European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

AVILAMYCIN

SUMMARY REPORT

1. Avilamycin is an orthosomycin antibiotic complex produced by the fermentation of *Streptomyces viridochromogenes*. Orthosomycin antibiotics are divided into two groups: those that contain an aminocyclitol residue and those that are esters of dichlorisoeverninic acid. Avilamycin falls into the latter group as do the evernimicins. Avilamycin is primarily active against gram-positive bacteria. The fermentation product comprises one major factor, avilamycin A, and 15 minor factors: avilamycin A', B, C, D1, D2, E, F, G, H, I, J, K, L, M and N. Specifications for purity of the dried fermentation product are: avilamycin A greater or equal to 60%, avilamycin B less than 18% (avilamycin A plus B greater or equal to 70%) and the other single factors less than 6%. The safety studies were carried out using avilamycin as fermentation product, with different degrees of purity or as pure (crystalline) product. The factor composition and purity of avilamycin differed between studies.

   Avilamycin is intended for use as a veterinary medicine in chickens, turkeys, pigs and rabbits to control bacterial enteric infections. It is intended to be administered to chickens, turkeys and pigs orally at a dose of 100 mg/kg feed for 21 days. In rabbits it is intended to be administered orally at a dose of 80 mg/kg feed for 28 days. Avilamycin was previously authorised as a feed additive for growth promotion in accordance with Council Directive 70/524/EEC; the substance was incorporated in pig feedstuffs at a concentration of 20 mg/kg feed for animals up to 6 months of age and 40 mg/kg feed for animals up to 4 months of age. In addition it was incorporated into chicken and turkey feedstuffs at a concentration of 10 mg/kg feed. The use of the substance as additive was discontinued in the EU from 1 January 2006.

2. Avilamycin inhibits protein synthesis. It is thought to bind to 50S ribosomal subunit. It prevents the association of IF2 which inhibits the formation of the mature 70S initiation complex and the correct positioning of the tRNA in the aminoacyl site. The binding site on the ribosome that is utilized by avilamycin appears to be different from those utilized by other protein synthesis inhibitors, which explains the lack of cross-resistance with orthosomycin-resistant mutants and other antibiotics. Some pharmacology testing was reported. It was not possible to establish a pharmacological NOEL or ADI on the basis of the data provided. However, the submitted summarised data indicated no effect at the lowest dose level of 1500 mg/kg, except on small intestinal transport in mice (reduction in charcoal transit time). From the toxicity studies (at much lower dose levels), there is considerable assurance that the reduction in small intestinal transport did not occur, or even if it did, resulted in no clinico-toxicological or pathological consequences. It is considered that a pharmacological ADI would not be lower than the toxicological equivalent.

3. The oral bioavailability of avilamycin in pigs, chickens and rat was low. This limited the capability to quantitatively demonstrate the distribution of avilamycin to tissues. Thus standard pharmacokinetic data were not available. Pharmacokinetics of a related compound, evernimicin, could be indicative of the behaviour of avilamycin *in vivo*. After intravenous administration, elimination half-life of evernimicin varied from 3 to 8 hours for mice, rats and rabbits and 24 hours for monkeys. Clearance was higher for smaller animals and the AUC was dose

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proportional in mice and rabbits. Evernimicin was found to be highly bound to plasma proteins (about 96% in humans and mice) and excreted primarily via the kidneys, with urinary excretion accounting for approximately 5 to 7% of the dose.

