminase activities in treated groups but this did not correlate with any gross or microscopic pathological changes at termination of the study. Daily oral doses of 0, 30, 100 or 300 mg/kg bw/day were administered to groups of 2/sex/dose dogs in a 6-month study. Adrenal weights were significantly increased in the 300 mg/kg bw group but there were no corresponding histopathological changes in the adrenals. Two dogs receiving 300 mg/kg bw had bilateral lymphocytic thyroiditis. The NOEL was 100 mg/kg bw/day.

6. Lincomycin was shown to be well tolerated when administered at the recommended doses to the target species.
7. In a 3-generation reproduction study, groups of Sprague-Dawley rats were fed diets containing lincomycin hydrochloride, premix grade, at concentrations calculated to provide 0, 0.375, 0.75 or 1.5 mg/kg bw/day. Two further groups were given feed containing 1.5 or 100 mg/kg bw/day of US Pharmacopoeia grade lincomycin. There were no adverse effects on any parameters at any dose level. In a 2-generation reproduction study, groups of Sprague-Dawley rats were given daily oral (gavage) doses of 0, 100, 300 or 1000 mg/kg bw/day of lincomycin hydrochloride, agricultural grade. The fertility index for F0 females was reduced in comparison with the controls though the fertility index for F1 females was unaffected by treatment. A NOEL of 300 mg/kg bw/day was retained for this study.

8. In a study of teratogenic potential, lincomycin was administered as a single subcutaneous injection of 300 mg/kg bw to female Sprague-Dawley rats. Groups of 4 or 5 pregnant rats were treated on one specified day between days 7 and 16 of gestation. Although there did not appear to be any evidence of teratogenicity, no overall conclusions could be drawn due to the inadequate study design. In another study, groups of 24 female Sprague-Dawley rats were given daily oral doses of 0, 10, 30 or 100 mg/kg bw/day from days 6 to 15 of gestation. There was no evidence of teratogenicity at any dose level. At 100 mg/kg bw, the incidence of resorptions was statistically significantly increased and there was a corresponding decrease in the numbers of live fetuses. The NOEL for foetotoxicity in this study was 30 mg/kg bw/day.

Eleven pregnant Beagle dogs were given daily intramuscular injections of 50 mg/kg bw lincomycin throughout gestation, beginning 2 or 3 days after mating. Six control dogs were injected with the vehicle (saline). The dams were allowed to deliver naturally and the pups were examined for external malformations when they were 2 weeks old. There was no evidence of teratogenicity. Due to the study design, no conclusions could be drawn regarding a NOEL for foetotoxicity.

9. Lincomycin gave negative results in:

- two *in vitro* assays for gene mutation in *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537 and TA1538)
- two assays for gene mutation in Chinese hamster V79 cells
- a DNA/alkaline elution assay using Chinese Hamster lung fibroblasts
- two *in vitro* unscheduled DNA synthesis (UDS) assays in primary rat hepatocytes (a third assay gave a positive result which probably arose from inadequate methodology)
- two *in vivo* micronucleus tests (one in rats, one in mice)
- an *in vivo* sex-linked recessive lethal assay in *Drosophila melanogaster*

It was concluded that lincomycin was not genotoxic.

10. In a combined chronic toxicity and carcinogenicity study, groups of 60/sex/dose Sprague-Dawley rats were fed diets equivalent to oral doses of 0, 0.375, 0.75 or 1.5 mg/kg bw/day of lincomycin hydrochloride premix grade. Further groups were fed diets containing 1.5 or 100 mg/kg bw/day of US Pharmacopoeia grade lincomycin. The rats were selected from the offspring of a multigeneration study and had been exposed to the same concentrations of the substance in utero. The study was terminated when the mortality in the control groups reached 80%, after 25 to 26 months. There were no treatment-related effects on ophthalmoscopy, haematology, clinical chemistry or urinalysis values. There appeared to be significant increases in the incidence of acute prostatitis in the 1.5 mg/kg bw premix-grade (40 out of 60 examined) and the 100 mg/kg bw US Pharmacopoeia grade (31 out of 59 examined) groups compared with the controls (21 out of 59 examined). The incidence of thyroid C-cell hyperplasia was increased in treated groups in comparison with the controls but the increase was not dose-related. Due to problems with the study designs, including the use of different grades of lincomycin and the fact that not all groups were given a full histopathological examination, it was not possible to draw any conclusions regarding NOELs. Despite the limitations of the study, it was concluded that there was no evidence of carcinogenicity.
11. Guinea pigs were given subcutaneous doses of 30, 75 or 300 mg/kg bw on alternate days. Over the 2-week period of the study all the animals died except for one given 30 mg/kg bw. Because of this excessive mortality, no evaluation of the contact sensitisation potential in guinea pigs was possible.
12. In a special study to investigate potential ototoxicity, lincomycin hydrochloride was given by intramuscular injection to groups of cats at doses of 0, 30 or 60 mg/kg bw/day for 2½ months. Cochlear and vestibular functions of the eighth cranial nerve were evaluated twice weekly, before and during treatment and for 5 weeks after the end of treatment. There was no evidence of eighth cranial nerve damage.
13. A toxicological ADI of 300 µg/kg bw/day was calculated by applying a safety factor of 100 to the NOEL of 30 mg/kg bw/day for foetotoxicity in the rat.

