European Medicines Agency
Veterinary Medicines and Inspections

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WITHDRAWAL ASSESSMENT REPORT

FOR

KEXXTONE

International Non-proprietary Name: avilamycin

Procedure No. EMEA/V/C/131

Withdrawal at Day 120

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1. SUMMARY OF THE DOSSIER

On 17 April 2007 Eli Lilly and Company Ltd submitted to the EMEA an application for the granting of a Community marketing authorisation for Kexxtone.

The active substance of Kexxtone is avilamycin, an oligosaccharide antibiotic which acts by inhibiting the protein synthesis of susceptible micro-organisms. The product is presented as a premix for medicated feeding stuff.

The product was intended to be used in rabbits in cases of enteritis at the group level, prevention of mortality and digestive signs due to *Clostridium perfringens* susceptible to avilamycin. The proposed dosage is 7 mg avilamycin/kg bodyweight, which corresponds to approximately 80 mg avilamycin/kg feed for 4 weeks. The proposed withdrawal period was zero days.

The CVMP on the basis of quality, safety and efficacy data submitted, considered that the product was not approvable at Day 120 since major objections had been identified regarding the quality of the active ingredient. Other points of concern related to the proposed incorporation rate in the feed, potential risks to the user, potential risk to the environment and providing adequate justification for the proposed dose regimen.

On 8 September 2008 Eli Lilly and Company Ltd withdrew the application at day 120 of the procedure.

2. QUALITY ASSESSMENT

Composition

The product contains granular avilamycin as active ingredient in a percentage sufficient to achieve 20% w/w avilamycin activity. The quantity of granulated avilamycin varies according to its potency. The granules have a concentration of about 270 mg avilamycin activity per gram. The remaining excipients are included in the formula as diluent and anti dusting agents.

Container

The premix is packed in 25 kg bags.

Development Pharmaceutics

Development of this product has been mainly performed in its former use as a feed additive and there was no evidence that the use of the product as a premix was taken into consideration. The terms described in the guideline “Additional Quality Requirements for Product Intended for Incorporation into Animal Feedingstuffs” (EMEA/CVMP/080/95) were not been taken into account. The chosen active ingredient concentration leads to an incorporation rate which was not justified as per the requirements of the Ph.Eur. monograph on ”Premixes for Medicated Feedingstuffs for Veterinary Use”.

Method of manufacture

The active substance is produced by fermentation of a strain of *Streptomyces viridichromogenes*. No purification steps are carried out, so the active ingredient is presented as granular avilamycin that contains mycelial solids and excipient.

The method of preparation is adequately described, although some changes are necessary to avoid potential problems concerned with traceability of batches or stability in the final product.

Control of starting materials

Active substance

Avilamycin is an antibiotic complex not described in any major pharmacopoeia (Ph.Eur., USP, JP), neither in its pure state nor in the granular form. The in-house standard quality used in Kexxtone is not intended to be pharmaceutical grade, but feed grade.

The absence of a pharmacopoeial monograph and the fact that the final product is mixed into feed, are considerations for accepting a non-purified avilamycin. However, the applicant failed to provide additional supportive data in order to achieve a finished product of a consistent quality (including a standardised formula of manufacture)

Excipients

None of the excipients is described in European Pharmacopoeia and were thus of an in-house standard. Insufficient information on the excipients was provided.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

No materials of animal origin are used in the manufacture of granular avilamycin or in the manufacture of the product.

Control tests during production

There are no intermediate products, being the granular avilamycin considered as raw material.

Control tests on the finished product

The finished product is tested for loss on drying, particle size, physical appearance and avilamycin content. Two HPLC methods have been described for identifying avilamycin content. The range for avilamycin activity content at release are in accordance with the current guidelines. The data submitted were within the acceptable limits.

No proposals for the impurities profile, neither for the limits for degradation products, were provided. A specification in order to confirm the homogeneity of content in the batches was also lacking. Data obtained in the batches tested did not justify the proposed limit for particle size.

The end product is not microbiologically tested. Although the monograph for veterinary premixes has no specific requirements on microbiological quality, this aspect cannot be ignored because:

a) premixes are considered pharmaceutical preparations, and no exceptions have been introduced in the chapter 5.1.4 of the Ph.Eur.
b) also feedstuffs have to comply with requirements on microbiological quality.

For these reasons, microbiological quality should have been stated as a finished product specification.