Most distribution studies were performed in pigs and chickens using radiolabelled avilamycin. A comparative study indicated that in rat the metabolism and distribution was comparable to pigs. Avilamycin is extensively metabolized and exhibits low tissue residues levels when administered orally to rats or swine. Therefore metabolites were only characterized in liver, due to low residue levels in the other tissues. Flambic acid is the primary metabolite in liver (15 to 20%) and faeces (40 to 50%). In liver no microbiologically active residues were detected. In chicken radioactive residues could not be characterized due to low levels of residues in the tissues and excreta. No metabolism data are available for rabbits or turkeys. When administered orally to swine, avilamycin was primarily excreted via the faeces (95%). The bulk of the radioactivity is excreted within the first week. Biliary excretion is of minor importance (estimated to be 7% of orally administered dose). Elimination data in turkeys and rabbits are not available. Limited data on evernimicin suggests that in rabbits this drug is eliminated from plasma via the kidneys with a half-life of 3 to 8 hours for intravenous doses of 3 or 15 mg/kg respectively. Clearance remained constant at 43 ml/h/kg for both doses. Pharmacokinetic data of pigs and chickens could be extrapolated to the minor species rabbit and turkey.Therefore, there is no need for additional pharmacokinetic studies in rabbits or turkey.

4. Avilamycin was shown to be of low acute oral toxicity. In GLP compliant studies in rat and mouse in which the fermentation product was used as test article determined LD_{50} ranged from more than 390 to more than 12000 mg/kg bw. The tests in which the crystalline form was used were non GLP and non OECD compliant.

5. Several repeat-dose studies were conducted in rat and mouse. All studies were carried out some years ago and are not to current standards. In rats exposed to daily oral doses of 0 to 1280/1205 (male/female) mg mycelium avilamycin/kg bw for 14 days no treatment related effects were observed. Discoloration of the paper in the trays below the cages was observed in all treatment groups. This may have been due to a photosensitive metabolite of avilamycin (as urine in bladders at necropsy was yellow). A statistically significant decrease in erythrocyte mean cell volume was observed in males of the highest dose group. Statistically significant increases in blood urea nitrogen and creatinine were observed in males of the highest two dose groups and in total bilirubin in highest dosed females. These effects were considered not toxicologically relevant and therefore a NOAEL of 1205 mg/kg bw/day was established. In another 14-day rat study, rats were orally dosed with 250/230 (male/female) to 5291/4652 mg crystalline avilamycin/kg bw/day, no treatment related effects were observed. Discoloration of the waste paper in the trays was observed in all treatment groups. Increased alanine aminotransferase activity was observed in two highest dosed female groups but without further signs of hepatotoxicity. A NOAEL of 4652 mg/kg bw/day was established. In addition some 28-day studies in rats were provided, due to lack of quality of design and reporting, it was not possible to derive a NOEL from these studies.

6. Some long-term repeat-dose studies were conducted in rats and dogs. In a 6-month dog study, daily oral doses of 0 to 178 mg mycelium avilamycin/kg bw caused no treatment related effects on mortality, clinical signs, body weight and histopathology. Reticulocyte count was increased in males of the highest dose group only at day 119. Alanine aminotransferase levels were significantly increased in 1 male and 2 females of the highest dose group at interim and at terminal sampling but without further signs of hepatotoxicity. The measurements of organ weights and histopathology were not in full compliance with the guidelines. Nevertheless a NOAEL of 178 mg/kg bw/day was established.

   In addition a 2-year rat carcinogenicity study was performed that included a satellite group in which additional toxicological parameters were monitored. No toxicological relevant effects were observed. Based on this study a NOAEL for repeated dose in rat of 120 mg/kg bw/day can be retained.

7. A non GLP tolerance study was provided in which pigs were fed a diet containing mycelial avilamycin resulting in a mean intake of 1 to 100 mg/kg/day for 21 weeks. No biologically relevant effects were observed. In chickens fed a diet containing mycelium avilamycin resulting in a mean intake of 2 to 216 mg/kg bw/day during 61 days also no biologically relevant effects were observed. In a GLP-compliant study, turkeys were fed a diet containing 20 or 100 mg avilamycin/kg feed for 16 weeks, no biologically relevant effects were noted.

