



**Australian Pesticides &
Veterinary Medicines Authority**

**THE RECONSIDERATION OF THE REGISTRATION OF PRODUCTS
CONTAINING VIRGINIAMYCIN AND THEIR LABELS
(DRAFT REVIEW REPORT)**

March 2003

Australian Pesticides & Veterinary Medicines Authority

**Canberra
Australia**

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FOREWORD

The Australian Pesticides & Veterinary Medicines Authority (APVMA) is the National Registration Authority for Agricultural and Veterinary Chemicals. When reading this document, "APVMA" means "NRA". For information regarding this name change, please visit www.apvma.gov.au.

The APVMA is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals in Australia. Its statutory powers are provided in the *Agricultural and Veterinary Chemicals Code Act, 1994* (Agvet Codes).

The APVMA can reconsider the approval of active constituents, the registration of chemical products or the approval of labels for containers of chemical products at any time. This is outlined in Part 2, Division 4 of the Agvet Codes.

The basis for the reconsideration is whether the APVMA is satisfied:

- that the continued use of the products will not pose an unacceptable risk to people, the environment or trade;
- that the products are effective for the purposes claimed; and
- that the product labels contain adequate instructions.

A reconsideration may be initiated when new research or evidence has raised concerns about the use or safety of a particular chemical, a product or its label.

The process for reconsideration includes a call for information from a variety of sources, a review of that information and, following public consultation, a decision about the future use of the chemical or product.

In undertaking reviews, the APVMA works in close cooperation with advisory agencies including the Department of Health and Ageing, Environment Australia, the National Occupational Health and Safety Commission, State Departments of Agriculture as well as other expert advisors, as appropriate.

The APVMA has a policy of encouraging openness and transparency in its activities and community involvement in decision-making. The publication of review reports is part of that process.

The APVMA also makes these reports available to the regulatory agencies of other countries as part of bilateral agreements or as part of the OECD *ad hoc* exchange program. Under this program it is proposed that countries receiving these reports will not utilise them for registration purposes unless they are also provided with the original data from the relevant applicant.

This draft report outlines the APVMA's review of products containing the antibiotic virginiamycin and their labels. It includes information on the reasons for, as well as the scope of, the review. The review's findings and proposed regulatory approach are based on information collected from a variety of sources, including data packages and information submitted by the registrants, information submitted by members of the public including users/industry groups and government organisations, and literature searches.

The information and technical data required by the APVMA to review the safety of both new and existing chemical products must be derived according to accepted scientific principles. This also applies to the methods of assessment. Details of the APVMA's data requirements for registration are outlined in various publications, which can be purchased or obtained by contacting the

APVMA. Among these are the APVMA's *Guidelines for Registering Agricultural/Veterinary Chemicals*.

ABBREVIATIONS, ACRONYMS AND GLOSSARY

Agvet Codes	<i>Agricultural and Veterinary Chemicals Code Act, 1994</i>
APVMA	Australian Pesticides & Veterinary Medicines Authority
DANMAP	Danish Integrated Antimicrobial resistance Monitoring and Research Programme
EAGAR	National Health & Medical Research Council Expert Advisory Group on Antimicrobial Resistance
FCE	Feed conversion efficacy
HACCP	Hazard Analysis Critical Control Point
JETACAR	Joint Expert Technical Advisory Committee on Antibiotic Resistance
Kg	Kilogram
MIC	Minimum Inhibitory Concentration
MRL	Maximum Residue Limit
NCCLS	National Committee for Clinical Laboratory Standards
NDPSC	National Drugs and Poisons Scheduling Committee
ppm	parts per million
Registrant	The entity or person in whose name the notice of registration is published (the "interested person")
QD	Quinupristin/dalfopristin – structural congener of virginiamycin for human use.
R	Resistant
S	Susceptible
Susceptible humans	Those humans most likely to succumb to an infection cause by a relevant microorganism.
SUSDP	Standard for Uniform Scheduling of Drugs and Poisons

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EXECUTIVE SUMMARY

Introduction

Virginiamycin is an antibiotic of the streptogramin class. Products containing virginiamycin are registered for in-feed use in poultry, pigs, cattle, sheep and horses. The approved uses include improvement of the growth rate and feed conversion efficiency in growing pigs, poultry (broilers) and turkeys, and reduction of the risk of acidosis in cattle and sheep. Veterinarians also prescribe products containing virginiamycin for the prevention of necrotic enteritis in chickens. In horses products containing virginiamycin are used to reduce the risk of laminitis.

Products containing virginiamycin that are used in food-producing animals are under review due to specific concerns about the potential to impair the efficacy of other therapeutic antibiotics for human infections through the development of resistant strains of organisms. The review does not include the use of virginiamycin in horses. Horses are not considered to be a food-producing species and, at this time, there is no concern about the development of resistant strains of organisms or other aspects of safety and efficacy related to this use.

The review focuses on whether continued use of the products would be likely to have an effect that is harmful to human beings, whether the products are effective for the purposes claimed and whether the labels contain adequate instructions. The primary purpose of the review is to undertake an assessment of the current recommendations for use for the products and to make objective, scientifically based recommendations about the future registration of the products. Recommendations take into account the recommendations of the Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR) report released in September 1999.

Public Health assessment

In accordance with the JETACAR report the public health risk assessed in this review is the probability of disease due to infection in susceptible humans with resistant pathogens arising from the use of virginiamycin in food producing animals, **and** the consequences of such disease.

The risk assessment incorporates an evaluation of local and international data on resistance to virginiamycin and quinupristin-dalfopristin (QD) in animals and humans, and published evidence regarding the risk of transmission of virginiamycin/ QD resistance from animals to humans. The assessment is based on the Part 10 of the APVMA Vet Requirement Series (Special data requirements). Part 10 provides for a qualitative risk assessment of the possible contribution of the use pattern to antibiotic resistance in food-borne microorganisms and human pathogens, and consequent disease in susceptible humans. Part 10 shares common approaches to risk assessment and management with other international models (OMAFRA 1996; AS/NZS 1998).

The risk assessment approach includes consideration of the following:

- the hazard
- the exposure
- the impact
- uncertainty of data used in risk assessment
- the current use pattern for virginiamycin in animal production and therapy in Australia
- the risk

Draft report of the review of virginiamycin

The hazard considered was the selection of resistance genes to virginiamycin in *Enterococcus faecium* of animals. In characterizing the hazard it was noted that:

- Virginiamycin use in food producing animals can select for *E. faecium* possessing either the *vat*(D) or *vat*(E) genes, which encode for production of a streptogramin A acetyltransferase (an inactivating enzyme), resulting in virginiamycin-resistant *E. faecium* .
- Production of streptogramin A acetyltransferases confers resistance to another streptogramin - QD.
- Virginiamycin-resistant *E. faecium* found in food producing animals and their commercial products can be co-resistant to other antimicrobials, including vancomycin.

The main exposure to the hazard was considered as the transfer of streptogramin resistant *E. faecium* to humans. Based on overseas findings, the following factors were considered:

- Virginiamycin resistant enterococci also resistant to vancomycin have been cultured from minced beef and pork.
- Colonisation of humans by animal *E. faecium* and/or transfer of resistance to human *E. faecium* strains may occur.
- Virginiamycin-resistant *E. faecium* of animal origin given to human volunteers can survive gastric passage, multiply, and be cultured for up to 14 days from their stools. Intestinal transit did not result in disease.