Stability

Active ingredient

The dossier includes data of an on-going study performed in three batches of granular avilamycin packaged in simulated commercial containers. The samples were stored in accordance with the VICH conditions. Only data for up to 6 months were available at the time of submission of the application for marketing authorisation. Despite some decreases in avilamycin activity, the specification of not less than 210 mg activity/gram was met during all the tested period.

Stress tests, to reveal the possible degradation of the product, were not been presented by the applicant. Studies on photostability of pure avilamycin were in progress, and only the protocol was

provided. No information on degradation products for either avilamycin or granular avilamycin was submitted. The suitability of the methods of analysis to control these products was not justified.

Finished product

Proposed ranges for avilamycin activity content during the shelf life were not in accordance with the current guidelines, and should have been reduced to 90-105% unless justified.

The applicant did not consider the guideline EMEA/CVMP/453/01 (Note for Guidance on Start of Shelf-Life of the Finished Dosage Form) to establish the expiry date of a production batch.

A shelf life of 24 months for the finished product was proposed, based on the stability data presented in the dossier. Three batches stored in the commercial pack were tested. The storage conditions were according VICH. Data up to 6 months were provided at 40ºC/75% RH. At the time of submission, the study at 25ºC/60% RH was ongoing, and data in the dossier were up to 12 months.

Avilamycin activity decreased during the tested period. The primary package did not protect the product when stored at 40ºC/75% RH, but changes in loss on drying observed in batches stored at 25ºC/60% RH were reasonable.

Two batches were tested after room temperature storage in broached bags. Data submitted justify the proposal of 14 days after first opening.

Homogeneity after transportation in a bag of 25 kg has been tested. The protocol of the study has been designed according the Eur. Pharm. test 2.9.40. The study demonstrated that the homogeneity is maintained after the transportation of the 25 kg-bag of the product.

In-feed studies – homogeneity and stability

Avilamycin content in the feed was determined by HPLC.

Study reports were provided on the behaviour of avilamycin feed additives in poultry and pig feedstuffs. Although the products tested were very similar to Kexxtone (e.g. same quantitative composition), the incorporation rate was different, and the types of feed tested were not the same as for the target species. No information could be obtained from these studies to make recommendations for the feed mill for the production of medicated rabbit feed. Nevertheless, these studies could be taken as “supporting data”.

Stability towards steam conditioning and pelleting was proven, and homogeneity was demonstrated at 5, 10, 20 and 40 mg/kg feed in the type of feeds tested under the conditions applied in these tests, except for turkey feed. The difference in variation coefficients for content of avilamycin in turkey feed versus broiler and pig feed should have been discussed by the applicant.

A study was performed to justify the use of Kexxtone in rabbit feed at a dosage of 125 mg avilamycin activity per kg feed, different from the proposed dosage (80 mg/kg feed). One batch of medicated rabbit feed (pellets) was assayed for potency and homogeneity. Pelleting conditions were not stated. The uniformity of content assay was not performed according to the pharmacopoeial method, as only six samples of the medicated feed batch were assayed. In addition, the proposed acceptable range specified in the protocol of 80-110% of the nominal range for the mean was not considered suitable. Therefore, the medicated feed assayed (with a mean of 104 mg/kg feed) has to be considered as under dose. Although uniformity of dosage can be confirmed, the mean content of the active ingredient found in the medicated feedstuff did not comply with the proposed rate (125 mg/kg feed ± 10%). Therefore, the applicant should have provided:

- A justification for applying a different incorporation rate in the study compared to the proposed dosage.
- A description of the preparation of the medicated feed (composition of the feed, incorporation, pelleting conditions).

- A statement on the storage conditions of the medicated feed up to the 36-day assay.
- A justification for the under dosage of the medicated feed observed in the study. The acceptable range should have been stated as 125 mg/kg feed ± 10%.

Recommended pelleting conditions should have been included in the product literature. The upper and lower limits of active ingredient in the final feed, which were considered acceptable with regard to safety and efficacy, should have been indicated.

Additional studies to document the behaviour of the premix in rabbit feed should have been submitted according guideline EMEA/CVNP/080/95.

The dusting potential of the product was tested using Stauber-Heubach technique, which gave good results between 0.075 and 0.255 g/m^3.

OVERALL CONCLUSION ON QUALITY

Kexxtone contains avilamycin at a concentration of 200 mg avilamycin activity per gram. It is intended to be used in pelleted feed for the prevention of mortality and digestive signs due to *Clostridium perfringens* susceptible to avilamycin. The proposed dosage is 7 mg avilamycin/kg bodyweight, which corresponds to approximately 80 mg/kg feed in the complete feed (0.4 kg of product per tonne of feed) for 4 weeks. This formulation had been marketed in the EU as a Feed Additive for pigs and poultry until 2005.