8. A 3-generation study (two litters per generation) was conducted in rats fed diets containing 0, 30, 300, and 3000 mg/kg mycelial avilamycin (derived from 7% raw material), or 3000 mg/kg purified avilamycin (100%) in the diet equivalent to 0 ,1, 11.5, 120 (7%), 120 (100%) mg/kg bw. Occasional deaths in F0 and F1B parents were unrelated to treatment. Body weight increased slightly in treated groups, especially in the 300 mg/kg group. Total litter losses before and after parturition were observed throughout various groups, they were not treatment related. Litter weight of the F0 generation increased at 3000 mg/kg (100%) and at 30 mg/kg and 300 mg/kg (7%) at birth and at day 4 and 8 in the first mating and in the second mating at 300 mg/kg (7%) at day 4 and at 300 mg/kg (7%) and 3000 mg/kg (100%) at day 21 post partum. F2A generation: lower litter size and weight at 3000 mg/kg (7%) from birth to day 21 in the first mating. In the second mating the cumulative litter loss increased at day 12 and day 21 post partum at 30 mg/kg. Liver weights increased slightly but significantly in unmated adult F2A females at 3000 mg/kg (100%) and 300 and 3000 mg/kg (7%) and male weanlings (F3B) at 3000 mg/kg (7% and 100%). At necropsy only occasionally abnormalities were observed, not related to treatment. Also in offspring no treatment related external, visceral or skeletal abnormalities were found. The NOEL in this study was 300 mg/kg in feed equivalent to 11.5 mg/kg bw/day. The incidental finding of increased liver weight in unmated adult F2A females at 11.5 mg/kg bw/day were considered to be of no toxicological relevance. The effect at this dose was only seen in this generation and sex, and the weight effects were not accompanied by other signs of liver toxicity.

9. A developmental toxicity study in rats dosed orally with 0, 500 1000 and 2000 mg 26.4% granular avilamycin/kg feed (equivalent to 0, 119, 238, 475 mg avilamycin/kg bw/day) caused no treatment related maternal effects on general maternal health (survival, clinical signs or gross internal examinations). One female in the 1000 mg/kg group was killed on gestation day 18 due to a gavage error. Red urine (in trays) was observed in animals of all dose groups (not controls) (4, 4, and 1 at doses of 500, 1000 and 2000 mg/kg respectively). Female body weights were not affected by treatment, as were maternal reproductive parameters. In foetuses a higher incidence of kidney cavitations and dilated ureters was observed in all treatment groups (but within historical control range). Foetal malformations, deviations and variations were not increased by treatment. NOELs for maternal and foetal toxicity and teratogenicity were retained at 475 mg avilamycin/kg bw/day. In addition a teratology study in rabbits dosed with 0, 44, 127 and 356 mg avilamycin/kg bw/day caused some maternal and foetal effects. Diarrhoea in 2, 2 and 4 dams of the low, mid and high dose groups was observed. One rabbit of the high dose group died on gestation day 17. Abortions occurred in 4 animals either anorexic or having diarrhoea. Maternal body weight gain was decreased at 127 and 356 mg/kg bw/day, and food consumption was decreased in all treated groups. The number of male foetuses was slightly increased in the 127 and 356 mg/kg bw dose groups. No other relevant effects were noted. The NOAEL in this study was 356 mg/kg bw/day. Diarrhoea in rabbits is a common phenomenon after treatment with antimicrobials, therefore the NOAEL for maternal toxicity was set at the highest dose level.

10. Avilamycin was tested for genotoxicity in 9 *in vitro* and 3 *in vivo* assays. Some of the studies were carried out some years ago and are not in accordance with modern standards, but the more recent studies were in full compliance with the current guidelines. These recent studies included an Ames test, test for the induction of forward mutation, *in vitro* chromosome aberration test, and an *in vivo* micronucleus tests. In none of the tests positive results were observed. Therefore there is no evidence that residues of avilamycin are likely to pose a significant mutagenic risk to consumers.