The impact was defined as disease due to infection in susceptible humans. Susceptible humans are those humans most likely to succumb to an infection with a relevant microorganism. Factors considered included:

- infection of humans with streptogramin resistant *E. faecium*,
- infection with *E. faecium* resistant to vancomycin,
- disease due to infection with *E. faecium* resistant to vancomycin and streptogramins, and
- treatment failure attributable to acquisition of streptogramin resistant *E. faecium* from animals.

In assessing the above steps, the following were taken into account:

- Conclusive evidence of human infection with animal derived streptogramin resistant *E. faecium* is lacking.
- Vancomycin- resistant enterococci have a high propensity to cause outbreaks in hospitals.
- While the number of infections resulting from colonisation with vancomycin-resistant enterococci is low, these strains spread easily to other patients, resulting in significant numbers of infections.
- Recent Australian studies have demonstrated no resistance to quinupristin/dalfopristin in human clinical isolates.
- Septicaemia from *vanA*-type vancomycin resistant *E. faecium* mostly occurs in highly vulnerable patients who have multiple medical problems. Failure of therapy in these patients would result in significant mortality, or prolonged treatment. Currently these patients are treated with a streptogramin – QD, or the newer antibiotic linezolid.
- The impact of antibiotic failure on more minor infections such as wound infections and urinary tract infections is small.

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The quality of the data available was also taken into account, including uncertainty due to inherent variability and measurement error, as well as uncertainty due to lack of information or understanding. Data on the prevalence of virginiamycin and vancomycin-resistant *E. faecium* in food animals and the incidence of human infections in Australia are lacking. There are limited data on the importance of virginiamycin in the selection of enterococci co-resistant to both virginiamycin and vancomycin in food animals.

Acceptable risk was considered to be:

- low probability of disease due to infection in susceptible humans; and
- impact which is unlikely to significantly compromise the treatment of bacterial infections in susceptible humans.

The conclusions are that the probability of disease due to infection in susceptible humans due to exposure to streptogramin resistant *E. faecium* of animal origin is low, and the severity of impact in susceptible humans is high.

Regarding the risk to the general population, the probability of disease due to infection due to exposure to streptogramin resistant *E. faecium* of animal origin is low, and the severity of impact in the general population is low.

Efficacy assessment

The outcome from studies examining the efficacy of virginiamycin in improving rates of growth and feed conversion efficiency in poultry and pigs was variable. While most studies demonstrated that recommended use rates of virginiamycin had a significant effect on weight gain and/or feed conversion efficiency, some studies were unable to demonstrate a measurable response. In the absence of contemporary Australian studies corroborating the efficacy of virginiamycin and recognising that substantial changes in the management of pigs and poultry, improvements in diet formulation and advances in the genetic selection of both species have occurred since virginiamycin in feed as a growth promotant in pigs and poultry is considered not to be supported.

In relation to preventative/therapeutic uses, data support the use of virginiamycin as an aid to reduce the risk of lactic acidosis in sheep fed grain on a weekly or twice weekly basis, and as an aid to control lactic acidosis in the initial month of adaptation of feedlot cattle to a high percentage grain diet. Instructions on currently approved labels are inadequate in this respect. The labels must provide more specific instructions with regard to the circumstances in which the product should be used, how the product should be used, the period for which the product should be used and the frequency of product use.

Necrotic enteritis is a common, frequently fatal gastrointestinal disease problem in poultry induced by toxigenic strains of *Clostridium perfringens*. It is particularly prevalent in broiler chickens on wheat-based diets. The data indicate that registered products containing virginiamycin are efficacious in controlling necrotic enteritis in chickens.

Recommendations

Based on the information assessed and as outlined, the APVMA cannot be satisfied that currently approved labels carry adequate instructions with regard to:

- the circumstances in which the products should be used;
- the times when the products should be used;
- the duration of any treatment using the product; and
- the frequency of the use of the product.

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However, the APVMA can be satisfied that the conditions to which the approval of labels for products for therapeutic uses in sheep, cattle and chickens are currently subject can be varied in such a way that the requirements for continued approval of the labels will be complied with.

The APVMA can be satisfied that the continued use of products containing virginiamycin for prophylactic/therapeutic uses in sheep, cattle and chickens in accordance with varied conditions of label approval:

- would not be likely to have an effect that is harmful to human beings; and
- are effective for the purposes claimed.

The APVMA cannot be satisfied that the conditions of product registration and label approval can be varied for products that are registered for improvement of growth rate and feed conversion efficiency in pigs, poultry and turkeys. There is insufficient data to qualify the circumstances in which the products should be used, the times when the product should be used or the duration of the treatment.

The APVMA proposes to address the potential risks identified in this report using the following regulatory actions.

(i) Vary conditions of label approval for products containing virginiamycin registered for the prevention of lactic acidosis in sheep and cattle and the prevention of necrotic enteritis in chickens by:

- the deletion of all label claims, indications and use patterns that pertain to growth promotion, and/or improved feed efficiency in pigs, poultry and turkeys; and
- the addition of more specific instructions and restraints statements pertaining to their use for the prevention of lactic acidosis in sheep and cattle and necrotic enteritis in chickens. The proposed label instructions relate to the circumstances in which the product should be used, how the product should be used, the times when the product should be used, the duration of any treatment using the product and the frequency product use. The proposed label restraints constrain veterinarian rights to prescribe the antibiotic for periods longer than specified on the label or to prescribe repeat treatments. Should situations arise where it is deemed to be necessary to prescribe the products for a period longer than specified on the label or to prescribe a repeat treatment (for example, for animal welfare reasons) the APVMA may issue a permit to allow a person or organisation to use a product containing virginiamycin in a way not authorised by the approved label.

(ii) Affirm registration of products containing virginiamycin registered for the prevention of lactic acidosis in sheep and cattle and the prevention of necrotic enteritis in chickens.

(ii) Cancel the registration and label approvals of those products that have label claims pertaining to growth promotion and/or improved feed efficiency only. The APVMA is not satisfied that these products are effective for this purpose or that conditions of registration or approval can be varied to satisfy the APVMA's concerns.

SECTION A: REASONS AND SCOPE OF THE REVIEW

1 Current use of virginiamycin in animal health in Australia

Virginiamycin is an antibiotic of the streptogramin class. Products containing virginiamycin are registered for in-feed use in poultry, pigs, cattle, sheep and horses. The approved uses include improvement of the growth rate and feed conversion efficiency in growing pigs, poultry (broilers) and turkeys, and reduction of the risk of acidosis in cattle and sheep. In horses products containing virginiamycin are used to reduce the risk of laminitis.

Products containing virginiamycin may fall into one of two poisons schedule classification, which determines whether they are freely available to users or require prescription. Animal feed premixes or animal feed additives containing 2 per cent or less of virginiamycin or when mixed in individual sachets containing 20g or less of virginiamycin are classified in Schedule 5 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). Schedule 5 is for substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label. Five of the six virginiamycin products under review are currently classified under Schedule 5 (see Table 1).

All forms of virginiamycin other than those that fall into Schedule 5 and other than animal feeds for growth promotion containing 50mg/kg or less of antibiotic substance are classified in Schedule 4 of the SUSDP. Schedule 4 is for substances requiring prescription by qualified practitioners (in this case veterinarians). One of the products under review contains 500g/kg virginiamycin and is a Schedule 4 product (see Table 1).