14. In vitro minimum inhibitory concentration (MIC) values were obtained for a range of micro-organisms representative of those in the human gut. The most sensitive, predominant species was *Fusobacterium*, with MIC₅₀ values of 0.2 to 0.4 µg/ml. In a study involving 5.5% of the larger (more than 100 beds) acute care hospitals in the USA between 1971 and 1983, reported that the pattern of susceptibility to lincosamides of Gram-positive aerobic and anaerobic bacteria had changed little over the survey period. Furthermore, many anaerobic populations showed a higher percentage of susceptibility in the most recent year of the survey. In addition, most of the more than 1100 strains of coagulase-positive *Staphylococci* isolated from cattle, pigs and poultry from 1970 to 1980 were sensitive to lincomycin, and the percentage of sensitive isolates were similar in 1980 to 1970. When lincomycin was introduced into the rumens of five cattle at concentrations of 0.9 ± 0.3 µg/ml, there were no detectable changes on the numbers of anaerobic bacteria, numbers of aerobic bacteria, numbers of spore-forming bacteria, numbers of protozoa, ruminal pH, and production of bacterial fermentation acids. Lincomycin was not tested at higher concentrations *in vivo* and precise MIC₅₀-values for the predominant strains were not determined. In a hamster model of antibiotic-associated colitis, a NOEL of 0.1 mg lincomycin/kg bw subcutaneously was identified. The route of administration used prevents the direct establishment of an ADI from these data, but indicates that the effects of lincomycin on gut bacteria *in vivo* are considerably less than *in vitro*. Similarly, in a study to investigate the effects of lincomycin on faecal *Salmonella thyphimurium*-shedding in pigs, an oral dose of 14 mg/kg bw for 53 days, produced no effect on *Salmonella thyphimurium* shedding compared to controls. Again, limitations in the study prevented the direct derivation of an ADI, but provided further support for adopting a higher CF2.

A microbiological ADI was calculated as follows:

$$\text{ADI} = \frac{\text{MIC}_{50} \text{ most sensitive organism} \times \text{CF2}}{\text{CF1}} \frac{(\mu\text{g/ml}) \times \text{daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}} \quad (\mu\text{g/kg bw})$$

Based on the above formula, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{0.2 \times 10}{1} \times 150 = \frac{300}{0.5 \times 60} = 10 \mu\text{g/kg bw i.e.} = 600 \mu\text{g/person}$$

The following assumptions were made:

- CF1 = 1 the most sensitive, predominant species was used, and evidence was provided that emergence of resistance is neither common nor rapid,
- CF2 = 10 to account for the evidence from *in vivo* studies that lincomycin is less potent under *in vivo* conditions compared with *in vitro* conditions,
- 150 g was the weight of the daily faecal bolus,
- oral bioavailability in humans is around 25 to 52%. A factor of 0.5 represents the extreme range of dose available to microorganisms.

In addition to the parent compound lincomycin, around 16 metabolites have been detected. Of these metabolites, three have been identified as being lincomycin sulphoxide, N-desmethyl lincomycin and N-desmethyl lincomycin sulphoxide. Compared to the parent compound none were found to have had any significant antimicrobiological activity. Both N-desmethyl and lincomycin sulphoxide have 15 to 100 times less antimicrobiological activity than the parent lincomycin. There was no evidence that the remaining metabolites, have any antimicrobiological activity.