11. A carcinogenicity study was carried out in rats. F_1 offspring of animals fed avilamycin for 1 week prior to mating were fed diets containing 0, 30, 300 or 3000 mg/kg in feed avilamycin activity derived from 7% raw material or 3000 mg/kg pure avilamycin (equivalent to 0, 1, 11.5, 120 mg/kg bw (7%) and 118 mg/kg bw (100%)). Total duration of exposure was 104 weeks. No overall treatment-related effects were seen on food consumption and urinalysis. Haematology analysis indicated that thrombotest times were above the normal upper limit of 30 seconds at weeks 13, 26, 52 and 78 in control males and males receiving the pure compound or 30 mg/kg mycelial cake. Thrombotest times of rats in the 300 mg/kg mycelium cake group was lower than normal at weeks 13, 26 (significantly) and 52. Values of the 3000 mg/kg mycelium cake group were considered as normal (significantly lower than the control group). A decrease in alanine aminotransferase was observed in all treated groups from weeks 13 to 52. In other parameters incidental statistical significant changes were observed, none of them were considered to be treatment related. Post mortem studies indicated that after 104 weeks of treatment the incidence of cortical scarring of the kidney increased in all male groups and the incidence of subcutaneous masses and haemorrhagic pituitary gland increased in all female groups. These findings are normal findings of senescence, thus considered not to be treatment related. Only minor differences in liver weight between controls and female animals treated with the mycelial cake at 300 mg/kg were observed. Higher incidence of pancreatic exocrine adenomas are observed in male rats of the 300 and 3000 mg/kg mycelial cake groups (incidence: 0, 1, 0, 2, and 4 for 0, 2000 (100%), 30, 300 and 3000 mg/kg (mycelial cake) respectively). Higher incidence of parafollicular cell carcinoma were observed in male rats of the 3000 mg/kg mycelial cake groups (incidence: 1, 0, 1, 3, and 4 for 0, 2000 (100%), 30, 300 and 3000 mg/kg (mycelial cake) respectively). The incidences observed were reported to be within historical control range. In females mammary and pituitary tumours were observed, but incidences were within range of historical controls (expected tumour profile).

    A second carcinogenicity study was carried out in mice. Mice were fed a diet containing 0, 30, 300 and 3000 mg avilamycin activity/kg, derived from raw material with a 7% activity or 3000 mg/kg, purified material (equivalent to 0, 3.2, 30.5, and 308 (7%) and 310 (100%) mg/kg bw/day) for 104 weeks. No treatment related clinical findings were observed, the number of male survivors decreased slightly in comparison with the female survivors, but this was not due to treatment. A slight reduction in body weight gain was observed in the first 26 weeks of study in males and females treated with 3000 mg/kg (100%) avilamycin, this reduction remained in females of this group during the whole study, but changed in males in a body weight increase at the end of the study. Changes in food consumption were not treatment related. Females treated with 3000 mg/kg (100%) avilamycin showed an increased incidence of reticulum cell sarcoma, but there was no effect on the incidence of total lymphoreticular neoplasms. When 3000 mg/kg was given as raw material with 7% activity, no effects on tumour incidence were observed. Females treated with 7% and 100% avilamycin at 3000 mg/kg showed a slight increase in pituitary adenomas (not considered to be treatment related). The incidences of other neoplasms were within the normal tumour profile of mice of this strain. The observed increased incidence of reticulum cell sarcoma was not considered to be relevant, because lymphoreticular neoplasms are frequently found in mice and the distinction between the different types is often disputable.