The products are generally used in long-term low dose regimens. They are used continuously in the feed of broilers, turkeys, pigs, cattle, and sheep at the inclusion rates of 5-20g/tonne of feed. Instructions for use on product labels provide no direction with regards to the period for which the product should be used or the frequency of product use.

Veterinarians also prescribe products containing virginiamycin for the prevention of necrotic enteritis in chickens. A claim for a 21 day treatment regimen has recently been approved and is now included on the label of one registered product (requiring veterinary prescription).

2 Reasons for the reconsideration of registrations and approvals relating to virginiamycin

The decision to review products containing virginiamycin stems from concerns over human health.

The Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR) in its report ‘The use of antibiotics in food producing animals: antibiotic-resistant bacteria in animals and humans’ (JETACAR 1999) recommended that the use of virginiamycin in food-producing animals be reviewed by the APVMA. JETACAR was established in 1997 by the, then, Minister for Health and Family Services and the Minister for Primary Industries and Energy. This committee broadly reviewed the use of antibiotics in food-producing animals and the occurrence of antibiotic resistance and its importance in human and veterinary medicine. The basis to the JETACAR recommendation for the review of virginiamycin in food-producing animals was evidence for:

- the emergence of resistant bacteria in humans and animals following antibiotic use;
- the spread of resistant animal bacteria to humans;

- the transfer of antibiotic-resistance genes from animal bacteria to human pathogens; and
- resistant strains of animal bacteria causing human disease.”

The APVMA decided in July 2000 to review products containing virginiamycin that are used in stockfeed for food-producing animals as a growth promotant and/or for disease prevention. The basis for its decision was concern that the continued registration of the products in accordance with the current recommendations for use would not be likely to have an effect that is harmful to human beings. An additional concern was whether the products were effective for the purposes claimed and that the labels contained adequate instructions.

The Commonwealth Government endorsed the JETACAR report and the APVMAs commitment to review antibiotic growth promotants in August 2000 (The Commonwealth Government response to the report of the Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR), August 2000).

3 Overseas regulatory status

3.1 Regulatory status in the EU

In the European Union virginiamycin was approved and regulated as a feed additive for growth promotant purposes in pigs and poultry. In 1998 the Council of the European Union withdrew the authorization for the in-feed growth-promotant use of several antibiotics including virginiamycin. This regulation did not affect any prophylactic or therapeutic uses of antibiotics in food animals, however virginiamycin is not approved for such uses in food animal species in Europe and does not have an established MRL.

The decision to withdraw the growth promotant use of virginiamycin was made despite advice from its scientific advisory committee that there was insufficient evidence regarding the transfer of bacterial resistance from livestock to humans. Pfizer Animal Health SA, as the only producer of virginiamycin in the world, challenged the Council's decision in European Courts.

The Court of Justice however upheld the decision in 2002 concluding that, despite uncertainty as to whether there was a link between the use of antibiotic additives and increased resistance to those antibiotics in humans, the ban on the products was not a disproportionate measure given the need to protect public health. In doing so the Court set out the conditions on which the precautionary principle may be applied.

3.2 Regulatory status in the US

In the US products containing virginiamycin are approved and regulated as feed additives and are used for therapeutic and growth promotant purposes in chickens, pigs, cattle and turkeys.

In April 2000 the Food and Drug Administration's Center for Veterinary Medicine decided to conduct a risk assessment on human health impacts associated with the use of virginiamycin in food-producing animals in particular in relation to the development of resistant organisms. Updates on the progress of that risk assessment have not been published to date.

However, in February 2002 the US Senate introduced a bill titled the "Preservation of Antibiotics for Human Treatment Act of 2002". The House of Representatives introduced its version of the bill in May 2002. The bill proposes to cancel the registration of non-therapeutic feeding of medically important antibiotics to livestock unless registrants can demonstrate that these uses do not

contribute to the development of antibiotic resistance affecting humans. The bills have been sent to committees for consideration.

3.3 Regulatory status in New Zealand

In New Zealand virginiamycin is approved for use in horses only and is classified as a prescription drug. New Zealand have completed a review of virginiamycin and concluded that mass medication of food producing animals for therapeutic use could only be considered if it is substantiated that the use is essential for the welfare of animals.

4 The scope of the reconsideration of registrations and approvals relating to virginiamycin

The JETACAR report recommended that the use of products containing virginiamycin be reviewed in terms of their impact on human and animal health, using a risk analysis approach, including cost-benefit analysis. The report offers criteria whereby antibiotics used in stockfeed as a growth promotant or in routine uses of similar duration and dose (such as for disease prevention) would not be likely to have an effect that is harmful to human beings. These criteria are that the products:

- are of demonstrable efficacy in livestock production under Australian farming conditions;
- are rarely or never used as systemic therapeutic agents in humans or animals, or are not considered critical therapy for human use; and
- are not likely to impair the efficacy of any other prescribed therapeutic antibiotic or antibiotics for animal or human infections through the development of resistant strains of organisms.

The review covers the human health aspects as well as the efficacy of products containing virginiamycin. The review covers those products that are used in food-producing species and product labels. The scope of the review does not include the use of virginiamycin in horses. Horses are not considered to be a food-producing species and at this time there is no concern about the development of resistant strains of organisms or other aspects of safety and efficacy related to this use.

The registered products subject to reconsideration and review are shown in Table 1.

Table 1 Registered products and their labels subject to reconsideration

PRODUCT NUMBER	PRODUCT NAME	REGISTRANT	LABEL CLAIMS and POISONS SCHEDULE CLASSIFICATION
38329	Stafac 20 Feed Premix	Phibro Animal Health Pty Ltd	For broilers, turkeys and growing pigs to improve growth and feed conversion efficiency. Schedule 5
41286	Virginiamycin 20	Agribusiness Products Pty Ltd	Improve growth rate and feed conversion efficiency in growing pigs, broilers and turkeys Schedule 5
41476	Virginiamycin 20	Lienert Australia Pty Ltd	Growth promotion and improved feed conversion efficiency in growing pigs Schedule 5
46049	Eskalin Feed Premix for Cattle	Phibro Animal Health Pty Ltd	Reduce risk of acidosis in cattle Schedule 5
49111	Eskalin Wetable Powder Spray-on Feed Premix	Phibro Animal Health Pty Ltd	Reduce risk of acidosis in cattle and sheep Schedule 5
51354	Eskalin 500 Feed Premix	Phibro Animal Health Pty Ltd	Reduce risk of acidosis in cattle and to improve growth and feed conversion efficiency in broilers, turkeys and growing pigs Schedule 4

SECTION B: PUBLIC HEALTH ASSESSMENT

5.1 Introduction

The purpose of this public health risk assessment is to determine the risks to human health with respect to antimicrobial resistance posed by the current use of virginiamycin in Australian livestock.

In conducting the public health assessment, the APVMA sought advice from the Expert Advisory Group on Antimicrobial Resistance of the National Health and Medical Research Council within the Department of Health and Ageing (EAGAR).