The active substance is produced by fermentation of a strain of *Streptomyces viridichromogenes*. No purification steps are carried out, so it is presented as granular avilamycin, containing mycelial solids and a diluent. The product is formulated by blending the granular avilamycin with diluent and mineral an anti-dusting agent. None of the components is described in a major Pharmacopoeia (Eur. Pharm., USP or JP). Additional information was requested to determine whether the quality of the raw materials is suitable to produce a pharmaceutical product of consistent quality.

The method of preparation was adequately described, although some changes are necessary to avoid potential problems concerned to traceability of batches or stability in the final product.

The testing monographs for the final product contain specifications and tests for appearance, identification for avilamycins A and B, factor composition, loss on drying and particle size. Avilamycin activity is measured by means of a HPLC method and applying conversion factors of bio-potency obtained from the analysis of isolated fractions of avilamycin A and B. The methods used to control the finished product specifications are considered suitable. These methods are closely similar to those used in the active ingredient. Some additional parameters should be included to confirm the quality of the product, and some of the established parameters have to be tightened or clarified. Additional information on the impurity profile should also be submitted.

There are concerns about the stability of the active ingredient and finished product, because the degradation of the active ingredient has not been documented. Additional information is required to establish a shelf-life period.

There are concerns regarding the avilamycin homogeneity in medicated feed. The behaviour of the product when mixed with pigs and poultry feed is reported in the dossier. However, the development pharmaceutics should be completed with data relevant to the suitability of use of the product mixed in rabbit feed. Such studies are to be performed in accordance with the European guideline “Additional Quality Requirements for Product Intended for Incorporation into Animal Feedingstuffs”.

The incorporation rate should have been justified as per Ph.Eur. requirements.

3. SAFETY ASSESSMENT AND RESIDUES

A. SAFETY ASSESSMENT

Pharmacokinetics

Avilamycin's poor absorption after oral administration has been demonstrated in several studies conducted in pigs, chickens and rats using radiolabelled avilamycin. In all species, almost all the radioactivity (>95%) was excreted in the urine and faeces shortly after administration. Avilamycin is extensively metabolised and exhibits low tissue residues when administered orally to rats or swine. Flambic acid is the primary metabolite in liver (15 to 20%) and faeces (40 to 50%).

Toxicology

Avilamycin has a low acute oral toxicity. Repeated dose toxicity indicates that the lowest NOAEL in rat is 1205 mg/kg/d (14 days) and 120 mg/kg/d (2 years); in dogs a NOAEL of 178 mg/kg/day (6 month) was established.

In a 3-generation study conducted in rats the only significant effect was the effect on liver weight in F2A adult females and F3B weaned males. The NOEL in this study was 11.5 mg/kg bw/day. Avilamycin has no developmental toxicity in two GLP studies (in rabbit and rat); no maternal or foetal effects of avilamycin were observed. The lowest NOEL (for maternal and foetal toxicity and teratogenicity) was 356 mg/kg bw/day, based on the highest dose tested in the rabbit study.

Avilamycin is not mutagenic according to twelve studies, three were *in vivo* and nine *in vitro*, with and without metabolic activation. All results were negative. In light of the negative mutagenicity data, the absence of any structural alerts and the absence of carcinogenic effects in the carcinogenicity studies, avilamycin can be considered as being non-carcinogenic.

User safety

A user safety assessment was provided including four potential user exposure scenarios, which in general followed the guideline on user safety.

The main routes of exposure of the user are the direct exposure to (unprotected) skin and eyes as well as indirect exposure through hand-mouth contact. It was considered that feed mill workers are more likely to have direct exposure to the product, whilst exposure to farmers was anticipated to be negligible. In addition, as the premix is intended for use in a minor species, a low exposure frequency to farmers was assumed.

Taking also into account the relatively low toxicity of the active substance, the applicant concluded that the proposed user warnings in the SPC and product literature would be sufficient to address user safety.

The CVMP, however, noted a number of shortcomings in the user safety assessment, in particular the risk assessment, and the applicant was asked to provide a revised user safety assessment, before final conclusions on user safety could be taken.

Environmental safety

Environmental exposure from the proposed use of avilamycin is expected via the use of contaminated rabbit manure as fertiliser.