12. Several immunotoxicity studies were carried out. A GLP compliant local lymph node assay was conducted in mice. Avilamycin at doses of 5, 10, 25, 50 and 100% caused no clinical signs or mortality. No local irritation reactions or significant increase in ear thickness were observed at any of the tested concentrations. In addition, no significant lymph node proliferation was noted at any of the tested concentrations. Secondly, the allergic contact sensitization potency of avilamycin in by using a modified Buehler topical patch method was evaluated. Mycelial avilamycin was used for the induction and challenge, with appropriate controls. No reactions were observed in the avilamycin-treated groups. Finally, in a combined GLP compliant study the acute dermal, ocular and inhalation toxicity of mycelial avilamycin was evaluated. In the ocular irritation test 10 mg mycelium avilamycin was applied and induced coronal dullness, slight iritis and slight conjunctivitis in all treated eyes within one hour of treatment. All treated eyes cleared within 7 days after treatment.

13. As avilamycin is not used in human medicine, no data relating to the use of this molecule in humans were available.

14. A toxicological ADI of 115 µg/ kg bw was calculated by applying a safety factor of 100 to the NOAEL of 11.5 mg/kg bw which was established in the 3-generation reproduction study in rat. This NOAEL was based on increased liver weights in offspring.

15. According to published literature, resistance development to avilamycin occurs by both transferable and non-transferable mechanisms. In these cases avilamycin resistant isolates were found to carry the emtA resistance gene, this element is localized on plasmids (that are mobile genetic elements). Avilamycin has a long history of use as a feed additive. While *in vitro* transmission is likely to occur, no evidence of *in vivo* transfer of resistance has been found. There is no human reservoir of avilamycin resistance. Avilamycin has a unique mode of action and shows no cross-resistance with other antibiotics. For these reasons a correction factor of 1 is proposed in the calculation of the microbiological ADI.

    A faecal binding study was conducted with avilamycin. The effect of faecal binding on the antimicrobial activity of avilamycin was studied by incubating selected avilamycin concentrations (0 to 100 µg/ml) with sterile pooled human faeces at concentrations of 0 to 50% w/v. After incubation of each combination for up to 8 hours, faecal solids were removed by centrifugations and the supernatant liquid inoculated with a avilamycin-susceptible *Enterococcus faecalis* strain to assay the antimicrobial activity of the free drug. Incubation in faecal dilutions of 10% and 25% (w/v) resulted in varying binding effects of avilamycin. Avilamycin was bound to faeces at levels between 60% and 95%, binding at the low faecal dilutions was time dependent. In the presence of 50% faeces avilamycin was rapidly an irreversibly bound to each of the three samples tested. After 24 hour incubation of the inoculated supernatants binding was greater than or equal to 99%, 98% and 95% (resulting in a conservative estimate of 97.3% mean binding, thus an available fraction of 0.05). As there are presently no accepted and validated protocols for this type of investigation and given the lack of chemical data in the dossier, it is not possible to estimate the appropriateness of the approach using microbiological testing of avilamycin added to sterile faecal slurries. Therefore, the fraction of the dose available to gut organisms is established at the default value of 1.

    The Minimum Inhibitory Concentrations (MIC) of avilamycin against 100 bacterial strains representative of human gut microbiota were determined. Ten strains of *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Peptostreptococcus*, *Lactobacillus*, *Enterococcus*, *Bacteroides fragilis*, other *Bacteroides* spp., *Fusobacterium* and *Escherichia coli* were tested. The methodology employed was MIC agar dilution as described by the NCCLS. With the aim of the determination of an overall microbiological ADI, the MIC data for *Escherichia coli* were excluded due to lack of sensitivity to avilamycin. For the calculation of the MIC_{50} the low inoculum determinants were used. The lower 10% confidence limit ($CL10\%_{lower}$) of MIC_{50} was 0.378 µg/ml. To correct for the lower density in strains used in the test system compared to the higher density in the gut a correction factor of 2 is proposed.