15. The effect of the presence of lincomycin in milk on the performance of bacterial starter cultures used in the manufacture of Italian cheese, yoghurt and buttermilk/sour cream was investigated. For each starter culture, the four-parameter Weibull growth curve was used to model the pH as a function of time. No significant effects were observed at concentrations up to 160 µg/l.

16. The reported adverse effects include hypersensitivity reactions and diarrhoea. Pseudomembraneous colitis is most common after oral administration of lincomycin and occurs in 1 to 10% of patients.

In a published study, 302 human patients with cervicitis or vaginitis, approximately 100 in each trimester of pregnancy, were treated orally with 2 g lincomycin per day for 7 days. The offspring were compared with a group of 559 babies delivered at the same time and were followed up for 7 years after birth. No adverse effects attributable to treatment of the mothers were identified.

17. The following pharmacokinetic studies were carried out in target species:

In chickens (1/sex/time point) given twice daily oral boluses of ¹⁴C-lincomycin (simulating a dietary dose of 1 mg/bird/day) for 35 days approximately 75% of the administered dose was radiometrically detected in the excrement 1 to 3 days after dosing while approximately 30% was detectable by microbiological assay. When chickens (2/time point) were fed a diet containing 11 mg ¹⁴C-lincomycin/kg for 35 days then 2 times 0.5 mg ¹⁴C-lincomycin/day by oral bolus, the 'total' tissue residue concentrations in bile ranged from 5 194 to 10 µg equivalents/kg after 1 hour to 3 day withdrawal periods, respectively.

In pigs, oral doses of 4.4, 11 (the therapeutic dose), and 22 mg lincomycin/kg bw resulted in therapeutic serum concentrations being reached by 0.5 hour after dosing. Peak serum concentrations of 1.8, 3.9 and 5.1 mg/ml were reached within an hour and less than 4% of the plasma lincomycin was protein bound. In studies where pigs were administered lincomycin by intravenous or oral routes the distribution of liver metabolites were determined by thin layer chromatography (TLC) to have been quantitatively the same. An oral bolus or intravenous injection of 10 mg lincomycin/kg bw resulted in plasma t_{1/2} values of 3.4 and 2.0 hours, respectively. In another pig study, the half-life for liver and kidney tissues after oral administration of lincomycin was reported to have been 24 and 29 hours, respectively.

In sheep, intramuscular injection of 20 mg lincomycin/kg bw resulted in a plasma C_{max} of 12.3 µg/ml after 1 hour and a milk C_{max} of 25 200 µg/l after 2 hours.

The results of trials were reported in which lactating cattle were dosed with 11 mg lincomycin/kg bw and 32% of the lincomycin dose appeared in the urine independent of the route of administration. 1.5% of an intravenous dose appeared in urine while 85 to more than 95% of an intramammary dose was reported to have been eliminated in milk. Pharmacokinetic data in dry cows were not presented.

18. When chickens (2/time point) were fed a diet containing 11 mg lincomycin/kg for 35 days then 2 times 0.5 mg ¹⁴C-lincomycin/day by oral bolus for 12 days, followed by 1 hour, 1, 2 and 3 day withdrawal periods, 'total' tissue residue concentrations were highest in the liver (mean values 165, 30, 13, and 5 µg equivalents/kg, respectively). Kidney tissues contained mean residue concentrations of 101, 21, 8, and 4, respectively. Skin and fat tissues all contained total residue concentrations below 5 µg equivalents/kg.

In another radiometric chicken study (3/sex/time point), birds were dosed with 5.1 to 6.6 mg ¹⁴C-lincomycin/kg bw/day for 7 consecutive days in drinking water. The mean total residue concentrations at 0, 0.5, 1, 2, 4 and 7 day time points after treatment were determined to have been: 1580, 503, 224, 107, 28 and 20 µg equivalents/kg in liver; 1260, 560, 230, 100, 30 and 10 in µg equivalents/kg in kidney; 52, 27, 27, less than 5, less than 5, less than 5 µg equivalents/kg in muscle and 132, 51, 65, 28, 17 and 5 µg equivalents/kg in skin plus fat, respectively. In this study, lincomycin in the liver accounted for 20 and 5 % of the total residues at 0 and 96 hour time points respectively. At the same time points, liver samples also contained lincomycin sulphoxide (40 and 6 %), N-desmethyl-lincomycin (4% to less than the limit of quantification), N-desmethyl-lincomycin sulphoxide (4% and 4%) and lincomycin-3-5'-adenylate (18 to 57%). The tissue residue distribution in this study was different from that found in the non-radiolabelled studies conducted in other species, hence was not used when elaborating MRLs.