An environmental risk assessment was provided. The Phase I assessment presented a PEC_{soil} calculation which exceeded 100 µg/kg. A Phase II assessment was therefore provided.

The Phase II assessment was not acceptable because the PECs values were not justified, and more importantly, because the physico-chemical properties, fate and effects data provided were not in accordance with the requirements of the VICH Phase II guidelines. In addition, the potential environmental impact of the metabolites excreted should be taken into account at Phase II.

B. RESIDUE ASSESSMENT

Maximum Residue Limit

A MRL has been established for rabbit tissues. However, no information was included on the status of the excipients in relation to Council Regulation (EEC) No. 2377/90. One of these, the diluent, is out of scope. Data about the composition and characteristic of the anti-dusting agent were requested in order to establish if the substance is already included in Annex II.

Withdrawal period

A study was conducted to determine the residue depletion kinetics of the marker residue in rabbits treated with avilamycin in feed for 28 days. The residue data indicated that the tissue concentration was well below the MRL at 0, 12 and 24 hours; the low concentration of marker residue in all tissues does not allow a valid statistical analysis. A withdrawal period of zero-days in meat was accepted.

Analytical method(s)

A validated analytical method is available for quantitative determination and confirmation of the marker residue in rabbit tissues.

OVERALL CONCLUSIONS ON SAFETY AND RESIDUES

Avilamycin has a low acute oral toxicity and is not considered to be carcinogenic. However, there were shortcomings in the user safety risk assessment for Kexxtone, and as a result it has not been demonstrated that the risk to the user is acceptable. Neither has the environmental risk assessment shown that the risk to the environment is acceptable.

4. EFFICACY ASSESSMENT

Pharmacodynamics

Avilamycin belongs to the orthosomycin family of antibiotics, and inhibits protein synthesis by a mechanism of action involving binding to the 50S ribosomal subunit and preventing the association of IF2, which inhibits the formation of the mature 70S initiation complex and the correct positioning of tRNA in the aminoacyl site.

Impact of resistance development to efficacy

Resistance to avilamycin in Gram-positive bacteria occurs exclusively by modification of the drug target; meaning that alterations in the antibiotic binding side of ribosome decrease the binding affinity of the drug.

No data were presented in compliance with the Guideline CVMP/VICH/644/01 on avilamycin susceptibility against enterococcal strains isolated from rabbits. However, a number of European surveys report on the activity of avilamycin against strains of enterococci recovered from other animal sources such swine, cattle or poultry were available. The data indicate that a base line level of

resistance among enterococcal isolates against avilamycin is present, but no there is no evidence of an over the time increasing number of isolates with reduced susceptibility to avilamycin.

Target animal safety

As per formal scientific advice received from CVMP a target animal safety study was not provided. To support target animal tolerance, reference was made to other studies (toxicology, dose determination), which is acceptable taking into account that rabbits are minor species.

The existing toxicity data showed a low toxicity, both acute oral and after repeated administration, of avilamycin in feed. In rabbits, only a few cases of diarrhoea were described at 44 mg/kg and the NOEL for maternity toxicity was established at 356 mg/kg bw/day.

Furthermore no adverse effects were reported at the dose determination study where rabbits received avilamycin at 1x, 1.5x and 2x the recommended dose during 28 days. In the clinical trial, decrease in appetite was observed in some animals in the avilamycin group.

The CVMP agreed that the data provided would satisfactorily describe target animal safety and concluded that the product is in general well tolerated by rabbits.

PK/PD analysis

Although the most frequent pathogen associated with epizootic rabbit enteropathy (ERE) is *Clostridium perfringens*, the MIC data of avilamycin to pathogenic strains of *C. perfringens* were only determined for strains isolated in Italy, France and Spain. According to guideline EMEA/CVMP/627/01 the strains isolated should be as representative as possible of a broad geographic area, so determination of MIC data would be required for pathogen strains from other relevant countries as well.

The applicant considered that the use of ratios such as AUC/MIC (AUCI) and C_{max}/MIC is not recommended for this antibiotic because these have not yet been validated for avilamycin. Because of this, a concentration of microbiologically active free avilamycin in the large intestine lumen is calculated on bases on different swine values and the equivalent for rabbit. The conclusion is the concentration of microbiologically active free avilamycin in the large intestine lumen are likely to be in the range 0.3-1.2 µg/ml after prolonged administration of avilamycin at a 80 mg/kg.