16. The microbiological ADI is derived as follows:

For the establishment of the microbiological ADI the following standard formula was used:

$$\text{ADI (µg/kg bw)} = \frac{\dfrac{\text{10\% lower conf. limit of } MIC_{50}\text{'s x CF2}}{\text{CF1}} \text{ (µg/ml)} \times \text{daily faecal bolus (220 g)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}}$$

And therefore the microbiological ADI was calculated as indicated below:

$$\text{ADI} = \frac{\dfrac{0.38 \times 2}{1} \times 220}{1} = 2.8 \text{ µg/kg bw i.e.} = 168 \text{ µg/person}$$

The following assumptions were made:

- CF1 = 1 because no evidence of *in vivo* transfer of resistance has been found, there is no human reservoir of avilamycin resistance and avilamycin has a unique mode of action and shows no cross-resistance with other antibiotics,
- CF2 = 2 to correct for the lower density in strains used in the test system compared to the higher density in the gut,
- 220 g was the weight of the colonic content,
- the fraction of the dose available to gut organisms is established at the default value of 1;

17. A microbiological ADI of 2.8 µg/kg bw was established. However, as described below, a bioautographic method to identify microbiologically active residues in all edible tissues from chicken and swine was provided. This method uses TLC for separation and a test organism seeded into an agar layer to determine the antimicrobial response. No microbiologically active residues could be detected in chicken and swine tissues.

18. Although the microbiological ADI is 40 times lower than the toxicological ADI of 115 µg/kg bw, taking into account the lack of microbiological activity of residues in the target tissues and that the pharmacological effects were considered of little relevance when compared with toxicological effects, the toxicological ADI was considered the relevant ADI for the safety assessment of avilamycin. Hence the most relevant ADI is derived from toxicological studies, namely 115 µg/ kg bw.

19. Several studies following administration of ^{14}C-avilamycin to pigs were submitted. In one GLP compliant study three gilts and two barrows were fed a diet containing 80 mg ^{14}C avilamycin/kg food (3 mg/kg bw/day) for 7 days. Animals were slaughtered 6 hours (n=1) and 3 (n=2) and 5 days (n=2) post treatment. Residues in liver, kidney and fat were respectively 147, 77 and 67 µg radioactive residues/kg at 6 hours; respectively less than 33 (limit of detection), 26 to 24 and 54 to 62 µg radioactive residues/kg at 3 days; respectively less than 33 (limit of detection), 25 to less than 17 (limit of detection) and 47 to 49 µg radioactive residues/kg at 5 days. In another GLP compliant study 4 barrows and 2 gilts received a diet containing 60 mg ^{14}C avilamycin/kg feed (2.3 mg/kg bw/day) for 10 or 14 days. Animals were slaughtered 6 hours post treatment. Residues in muscle, liver, kidney and fat were respectively 93, 554, 321 and 261 µg radioactive residues/kg after 10 days of treatment and respectively 153, 659, 340 and 551 µg radioactive residues/kg after 14 days of treatment. In this study relatively high levels of radioactive residues are reported because the label was distributed over all rings of avilamycin. In all other residue label studies the label was only incorporated into the dichloroisoeverninic acid

moiety. In a last GLP compliant study 5 barrows and 4 gilts were fed at 12 hour intervals for either 4, 7 or 10 days a diet containing 80 mg ^{14}C avilamycin/kg feed (3 mg/kg bw/day). After 10 days of feeding mean residues in muscle, liver, kidney and fat were respectively 16, 216, 102 and 20 µg radioactive residue/kg. No parent avilamycin could be detected (less than 50 µg/kg) in the tissues. In liver and kidney dichloroisoeverninic acid-related residues could be identified; in liver representing 50% or more of the total radioactive residue. A major metabolite was characterized in liver being flambic acid, comprising 15 to 10% of liver residue. In fat radiolabel was found to be incorporated in the fatty acid portion of the triglycerides.

20. Several studies following administration of ^{14}C-avilamycin to chickens were submitted. In one GLP compliant study 3 groups of 4 chickens (2 males and 2 females) were fed a diet containing 15 mg ^{14}C-avilamycin/kg feed (approximately 1 mg/kg bw/day) for 4, 7 or 10 days. After 10 days of treatment no radioactive residues expressed as avilamycin equivalents could be detected in muscle (less than 8 µg/kg) and kidney (less than 2.4 µg/kg). In liver, skin and fat mean residues were 22, 20.5, 25,8 µg/kg, respectively. Since radioactivity levels were low, no metabolites were characterised.