The risk assessment incorporates an evaluation of local and international data on resistance to virginiamycin and QD in animals and humans, and published evidence regarding the risk of transmission of virginiamycin/ QD resistance from animals to humans. The assessment is based on the Part 10 of the APVMA Vet Requirement Series (Special data requirements). Part 10 provides for a risk assessment (qualitative, semi-quantitative or quantitative) of the possible contribution of the use pattern to antibiotic resistance in food-borne microorganisms and human pathogens, and consequent disease in susceptible humans. Part 10 shares common approaches to risk assessment and management with other international models (OMAFRA 1996; AS/NZS 1998).

The public health risk assessed in this review is the probability of disease due to infection in susceptible humans with resistant pathogens arising from the use of virginiamycin in food producing animals, **and** the consequences of such disease.

Acceptable risk was considered to be:

- low probability of disease due to infection in susceptible humans; and
- impact which is unlikely to significantly compromise the treatment of bacterial infections in susceptible humans.

5.2 Terminology

The terminology used in this risk assessment is consistent with Part 10 of the APVMA Vet Requirement Series (Special data requirements).

Hazard is defined as: *antibiotic resistant microorganisms or their transferable genetic elements (that have the potential to transfer to humans) within an animal species, arising from the use of an antibiotic in an animal species.*

Exposure is defined as: *the amount and frequency of exposure of susceptible humans to antibiotic-resistant microorganisms (or their transferable genetic elements) from animal sources.*

Assessment of the uncertainty of the data used in risk assessment is:

- a) *Uncertainty due to inherent variability and measurement error.*
- b) *Uncertainty due to lack of information or understanding.*

Impact is defined as: *impact of infections (caused by antibiotic-resistant pathogens of animal origin) in susceptible humans.*

Risk is defined as: *Probability of disease due to infection in susceptible humans after exposure of humans to antibiotic-resistant microorganisms (and genetic material) of animal origin **and** the severity of the impact of exposure on susceptible humans.*

Susceptible humans are defined as: *Those humans most likely to succumb to an infection cause by a relevant microorganism.*

5.3 The methodology of assessment

The risk assessment includes consideration of information, studies or discussion (where relevant to the target animal species) of the following areas:

- the hazard
- the exposure
- the impact
- uncertainty of data used in risk assessment
- current preventative/therapeutic use of antibiotic in Australian animal health
- the risk

The methods of this risk assessment were to access information, studies or discussion, so as to :

- examine the current registered uses of virginiamycin in Australia
- examine available local and international data on resistance to virginiamycin and quinupristin-dalfopristin in animals and humans
- examine the published evidence regarding the risk of transmission of virginiamycin/ QD resistance from animals to humans
- make recommendations about virginiamycin products taking into account recommendations of the JETACAR report (JETACAR 1999).

5.4 Information sources

Information was sourced from refereed literature, personal communications, public submissions, and data provided by registrants. The data was assessed for adequacy by EAGAR. Incomplete information was assessed as such, with data uncertainty being assessed as part of the risk characterization process.

5.5 Assumptions

The assessment made the following assumptions:

- that the hazards, exposure, impact and risk characterization are essentially similar between the animal species.
- that genetic resistance material can transfer from animal enterococci to human enterococci.

5.6 Background to the risk assessment

5.6.1. Streptogramins

Streptogramins (A and B) include virginiamycin, pristinamycin and quinupristin-dalfopristin (QD, brand name Synercid®).

5.6.1.1 Mode of Action

All currently marketed products containing streptogramins are combinations of A and B components, which act synergistically at the ratios used in the formulations. Streptogramins are protein synthesis inhibitors, acting at the level of the 50S subunit of the ribosome. Streptogramins B act at the same site as macrolides and lincosamides, and can be affected by the same resistance mechanisms. Streptogramins A binding at a separate site on the ribosome have both intrinsic antimicrobial activity and enhance the effect of the streptogramin B component. More detailed information on the mode of action of streptogramins is provided in Appendix 1.

5.6.1.2 Spectrum of activity

Virginiamycin exhibits antibiotic activity against the following:

Gram-positive bacteria, aerobic and anaerobic except most strains of *Enterococcus faecalis* (and some uncommon enterococcal species), *Nocardia asteroides*, some viridans streptococci, *Streptococcus agalactiae* and *Clostridium fallax*.

Susceptible Gram-negative bacteria include most *Haemophilus* species, *Neisseria gonorrhoeae* and *N. meningitidis*.

Lawsonia intracellularis, *Brachyspira* species and some species of Mycoplasma are susceptible to virginiamycin.

5.6.1.3 Mechanisms of Resistance

Resistance to the streptogramins is mediated by enzymatic modification of the antibiotics, active transport/ efflux or alteration in the target site. There is a range of genes responsible for these mechanisms in both staphylococci and enterococci. Some mechanisms of resistance are poorly understood. Further information on the mechanism of resistance is provided in Appendix 1.

5.6.1.4 Streptogramin use in Humans

Only one streptogramin product for human use is registered in Australia, since 2000. The product is quinupristin-dalfopristin (QD, brand name Synercid® registered by Rhone-Poulenc Rorer (now merged as Aventis)), and is available in an injectable form only. The approved indications of QD in Australia are:

“Treatment of suspected or proven Methicillin Resistant Staphylococcus aureus or Vancomycin Resistant Enterococcus faecium infections requiring intravenous therapy where other antibiotics are inappropriate. Synercid can be used for the above indications in β -lactam, quinolone or glycopeptide intolerant patients.”

Although it is currently considered to be one of the drugs-of-choice for serious vancomycin-resistant *Enterococcus faecium* infections, Synercid® is not widely used. It has a very high acquisition cost, significant venous irritation almost mandating the use of a central line, and about a 15% incidence of myalgia, necessitating cessation of therapy in a proportion of patients (Aventis Pharma Pty Ltd, and Synercid® Product Information).

5.6.1.5 Other Related Human Agents

Pristinamycin has been a minor use oral antibiotic available in France for many years. This antibiotic is not registered for use in humans in Australia. However, occasional patients have been treated in Australia under the Special Access Scheme. It has principally been used for the treatment of patients with methicillin-resistant *S. aureus* infections when all other agents have been unsuitable.

5.6.1.6 Streptogramin use in Animals in Australia

Virginiamycin is the only streptogramin registered for use in cattle, sheep, horses, pigs and poultry.

5.6.2. Use Pattern including Indications and Species

Virginiamycin is used in long-term low dose regimens. Virginiamycin has been used as a growth promotant and prophylactic agent. It is used continuously in the feed of broilers, turkeys, pigs, cattle, and sheep at the inclusion rates of 5-20g/tonne of feed. Its primary uses include improving the growth rates and feed efficiency in pigs, broilers and turkeys, and preventing lactic acidosis in cattle and sheep, and laminitis in horses. Veterinarians also prescribe products containing virginiamycin for the prevention of necrotic enteritis in chickens.

Detailed assessment of whether the product are effective for the purposes claimed can be found in Section C - Efficacy assessment.

5.7 Hazard characterisation

5.7.1 Bacterial resistance

5.7.1.1 Resistance hazard

The principal resistance hazard is *E. faecium* resistance to virginiamycin and cross resistance to QD, via the *vat(D)* and *vat(E)* resistance genes. These genes encode for an acetyl transferase enzyme that inactivates the streptogramin A component of both virginiamycin and the dalfopristin of the quinupristin/dalfopristin combination (Werner *et al* 2000; Werner *et al* 1998; Aarestrup *et al* 2000.)