Dose determination/confirmation studies

A dose-determination study was conducted according to Good Clinical Practice. The study included four groups of rabbits; three avilamycin groups (80, 120 or 160 mg/kg feed) and one positive control group, tiamulin 40 mg/kg feed. The target avilamycin doses were 7 mg/kg bw (1x the recommended dose); 10.5 mg/kg bw (1.5x) and 14 mg/kg bw (2x); the primary efficacy parameter was morbidity during the treatment period.
The results showed that no advantage was gained by increasing the dose of avilamycin above 80 mg/kg feed. All three avilamycin doses demonstrated a statistically significant reduction in morbidity compared with the positive control and the lowest rate of morbidity was seen in the 80 mg/kg feed group. The conclusion is that a dose of 6 mg/kg/bw was the most effective dose.

Another study was conducted to confirm the treatment duration. Avilamycin was administered at the inclusion rate of 80 mg/kg feed for 14, 21, 28 and 35 consecutive days. Tiamulin was given for 28 days at 32 mg/kg feed to constitute a positive control group.

The primary efficacy parameter was mortality due to ERE associated signs during the treatment period. Morbidity characterised by the occurrence of one of three clinical signs (diarrhoea, tympany and borborygmus) were recorded too. The results obtained showed avilamycin 80 mg/kg feed for 28 and 35 days significantly decreased the number of rabbits that died with ERE-associated signs; besides significantly fewer clinical signs were reported. On the basis of the mortality rate, it was concluded that treatment duration of 28 days was the most appropriate therapeutic regimen for avilamycin at the

incorporation rate of 80 mg/kg feed (approximately 6 mg/kg/bw) when given in an ERE-infected environment.

Field trials

A GCP-compliant, randomised, blinded field trial was performed in Europe (2005) to assess the efficacy of the product administered in feed at a dose of 80 mg avilamycin/kg feed during 28 days for the control of enteritis caused by *Cl. Perfringens*. This was equivalent to a dose of 6.8 mg/kg/bw.

The primary efficacy parameter was morbidity; three clinical signs were taken into account to define an animal as "morbid": diarrhoea, abdominal distension and mortality due to clostridial enteritis. The secondary efficacy parameters were post treatment morbidity, proportion of the animals displaying morbidity on each day of the study, mortality, daily gain weight, feed conversion ratio and feed conversion index. The result showed a 31.7% morbidity rate in the avilamycin group and 50% in the tiamulin group.

The applicant concluded that the product at 7 mg/kg/bw for 28 days in cases of enteritis in rabbits at the group level, prevents mortality and digestive signs due to *Clostridium perfringens*. However, the CVMP acknowledged some clinical effect of the product in the control of the enteritis caused by *Clostridium perfringens*, but noted that there are several shortcomings in the study. In particular, concern was expressed about choice of efficacy parameters and the actual dose (inclusion rate) received by the treated animals.

OVERALL CONCLUSION ON EFFICACY

Avilamycin is an oligosaccharide antibiotic with primarily activity against Gram-positive bacteria. Resistance development and target animal safety were sufficiently addressed by the applicant and did not raise concerns.

The efficacy of the product in the proposed dose regimen required further justification.

5. BENEFIT RISK ASSESSMENT

Benefit

CVMP recognises that the product might be effective to maintain a low percentage of mortality, although it has not been clearly established for clinical signs (morbidity).

Risk

Risk relating to the quality

The quality of both the active substance and the final product has not been sufficiently justified in the dossier. This concern also includes the development of the product, the method of preparation and the stability studies. In addition, the behaviour of the product in the target feedstuffs has not been complemented according to the current guideline.

The main concerns are the proposed incorporation rate and the quality of the active ingredient. These are considered as major objections and preclude at present a positive opinion.

Risk relating to the safety

The user safety assessment has not demonstrated that the risk to the user is acceptable.

The environmental risk assessment has not demonstrated that the risk to the environment is acceptable.

Risk relating to the efficacy
The PK/PD approach is not sufficiently justified and the dose regimen needs more justification both *in vitro* and *in vivo*. A different dosage is obtained in each study and there is not adequate evidence to select a dose of 7 mg/kg/bw.

Benefit-risk balance

The CVMP could not give a positive opinion whilst there were still outstanding issues as detailed above.

6. OVERALL CONCLUSIONS

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP considers that the application for Kexxtone in the treatment of enteritis at the group level, prevention of mortality and digestive signs due to *Clostridium perfringens* susceptible to avilamycin is not approvable at the present time since major objections have currently been identified which preclude a recommendation for marketing authorisation.