19. In a non-radiolabelled chicken study, residues in tissues from birds (4/time point) dosed with 264 mg lincomycin/l in the drinking water for 7 days were determined by a microbiological assay. It was claimed that all tissues contained lincomycin concentrations below the limit of quantification (not given), between 0 and 48 hours after treatment, except for one liver (980 µg/kg at 0 hour withdrawal) and one kidney (850 µg/kg at 6 hour withdrawal) sample.
20. In laying hens (6/time point), oral boluses of 0.55 mg ¹⁴C-lincomycin every 12 hours for 12 consecutive days resulted in whole egg total residue concentrations ranging from 1.2 to 12.0 µg equivalents/kg during the treatment period and 1 to 4 µg equivalents/kg 3 days after treatment (the yolk concentrations were approximately 3 times those in egg white). In the same study, mean tissue residue concentrations at 4, 28 and 76 hours after treatment were found to be 141, 24 and 6 µg equivalents/kg in liver; 152, 21, and 6 µg equivalent/kg in kidney; 20, 13, and 10 µg equivalents/kg in muscle and 19, 14, and 3 µg equivalents/kg in skin/fat, respectively.
21. In pigs (3/time point) dosed intramuscularly with 11 mg ¹⁴C-lincomycin/kg bw/day for 3 days, mean tissue residue concentrations 12, 24 and 48 hours after treatment were determined by liquid scintillation counting (LSC) to have been 17 300, 13 600 and 3 840 µg equivalents/kg in liver; 12 000, 5 750, and 3 080 µg equivalents/kg in kidney; 393, 127, and 138 µg equivalents/kg in muscle, 590, 260, and 200 µg equivalents/kg in fat and 1 050, 863, and 585 µg equivalents/kg in injection site muscle tissue, respectively.

In another radiometric pig study (3/sex/time point), where animals were fed as diet containing 20 to 200 mg/kg lincomycin, a microbiological assay detected less than 10 % of the total residues determined by LSC.

22. In pigs were dosed via their feed (3/sex/time point: approximately 1.3 to 2.3 mg lincomycin/kg bw/day for 61 days) the lincomycin concentrations (microbiological assay) were highest in kidney tissues (maximum values of 280 µg/kg) at 0 withdrawal respectively). All other tissues contained lincomycin concentrations below the limits of quantification of the microbiological assay method (less than 100 µg/kg). In another non-radiolabelled pig study, the animals (3/sex/time point) were dosed via their drinking water: 7.8 to 10.7 mg/kg bw/day for 10 days. The lincomycin concentrations (microbiological assay) were highest in kidney tissues (maximum values of 250 µg/kg) at 0 days withdrawal. All other tissues contained lincomycin concentrations below the limit of quantification (50 µg/kg) of the analytical method. In both these studies the lincomycin concentrations determined by microbiological assay were the same as those later determined by GC/MS.

Following intramuscular administration of 11 mg lincomycin/kg bw to pigs (3/time point) all tissue sample were reported to have contained lincomycin concentrations below 100 µg/kg 1 day after treatment and at time points thereafter (method of analysis not given).

Three new non-radiolabelled residue depletion studies, using the updated and validated GC/MS routine analytical method were presented.

Pigs (4/time point) were intramuscularly injected, daily for 3 consecutive days, with 10 mg lincomycin/kg bw and sacrificed at 3, 6, 12, 24, 48 and 144 hours after treatment. Liver samples contained mean residues of 4 710, 4 860, 2 480, 552, 65 and less than 17 µg lincomycin/kg, respectively. Kidney samples contained 20 900, 18 400, 7 470, 1 360, 239 and less than 60 µg lincomycin/kg, respectively. Muscle samples contained 2 460, 1 840, 638, 85, less than 17 and less than 17 µg lincomycin/kg, respectively. Fat samples contained 468, 456, 204, 39, less than 17 and less than 17 µg lincomycin/kg, respectively. Twelve days after treatment the theoretical maximum daily intake of microbiologically active residue was 151 µg (25% ADI), these data were used in-part when determining MRLs for porcine tissues.