5.7.1.2 Secondary resistance hazard

Virginiamycin also confers cross resistance to the macrolides, lincosamides and the streptogramins by other mechanisms – so called MLS_B group (Leclercq and Courvalin, 1991 and 1991a). This cross-resistance, and the resistance genes involved are more fully described in Appendix 1.

5.7.1.3 Resistance Breakpoints

Enterococcal resistance to virginiamycin is currently defined as minimum inhibitory concentration (MIC) values of ≥ 4 mg/L. This value is based on population distribution of MIC values and is used for DANMAP resistance surveillance in Denmark (Aarestrup *et. al*, 2000). According to DANMAP 2001, the breakpoint is now ≥ 8 mg/L (DANMAP 2001). QD resistance breakpoints have been established by the NCCLS for enterococci at ≤ 1 mg/L susceptible, 2mg/L intermediate, and ≥ 4 mg/L resistant. NCCLS assigned the same values to staphylococci and streptococci. More recently, the British Society for Antimicrobial Chemotherapy has selected a breakpoint of ≤ 2 mg/L for susceptible, ≥ 4 mg/L for resistant. Some of the differences in selection of breakpoints by different authorities may be related to the use of different methods and media. Data from a variety of sources on MIC distributions for virginiamycin and QD are tabulated in Appendix 2. All resistant strains have an MIC above 2mg/L. The NCCLS QD breakpoints for *E. faecium* result in a proportion of possibly normal strains testing as intermediate (MIC = 2mg/L). It is less clear whether *E. faecium* strains with a MIC of 2mg/L are abnormal and harbour resistance determinants such as the *vat(D)* or *vat(E)* genes.

5.7.1.4 Bacterial species likely to be affected

It is possible for virginiamycin resistance to be selected in a wide range of intestinal Gram-positive bacteria. The most important bacteria with respect to human health that are likely to be affected are those in which multi-resistance is already present, especially vancomycin-resistant *E. faecium*. QD is currently considered the drug of choice for vancomycin-resistant *E. faecium* infections in humans. Therefore, selection of virginiamycin resistance in vancomycin-resistant *E. faecium* and transmission of such strains through the food chain could represent a significant risk to humans.

Co-selection of organisms having resistance to an unrelated drug is a well-described phenomenon of antimicrobial use. There is no evidence that virginiamycin use selects for vancomycin resistance unless vancomycin resistance is already present. Unrelated resistance genes located together on transmissible genetic elements have been described for many antimicrobials and have contributed to the persistence of resistance well after the withdrawal of an agent from use (Enne *et al.*, 2001).

5.7.1.5 Resistance data in targeted animal bacterial species

Resistance of animal strains of indicator organisms of relevance to humans to virginiamycin has only been studied in enterococci, in particular *E. faecium*. In countries where studies have been conducted such as Germany, Denmark and the US (Werner *et al*, 2000; Aarestrup *et al* 2000; Welton *et al*, 1998), resistance to virginiamycin appears to be very common in this species.

There are no data on virginiamycin resistance in lactobacilli, streptococci or *Clostridium perfringens* of animal origin.

One study has shown a relationship between use of products containing virginiamycin and resistance. In Denmark where products containing virginiamycin was used widely until 1998, a high proportion of *E. faecium* from broilers (70%) and pigs (49%) were resistant (Aarestrup *et al* 2000).

5.7.1.6 Resistance data in human bacterial species

Recent studies from Australia have demonstrated no acquired resistance to quinupristin/dalfopristin in human clinical isolates (Turnidge & Bell, 1999)

Species (number of isolates)	%Quinupristin/ Dalfopristin R	% Erythromycin R	% Clindamycin R
<i>S. aureus</i> – oxacillin S (150)	0	10.0	2.6
<i>S. aureus</i> – oxacillin R (101)	0	86.1	57.4
Coagulase-negative staphylococci – oxacillin S (67)	0	11.9	3.0
Coagulase-negative staphylococci – oxacillin R (97)	0	58.8	16.5
<i>Enterococcus faecium</i> (108)	0	82.4	NT (100)

S- susceptible

R- resistant

NT- Not tested

Data on QD resistance in *E. faecium* from 200 sites in North America in 1996 showed the following (Jones *et al.*, 1998):

Subset-N	Test Method	MIC (µg/ml)			Zone (mm)	Diameter Range	Number QD R** isolates
		50%	90%	Range	Median		
Vancomycin-susceptible							
306	MIC	0.5					1
186	Disc						0
Vancomycin-resistant							
326	MIC	0.5	1	0.12-2			0
193	Disc				25	15-32	1

** Interpretative criteria for resistance as described by NCCLS (1998)

No highly resistant strains of *E. faecium* were detected, instead there were a small proportion of strains with MICs 1-2 fold higher than the breakpoint MIC of 1mg/L, and only 2 authenticated resistant strains.

Several studies have noted that part of the difficulty of interpreting data on resistance in *E. faecium* relates to difficulties with identification. Routine biochemical tests can result in misidentification of *E. faecium* as *E. faecalis* and vice versa. As *E. faecalis* is naturally resistant to streptogramins, this can result in some resistance in *E. faecium* in published studies being due to the inclusion of misidentified *E. faecalis*.

The emergence of resistance to quinupristin-dalfopristin during treatment has been summarised (Dowzicky *et al*, 2000). Dowzicky *et al* studied the strains isolated pre- and post-therapy in the worldwide phase III studies of QD. One of 453 or 0.2% of strains in comparative studies (a strain of methicillin-resistant *Staphylococcus aureus*), and 6 of 338 or 1.8% of strains from non-comparative studies (all vancomycin-resistant *E. faecium*) became resistant during treatment.

5.7.1.7 Cross-resistance data in animal isolates

Data on cross-resistance in animal isolates are available for *Enterococcus faecium*:

Animal and raw meat quinupristin-dalfopristin resistant enterococci from Western Europe (n=22) were demonstrated to have *vat*(D) and *vat*(E) resistance genes, which encode for acetyl transferases for streptogramin A. *vat*(E) was detected on plasmids. The *erm*(B) gene, which alters the target site for streptogramin B, was detected in 82% of the isolates (Soltani *et al*, 2000).

In poultry from the eastern seaboard of the USA. - 21/27 (78%) of *E. faecium* isolated from poultry transport containers/ litter samples and 21/41 (51%) from cloacal swabs were resistant to QD (Hayes *et al*, 2001).

Three turkey flocks were sampled in the USA where virginiamycin was in use. 125 cloacal cultures were obtained. *E. faecium* was isolated from 22-38% of the cultures depending on the age of the flock. QD resistance increased from 23% in the youngest birds (24 days) to 100% in the oldest (130days) (Welton *et al* 1998).

Turkeys, pigs, sewage water treatment, broiler carcasses, and pork samples were sampled in Germany. Farms with and without the use of virginiamycin were sampled. No QD resistant enterococci were found despite enrichment techniques in the samples from the farms not using virginiamycin. All manure samples from the turkey (2) and pig (6) farms using virginiamycin yielded QD resistant *E. faecium*. 11/24 (46%) poultry carcasses were positive for QD resistant *E. faecium*. Only 1 isolate was found to be resistant from 10 samples of raw pork (Werner *et al*, 2000).