21. From the results of the above presented studies in pigs and chickens it can be concluded that avilamycin can not be used as the marker residue, since it could not be detected. Moreover a reference standard for its major metabolite flambic acid is not available. Instead dichloroisoeverninic acid, a moiety present in avilamycin and flambic acid and other possible metabolites, is proposed as the marker residue. Dichloroisoeverninic acid is not a common chemical structure and is likely to be an unique representative of avilamycin residues in animal tissues. Although the dichloroisoeverninic acid moiety can also be found in curamycin, flambamycin and everninomycin, none of them are veterinary drugs.

22. A GLP-compliant residue study in pigs was submitted. Twelve pigs (6 males and 6 females) were fed avilamycin at a nominal concentration of 150 mg/kg feed for 21 consecutive days. At the end of the exposure period animals were slaughtered at withdrawal intervals of 0, 6 and 24 hours. Avilamycin residues in swine tissues were detected as dichloroisoeverninic acid. All dichloroisoeverninic acid levels in muscle, fat + skin and kidney were below the limit of detection or quantification. In liver samples, dichloroisoeverninic acid residues were found in all swine samples at the 0 hour time-point with an equivalent avilamycin range of 75.2 to 138 µg/kg. At the 6 hour time-point the swine liver samples had an equivalent avilamycin range of 29.6 to 49.5 µg/kg. And at the 24 hour time-point all liver samples were below the limit of quantification, except for one liver sample having an equivalent avilamycin value of 32 µg/kg. Thus, liver is determined as the target tissue.

23. A GLP-compliant residue study in broiler chickens was submitted. Eighteen chickens (9 males and 9 females) were fed avilamycin at a nominal concentration of 150 mg/kg feed for 21 consecutive days. At the end of the exposure period animals were slaughtered at withdrawal intervals of 0, 6 and 24 hours. Avilamycin residues in chicken tissues were detected as dichloroisoeverninic acid. All dichloroisoeverninic acid levels in muscle, fat + skin and kidney were below the limit of detection or quantification. In liver samples, dichloroisoeverninic acid residues were found in 5 out of 6 chicken samples at the 0 hour time-point with an equivalent avilamycin range of 31.9 to 113 µg/kg. At the 6 hour time-point in only 1 out of 6 chicken liver samples residues were found with an equivalent avilamycin value of 29.8 µg/kg. At the 24 hour time-point all chicken liver samples were below the limit of detection or quantification. Thus, liver is identified as the target tissue.

24. Several other non radiometric non GLP compliant residue depletion studies were provided. However, the level and duration of dosing in these studies was lower and shorter than the recommended; in general no residues were detected in tissues. Therefore, these results are considered to be of limited use.

25. In a combined tissue and egg radiometric study 7 hens were fed a diet containing 30 mg ^{14}C-avilamycin/kg feed for 14 days. In egg yolk residues after 10 days were 199 µg/kg, after 12 days 213 µg/kg and after 14 days 213 µg/kg. In albumin residues could not be detected (less than 70 µg/kg). Residues were not characterized. No additional data for eggs have been submitted, an MRL for eggs was not requested.

26. A bioautographic method to identify microbiologically active residues in all edible tissues from chicken and swine was submitted. This method uses TLC for separation and a test organism seeded into an agar layer to determine the antimicrobial response. Samples were obtained from the residue studies in pigs and broiler chickens as described above. No microbiologically active residues could be detected in chicken and swine tissue at any time points.

27. For turkeys and rabbits residue studies demonstrate that the marker residue dichloroisoeverninic acid is detectable and quantifiable in turkey and rabbit tissues.