Pigs (4/time point) were fed a diet containing 220 mg lincomycin/kg for 7 days and sacrificed on days 3, 6, 12, 24 and 48 after the end of treatment. Liver samples contained mean residues of 272, 169, 75, 40 and less than 17 µg lincomycin/kg, respectively. Kidney samples contained 904, 427, 278, 108 and less than 60 µg lincomycin/kg, respectively. Muscle samples contained 74, 31, less than 17, less than 17 and less than 17 µg lincomycin/kg, respectively. Fat samples contained 31, 17, less than 17, less than 17, and less than 17 µg lincomycin/kg, respectively. Three days after treatment the theoretical maximum daily intake of microbiologically active residue was 96 µg (16% ADI), these data were used in-part when determining MRLs for porcine tissues.

Pigs (4/time point) were given water containing 66 mg lincomycin/l for 7 days and sacrificed on days 3, 6, 12, 24 and 48 after the end of treatment. Liver samples contained mean residues of 204, 105, 53, 17 and less than 17 µg lincomycin/kg, respectively. Kidney samples contained 647, 296, 161, less than 60 and less than 60 µg lincomycin/kg, respectively. Muscle samples contained 42, 28, less than 17, less than 17 and less than 17 µg lincomycin/kg, respectively. All fat samples contained less than 17 µg lincomycin/kg. Three days after the end of treatment the theoretical maximum daily intake of microbiologically active residue was 66 µg (11% ADI), these data were used in-part when determining MRLs for porcine tissues.

Data for porcine skin plus fat in natural proportions were not provided.

23. A new non-radiolabelled residue depletion study in sheep, using the updated and validated GC/MS routine analytical method was presented.

Sheep (5/time point) were intramuscularly injected with 5 mg lincomycin/kg bw (and 10 mg/kg spectinomycin) for 3 consecutive days and sacrificed at 8 hours, 7, 14, and 21 days after the last treatment. Liver samples contained mean residues of 4 340, 27, less than 17 and less than 17 µg lincomycin/kg, respectively. Kidney samples contained 9 150, less than 17, less than 17 and less than 17 µg lincomycin/kg, respectively. Residue depletion data for muscle and fat samples were not presented. Notwithstanding the absence of data for sheep muscle and fat samples, the MRLs proposed for bovine and porcine tissues were also proposed as provisional MRLs for ovine tissues as the relative distribution of lincomycin in the 8 hour liver and kidney samples were the same as that observed in bovine and porcine tissues at later time points.

24. Lactating sheep (n = 5) were intramuscularly dosed alternatively with 20 mg lincomycin/kg bw and 20 mg clindamycin/kg bw in a crossover regime at 3 week intervals, then with 15 mg lincomycin/kg bw or 15 mg clindamycin/kg bw in a crossover regime at 2 hour intervals. During the treatment period when a 20 mg lincomycin/kg bw dose was applied, milk concentrations reached a C_{max} of 25 200 µg/l after 2 hours.
25. In cattle dosed intramuscularly, with 15 mg lincomycin/kg bw (twice on first day then once/day for next 4 days) in total 6 administrations, tissue lincomycin concentrations (microbiological assay) 1 day after the final dose were 560 µg/kg in liver, 42 µg/kg in kidney, less than 100 µg/kg in muscle and fat and 260 µg/kg in injection site muscle tissues. At 7 and 14 day withdrawal time points the tissue residue concentrations were less than 100 µg/kg. One day after treatment the theoretical maximum daily intake of microbiologically active residue was 65 µg (11% ADI), these data were used in-part when determining MRLs for bovine tissues.

In another cattle study (5/time point), the same intramuscular dosing regime resulted in tissue lincomycin concentrations (GC/MS assay) 8 hours after the final dose of 295 µg/kg in liver, 3 340 µg/kg in kidney, 720 µg/kg in muscle, 97 µg/kg fat and 2 420 µg/kg in injection site muscle tissues. At 7, 14, and 21 day time points the tissue residue concentrations had depleted to less than 47 µg/kg. The residue concentrations determined in these studies are known to have been underestimated due to the poor recovery in the solvent extraction phases of the microbial and GC/MS assays methods. The 8 hour residue data equated to a theoretical maximum daily intake of microbiologically active residue of 4 452 µg (742% ADI), these data were used in-part when determining MRLs for bovine tissues.