5.7.1.8 QD cross-resistance data in human isolates

Data on QD cross-resistance in human isolates are available for *Enterococcus faecium*:

In Taiwanese data prior to clinical use of QD – (1287 clinical isolates), there were 100 isolates of vancomycin resistant *E. faecium* of which 51% were resistant (MIC \geq 8mg/L) and 15% (MIC 4mg/L) were intermediate for QD. Virginiamycin is used as a growth promoter in Taiwan. *E. faecium* was identified using biochemical tests – not molecular methods that are known to be superior in distinguishing *E. faecalis* from *E. faecium*. Resistance mechanisms were not determined (Luh *et al* 2000).

One hundred *E. faecium* (ID method not specified) clinical isolates from 10 North American medical centres were collected in 1996-97 (prior to registration of QD in USA). Fifteen percent were classified as either intermediate resistance (2mg/L – 9%) or resistant (\geq 4mg/L – 6%). Resistance mechanisms were not determined (Barry *et al*, 1998). In the United Kingdom, clinical isolates from 30 centres were tested in 1996-97. Thirty one *E. faecium* (mostly from blood cultures – ID biochemical) were tested of which one isolate (3.2%) was resistant ($>$ 2mg/L)(Andrews *et al* , 2000).

SENTRY is a global surveillance program that has included QD in testing using NCCLS methods since 1997 in North America, and since 1998 in the Western Pacific and Europe. SENTRY European data (1997-98) detected no resistance in 90 isolates of *E. faecium* from 20 University

hospitals (Schmitz *et al*, 1999). QD resistance developed in 6 vancomycin-resistant *E. faecium* (1.8% of evaluable cases) during the pre registration trials of QD (Dowzicky *et al*, 2000). SENTRY Data from the Western Pacific for 1998-2000 encountered 4 QD resistant strains among 121 strains of *E. faecium* (3.3%). None of these strains were from Australia (Jan Bell, personal communication)

5.7.1.9 Cross-resistance data in human isolates to other streptogramins

Pristinamycin has been used in France for more than 25 years. Resistance has been reported either in low levels ($\leq 5\%$) in resistance surveillance programs on MRSA (Schmitz *et al* 1999a) or in association with clonal spread during a hospital outbreak of staphylococcal disease (Arpin *et al* 1996). Reports on surveillance of enterococcal resistance (either screening or clinical infection) are not available in the literature.

5.7.1.10 Data on co-selection of resistance to unrelated antibiotics

Published data from Germany and Denmark shows that co-resistances to virginiamycin and other antibiotics in animal isolates of *E. faecium* are common, suggesting that co-selection of resistance is possible (Werner *et al*, 2000; Aarestrup *et al* 2000). Investigators in Denmark have monitored the prevalence of *vanA E. faecium* following the cessation of avoparcin use in 1995 and the subsequent cessation of use of virginiamycin in 1998 (Bager *et al*, 1999; Aarestrup *et al* 2001). They showed steady decline in *vanA E. faecium* in broiler chickens and pigs despite a four-fold increase in virginiamycin usage between 1995 and 1997. Virginiamycin did not result in persistence of *vanA E. faecium* despite increasing use.

Cessation of virginiamycin use was associated with reduced rates of macrolide resistant *E. faecium* in both chickens and pigs. Rates of resistance to virginiamycin have also declined, but not to the same extent.

5.7.1.11 Dose-response assessment

No information could be found describing the relationship between the frequency and magnitude of exposure of humans (dose) to antibiotic – resistant food-borne microorganisms and the severity and/or frequency of the impact (response). No information could be found estimating the critical threshold of exposure required to cause infection in susceptible humans.

5.8 Exposure

5.8.1. Exposure of animal bacteria to the antibiotic

5.8.1.1 Volume of streptogramin imports to Australia

Import volumes of streptogramins into Australia (in kilograms) for the financial years 1992-3 to 1996-7 were published in the JETACAR report. The figures are reproduced in Appendix 3 and range from 17000 kg in 1992-1993 to 36005kg in 1996-1997. The preliminary figure for the financial years 1997-98 is 59,172 kg, for 1998-99 0kg and for 1999-2000 65,000kg. Import volumes appear to be consistently increasing.

5.8.1.2 Volume of use in animals

There are no firm data about end usage in animals in Australia. It is understood that usage has been consistent and at a moderate level in meat chickens and feedlot cattle.

5.8.1.3 Use pattern in animals

Virginiamycin is used continuously in the feed of broilers, turkeys, pigs and cattle at the inclusion rates of 5-20g/tonne of feed.

5.8.1.4 Use pattern in animals with respect to potential to select for resistance

The use pattern in animals is such that resistance selection pressure is likely to be high. Continuous inclusion of virginiamycin in feed at rates of 5-20g/tonne is likely to generate gut levels that are close to the MIC of *E. faecium*. Such concentrations have been shown *in vitro* to be the more likely to select for resistance based on the standard laboratory technique for selecting resistant mutants.

5.8.2. Exposure of humans to resistant animal bacteria or transferable genetic elements

5.8.2.1 Likely routes of bacterial transmission to humans

It is possible that virginiamycin-resistant *E. faecium* could spread to humans via the food chain. It is not known whether the resistance is spread through colonisation of humans with resistant animal strains, or via the transfer of resistance genes from animal to human enterococci in the human gut. In Germany virginiamycin intermediate and resistant strains have been detected in enterococci also resistant to vancomycin cultured from minced beef and pork (Klein *et al*, 1998).

5.8.2.2 Viability of animal *E. faecium* strains in human intestine

A recent Danish study examined the effect on volunteer of a virginiamycin-resistant strain of *E. faecium* (6 subjects) as well as a vancomycin-resistant strain (6 subjects) (Sorensen *et al*, 2001). Resistant strains were detected in all subjects until day 5 after ingestion and in one subject at day 14. The authors concluded virginiamycin-resistant *E. faecium* can survive gastric passage, multiply, and be isolated for up to 14 days. Intestinal transit did not result in disease. This study did not examine long-term carriage or gene transmission.

A very recent study from China demonstrated the transmission of a pathogenic strain of *E. faecium* from pigs to humans, infection with this pathogen resulting in the deaths of thousands of pigs and twelve humans (Lu *et al*, 2002). Transmission was confirmed by 16S RNA gene sequencing and genomic DNA analysis by pulsed-field gel electrophoresis.

5.8.2.3 Mitigation procedures likely to reduce bacterial transmission to humans

Hazard Analysis Critical Control Point (HACCP) procedures in food processing reduce the likelihood of carcass contamination by resistant bacteria. High standards of food hygiene will reduce human exposure.

5.8.2.4 Residual concentrations in food

Virginiamycin is poorly absorbed from the gastrointestinal tract and concentrations in animal tissues for human consumption are undetectable or negligible by standard methods. Maximum Residue Limits (MRL's) for virginiamycin are published in the APVMA's MRL Standard (www.APVMA.gov.au). For some food commodities the MRL is set "at or about" the limit of analytical quantitation.

5.9 Impact

5.9.1 Importance of class of antibiotic in human medicine

Virginiamycin is not used in human medicine, however, a structurally related streptogramin, quinupristin/dalfopristin (QD) was introduced specifically for the treatment of multi-resistant Gram-positive infections. It is currently considered the drug of choice for the treatment of *vanA*-type vancomycin resistant *E. faecium*. Teicoplanin is the drug of choice for *vanB*-type *E. faecium*, although strains resistant to teicoplanin have occasionally emerged during treatment.