28. For routine monitoring of avilamycin as dichloroisoeverninic acid a validated analytical LC-MS/MS method for chicken and swine tissues was proposed. The method is specific with regard to possible interference of other commonly used veterinary drugs or substances. The method is validated across a range, including half and twice the MRL. The validations on accuracy, precision, applicability range and stability meet the demands of Volume 8 of the Rules Governing Medicinal Products in the European Union. The limits of quantification expressed as dichloroisoeverninic acid were 18 µg/kg in muscle, 44.5 µg/kg in fat + skin, 89 µg/kg in kidney, and 134 µg/kg in liver.

29. As dichloroisoeverninic acid levels in all edible tissues were very low, the MRLs are set at twice the limit of quantification, expressed as dichloroisoeverninic acid; these values were rounded.

30. The routine analytical method as developed and validated for chicken and swine is capable of detecting and quantifying residues incurred in tissues from turkeys and rabbits. Applicability of this method to other poultry species than chicken and turkey should not be problematic and therefore from this aspect extrapolation to the tissues of all poultry would be possible.

31. Avilamycin is extensively metabolised in all species studied, there were no indications of that metabolism varies significantly between the species. Residue studies demonstrate that the marker residue dichloroisoeverninic acid is detectable and quantifiable in turkey and chicken. The proposed MRLs for chicken and turkey are identical and so it was considered appropriate to recommend the extension of the MRLs so that that the same tissue MRL values would apply to all poultry species.

Conclusions:

Having considered that:

- a toxicological ADI of 115 µg/kg bw (i.e. 6900 µg/person) was established; a microbiological ADI of 2.8 µg/kg bw (i.e. 168 µg/person) was established,
- incurred residues of avilamycin show no antimicrobial activity, and therefore the toxicological ADI was considered more relevant than the microbiological ADI for the risk assessment,
- dichloroisoeverninic acid was retained as the marker residue; the marker residue is present in tissues of pigs, chickens, turkeys and rabbits,
- residue concentrations of the marker residue were below the limit of quantification or detection in muscle, fat + skin and kidney in pigs and chickens. Low residue concentrations were present in liver,
- a ratio of marker to total residue of 0.5 was established for liver and kidney, and a conservative ratio of 0.1 for muscle and fat + skin was retained,
- the limits of quantification expressed as dichloroisoeverninic acid were 18 µg/kg in muscle, 44.5 µg/kg in fat + skin, 89 µg/kg in kidney, and 134 µg/kg in liver,
- dichloroisoeverninic acid levels in all edible tissues were very low, MRLs can be set at twice the limit of quantification,
- MRLs will be expressed as the marker residue dichloroisoeverninic acid correcting for the molar ratio of avilamycin/dichloroisoeverninic acid and rounding the values,
- avilamycin is extensively metabolised in all species studied,
- MRLs set for pigs and chickens can be extrapolated to rabbit and poultry, respectively,
- a validated routine analytical method for the determination of the marker residue in edible tissues of pigs, chickens, turkeys and rabbits is available and the method is also considered to be applicable to all poultry species;

The Committee for Medicinal Products for Veterinary Use recommends the inclusion of avilamycin 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 tissue	Other provisions
Avilamycin	Dichloroisoeverninic acid	Porcine	50 µg/kg 100 µg/kg 300 µg/kg 200 µg/kg	Muscle Skin + fat Liver Kidney	
		Rabbit	50 µg/kg 100 µg/kg 300 µg/kg 200 µg/kg	Muscle Fat Liver Kidney	
		Poultry	50 µg/kg 100 µg/kg 300 µg/kg 200 µg/kg	Muscle Skin + fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption

Based on the MRL values, the theoretical maximum daily intake of dichloroisoeverninic acid will represent about 304 µg, equivalent to 1702 µg of avilamycin based on a molecular weight ratio of 5.6. This intakes corresponds to approximately 25% of the toxicological ADI.