26. In lactating cattle (n = 5) intramammary doses of 200 mg lincomycin/quarter after three consecutive milkings resulted in lincomycin concentrations (determined using a microbial assay of unknown limit of quantification) in the first 3 post-treatment milk samples of 115 000 (12 hours: 64 000 to 150 000), 17 800 (24 hours: 4 900 to 62 000), and 1 410 (36 hours: less than 200 to 3 950) µg/l. By the fourth post dose milking (48 hours) milk residue concentrations had depleted to below 200 µg/kg in all samples.

In another cattle study (n = 12) employing the same treatment and sampling protocol, quarter milk samples were reported to have contained the following lincomycin concentrations (determined by microbiological assay): 36 700 (2nd dose, ranging from 17 100 to 70 900), 41 400 (3rd dose, ranging from 23 200 to 66 400), 42 000 (12 hour, ranging from 22 800 to 60 100), 5 170 (24 hour, ranging from 1 890 to 13 600), 600 (36 hour, ranging from 60 to 2 340) and 130 (48 hour, ranging from 33 to 770) µg/l, respectively.

From the comparative data available for lincomycin residues in incurred milk samples it is evident that lincomycin itself is the only microbiologically active compound present in milk.

27. A routine analytical method for the determination of the marker residue, lincomycin, based on GC with MS detection and described in ISO 78/2 format, was proposed.

The analytical method is fully validated for all edible bovine tissues and milk. The limit of quantification of the method was 15 µg/kg for all bovine tissues and milk.

The routine analytical method is also validated for porcine liver and kidney (limit of quantification: 60 µg/kg), porcine muscle and fat (limit of quantification: 17 µg/kg) chicken tissues (limit of quantification: 17 µg/kg) and ovine muscle, liver and kidney (limit of quantification: 17 µg/kg). However, the routine analytical method was not fully validated for ovine fat and milk, porcine and chicken skin and fat in natural proportions and eggs. The limit of quantification for eggs was reported to be 30 µg/kg.

Conclusions and recommendations

Having considered that:

- a microbiological ADI of 10 µg/kg bw, i.e. 600 µg per 60 kg person, was established for lincomycin,
- lincomycin represents all of the microbiological activity of incurred residues in tissues, milk and eggs; the parent compound is therefore recommended as the marker residue in all target species,
- a validated routine analytical method is available for all edible bovine tissues and milk,
- the routine analytical method was not fully validated for ovine fat and milk, porcine and chicken skin and fat in natural proportions and eggs,
- the tissue distribution of lincomycin being the same in bovine, ovine and porcine species,
- the distribution of total residues in chickens indicated that the daily consumption of residues would be below the ADI at all time points after treatment,

the Committee recommends the inclusion of lincomycin in Annex I of Council Regulation (EEC) No 2377/90) in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Lincomycin	Lincomycin	Bovine	100 µg/kg 50 µg/kg 500 µg/kg 1500 µg/kg 150 µg/kg	Muscle Fat Liver Kidney Milk	

the Committee also recommend the inclusion of lincomycin in Annex III of Council Regulation (EEC) No 2377/90) in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Lincomycin	Lincomycin	Porcine	100 µg/kg 50 µg/kg 500 µg/kg 1500 µg/kg	Muscle Skin+fat Liver Kidney	Provisional MRLs expire on 1.1.2001
		Ovine	100 µg/kg 50 µg/kg 500 µg/kg 1500 µg/kg 150 µg/kg	Muscle Fat Liver Kidney Milk	
		Chicken	100 µg/kg 50 µg/kg 500 µg/kg 1500 µg/kg 50 µg/kg	Muscle Skin+fat Liver Kidney Eggs	

Based on these MRL values the maximum daily intake of microbiologically active residue will be between 330 and 383 µg/person/day equivalent to between 55 and 64% of the ADI.

In order to establish final MRLs for lincomycin in porcine, ovine and chicken tissues, in ovine milk and eggs, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The proposed routine analytical method should be validated for ovine fat, porcine and chicken skin and fat in natural proportions and eggs. The method should also be validated for ovine milk, taking into consideration the CVMP Note for Guidance on the Establishment of Maximum Residue Limits for Minor Species (EMEA/CVMP/153a/97-FINAL).