QD is not the sole agent for treating vancomycin-resistant enterococcal infections, a new agent, linezolid, has now been registered for human use in Australia, and appears to have equivalent

efficacy. Recently published studies from the United States demonstrating that enterococcal resistance to linezolid emerges comparatively rapidly (Gonzales *et al*, 2001; Jones *et al*, 2002), and a small hospital outbreak has just been described (Herrero *et al*, 2002). It appears unlikely that linezolid will completely replace QD in the short term, becoming the drug of choice for *vanA*-type *E. faecium*.

Three further novel agents for human use, oritavancin (a glycopeptide), tigecycline (a glycylcycline), and daptomycin (a lipopeptide) are currently in clinical trial stage and offer potential further options for the treatment of multi-resistant Gram-positive infections. None of these novel agents has an analogue used for veterinary or agricultural purposes.

5.9.1.1 Residual Importance of disease

Enterococcal infections infrequently cause disease (complicated urinary tract infection, and rarely, endocarditis) in the general human population. Enterococcal infections occur more frequently and importantly in hospital-acquired infections, affecting urinary tract infections, wounds (almost always associated with other bacteria) and may result in septicaemia.

Vancomycin-resistant enterococci emerged as causes of infection in Australia from 1994 (Bell *et al*, 1998). They are found most frequently in hospital-acquired infections and are prominent in immuno-compromised dialysis, haemato-oncology and liver transplant patients vulnerable to septicaemia (susceptible humans). Cross-infection is a major problem with these resistant strains, resulting from intensive use of selecting antibiotics such as the cephalosporins, glycopeptides and metronidazole. The natural resistance of enterococci, coupled with vancomycin resistance, sometimes leaves only one antibiotic for treatment.

There are four major varieties of vancomycin-resistant enterococci in humans, determined by the two most common resistance elements, *vanA* and *vanB*, and the two common species, *E. faecalis* and *E. faecium*. Vancomycin-resistant *E. faecium* strains are more commonly found in hospital patients than vancomycin-resistant *E. faecalis* strains, possibly due to the higher prevalence of other resistances harboured by *E. faecium*. All four common types behave similarly in terms of which patients are vulnerable and the types of infection caused.

Current evidence shows that only *vanA E. faecium* can be linked to food animals. There has been one isolate of *vanB E. faecalis* from an animal source in Australia, the significance of which remains uncertain (Butt *et al*, 1997). *vanA E. faecium* represent approximately 20% of all clinical isolates of vancomycin-resistant enterococci in Australia (Bell *et al*, 1998).

Vancomycin-resistant enterococci have a high propensity to cause outbreaks of hospital-acquired colonisation and infection. Outbreaks of vancomycin-resistant enterococci are being seen with increasing frequency in Australia, with most adult teaching hospitals having experienced at least one outbreak or several sporadic cases. When outbreaks do occur, infection control efforts are greatly intensified adding significant costs to the hospital. Furthermore, vancomycin-resistant enterococci have a considerable capacity to spread to other hospitalised patients. Consequently, although the proportion of colonised patients who actually become infected is low, the large number of colonised patients ensures that the number of infected patients is significant (J. Turnidge, *pers.comm.*)

5.9.1.2 Residual Impact of failure of antibiotic treatment in humans

Septicaemia from *vanA E. faecium* mostly occurs in highly vulnerable patients who have multiple medical problems. Aggressive early antibiotic therapy is considered mandatory for this infection. Failure of therapy in these patients will result in significant mortality, or prolonged treatment. The

impact of antibiotic failure on more minor infections such as wound infections and urinary tract infections is small.

5.10 Assessment of the uncertainty of the data used in risk assessment

5.10.1.1 Uncertainty due to inherent variability and measurement error.

It is not clear whether strains of *E. faecium* with so –called intermediate resistance (MIC 2mg/L) to virginiamycin are truly resistant and possess relevant resistance genes.

5.10.1.2 Uncertainty due to lack of information or understanding.

Accurate data on virginiamycin and vancomycin-resistant *E. faecium* prevalence in food animals and incidence of human infections in Australia are lacking. There are limited data on the importance of virginiamycin in the selection of enterococci co-resistant to both virginiamycin and vancomycin in food animals.

Transfer of resistant enterococci through milk is possible but has not been investigated. Enterococci are heat tolerant and are expected to survive standard pasteurisation.

5.11 Summary

The following table summarises the key steps in the risk chain from hazard (streptogramin resistant *E. faecium* in animals) via exposure to impact. Taking into account the uncertainty of data, and the preventative and therapeutic uses of the product, the probability and severity of the impact – the risk- is characterised.

Factors considered	Findings
Hazard	
Selection of resistance genes to virginiamycin in <i>E. faecium</i> of animals.	<ul style="list-style-type: none"> • Virginiamycin use in food producing animals can select for <i>E. faecium</i> possessing either the <i>vat(D)</i> or <i>vat(E)</i> genes, which encode for production of a streptogramin A acetyltransferase (an inactivating enzyme), resulting in virginiamycin-resistant <i>E. faecium</i> (VirREF). • Production of streptogramin A acetyltransferases confers resistance to another streptogramin - quinupristin/dalfopristin. • VirREF found in food producing animals and their commercial products can be co-resistant to other antimicrobials, including vancomycin.
Exposure	
Consumption of streptogramin resistant <i>E. faecium</i> by humans	<ul style="list-style-type: none"> • Virginiamycin resistant strains have been detected in enterococci also resistant to vancomycin cultured from minced beef and pork.
Colonisation of humans by animal <i>E. faecium</i> and/or transfer of resistance to human <i>E. faecium</i> strains	<ul style="list-style-type: none"> • Virginiamycin-resistant <i>E. faecium</i> of animal origin can survive gastric passage, multiply, and be isolated for up to 14 days from human volunteers.
Impact	
Infection of humans with animal derived streptogramin resistant <i>E. faecium</i>	<ul style="list-style-type: none"> • Conclusive evidence of human infection with animal derived streptogramin resistant <i>E. faecium</i> is lacking
Infection with <i>E. faecium</i> resistant to vancomycin	<ul style="list-style-type: none"> • Vancomycin- resistant enterococci have a high propensity to cause outbreaks in hospitals. • While the number of infections resulting from colonisation with vancomycin-resistant enterococci is low, these strains spread easily to other patients, resulting in significant numbers of infections.
Disease due to infection with <i>E. faecium</i> resistant to vancomycin and streptogramins	<ul style="list-style-type: none"> • Recent Australian studies have demonstrated no resistance to quinupristin/dalfopristin in human clinical isolates.
Treatment failure attributable to acquisition of streptogramin resistant <i>E. faecium</i> from animals.	<ul style="list-style-type: none"> • Septicaemia from <i>vanA E. faecium</i> mostly occurs in highly vulnerable patients who have multiple medical problems. Failure of therapy in these patients would result in significant mortality, or prolonged treatment. Currently these patients are treated with a streptogramin – quinupristin/dalfopristin, or the newer antibiotic linezolid. • The impact of antibiotic failure on more minor infections such as wound infections and urinary tract infections is small.

Factors considered	Findings
Assessment of data uncertainty	
Uncertainty due to inherent variability and measurement error. Uncertainty due to lack of information or understanding.	<ul style="list-style-type: none"> • It is not clear whether strains of <i>E. faecium</i> with so –called intermediate resistance (MIC 2mg/L) to virginiamycin are truly resistant and possess relevant resistance genes. • Data on virginiamycin and vancomycin-resistant <i>E. faecium</i> prevalence in food animals and incidence of human infections in Australia are lacking. • There are limited data on the importance of virginiamycin in the selection of enterococci co-resistant to both virginiamycin and vancomycin in food animals.
Preventative/ therapeutic uses	
	<p>Virginiamycin plays a role in the management of the following diseases in food producing animals:</p> <ul style="list-style-type: none"> • lactic acidosis in cattle and sheep given high grain diets • necrotic (clostridial) enteritis in chickens
Level of acceptable risk	
	That level of probability and severity of impact which, when weighed against preventative/therapeutic use in the target animal species, is not considered to significantly compromise the treatment of bacterial infections in susceptible humans.
Risk characterization	
Risk in susceptible humans	The probability of disease due to infection in susceptible humans due to exposure to streptogramin resistant <i>E. faecium</i> of animal origin is low, and the severity of impact in susceptible humans is high.
Risk in general population	The probability of disease due to infection in humans in the general population due to exposure to streptogramin resistant <i>E. faecium</i> of animal origin is low, and the severity of impact in humans in the general population is low.

5.12 Proposed recommendations for risk management

The primary strategy for the management of the potential risks identified in the public health assessment is to modify the exposure and duration of exposure of target animal bacteria to virginiamycin.

The role of virginiamycin in the prevention of animal disease in animal production is recognised. The outcomes of the efficacy assessment, described in Section C describe those uses where the products are considered to be effective for the purposes claimed. In such cases the products should only be used under professional veterinary management thereby minimising the exposure of animal bacteria to virginiamycin. The public health assessment therefore recommends that all uses of virginiamycin in animals be classified in Schedule 4.

With regard to the duration of exposure it is noted that current label instructions allow an unqualified and unquantified duration of use. The report therefore recommends that label instructions be modified so as to permit use of product for a defined period and in defined situations as supported by the efficacy data submitted.

Further specific details of risk management proposals can be found in Section D: Proposed Regulatory Approach.

SECTION C. EFFICACY ASSESSMENT

6.1 Introduction

The first recommendation of the JETACAR Report stated that, *inter alia*, in-feed antibiotics used in food-producing animals for growth promotant purposes, or other routine uses where duration and dose level are the same, or very similar, should not be used unless they are of demonstrable efficacy in livestock production under Australian farming conditions.

Consequently the efficacy of virginiamycin, pertaining to label claims and use patterns, was assessed. Further any uses of the antibiotic in Australian animal health are considered and are incorporated into the risk assessment.

The efficacy of virginiamycin was assessed at the time of its initial registration. This review reassesses the efficacy of virginiamycin for the claimed uses based on both the original data and more recent studies.

6.2 Methodology of assessment

The data assessed for the efficacy review is published and unpublished data provided by a registrant in support of the efficacy of virginiamycin.

In those studies where there were insufficient data provided to allow a full methodological and statistical assessment of the studies, the conclusions of the studies were not accepted as supporting efficacy.

Some studies included biochemical measurements that may be correlated with disease, such as rumen or blood lactate levels. Where these supported findings based on measurements of direct production or clinical relevance, they were accepted.

If a study was interpreted as not demonstrating efficacy, based on measurement of direct production or clinical relevance, such as weight gain or feed conversion efficiency, any effect observed on a biochemical measurement was not considered.

In some studies the responses to use of virginiamycin were inconsistent. Where no coherent explanation for this variation was provided, the studies were considered as failing to demonstrate efficacy. The user of a product should be able to reliably predict when the product will be efficacious, and the effect should be measurable.

6.3 Information sources

Both published and unpublished data was assessed. Data submitted included both complete descriptions of studies as well as a number of summaries of studies, which included very limited data.

6.4 Terminology

Efficacy

Producing or sure to produce the desired effect. The desired effect on a measurement of relevance in clinical disease or in production must be consistently demonstrated.

Growth promotion, feed conversion efficiency:

The use of substances to increase the rate of weight gain and/or the efficiency of feed utilisation in animals by other than purely nutritional means. The term does not apply to the use of antimicrobials for the purpose of inhibiting specific pathogens even when an incidental growth response may be thus obtained.

Therapy/ Therapeutic

The use of antimicrobials for the purpose of inhibiting a pathogen(s) which already infects the host; that is, initiating treatment because there is a disease condition.)

Prevention/Prophylaxis

The use of antimicrobials (by any route of administration) to prevent infection with a pathogen(s) that is anticipated to challenge the host during the treatment period; that is, initiating treatment in advance of an actual infection or disease condition because such a condition is expected to occur if treatment is withheld.

6.5 Summary of studies submitted in support of label claims.

Data was provided to support the various label claims for virginiamycin, including those for growth promotion, prevention of lactic acidosis, and resulting liver abscessation. In addition data was provided in support of virginiamycin use by veterinarians to prevent the disease conditions of necrotic enteritis in chickens. The studies were evaluated and a summary of that evaluation is provided below. A detailed evaluation of individual studies is provided in Appendix 4.

6.5.1 Growth promotion claims in pigs and poultry

The data from a large number of studies examining the efficacy of virginiamycin in improving rates of growth of feed conversion efficiency in poultry and pigs were provided for review. While some overseas studies demonstrated that treatment with virginiamycin at recommended dosages had a measurable effect on weight gain and/or on feed conversion efficiency, others, particularly more recent studies, do not show any significant growth promotion effect from treatment. There does not appear to be a clear explanation for this variation in response to treatment, suggesting that a user could not be certain of a predictable growth promotion effect when using the product. The sources of variation may include diet, environment, genetics and bacterial challenge. When originally registered in Australia, the efficacy data provided for virginiamycin supported the various growth promotion claims, and since that time there have been no reports of product inefficacy. Contemporary studies substantiating the efficacy of virginiamycin for growth promotion in pigs and poultry under Australian conditions have, to date, not been required or undertaken. Additionally substantial changes in the management of pigs and poultry, improvements in diet formulation and advances in the genetic selection of both species have occurred since virginiamycin was first registered in Australia.

6.5.2 Prevention of lactic acidosis claim in cattle and sheep

Lactic acidosis in ruminants is a consequence of feeding diets containing a high proportion of highly digestible grains. Rapid fermentation of such diets in the rumen results in ruminal acidosis and stasis, devitalisation of the ruminal wall, systemic acidosis and can ultimately result in death. In less severe cases animals may not feed well, lose weight, and in more chronic sequelae, develop liver abscesses. Highly digestible diets are commonly fed under drought conditions, in situations where animals are kept intensively, such as in feedlots and during live export, and in dairy cattle production. While lactic acidosis can be prevented by controlled introduction of the highly digestible diet over a prolonged period, it is common for errors to occur during this process, and in large groups of animals it is difficult to control the intake of individual animals. Reductions in the risk of lactic acidosis have a significant impact in many areas of sheep and cattle production.

Examination of the submitted data indicates that the products are effective in the control of lactic acidosis in sheep fed grain on a weekly or twice weekly basis and help prevent lactic acidosis in the initial month's adaptation of feedlot cattle to a high grain diet.

6.5.3 Use of virginiamycin to prevent necrotic enteritis in chickens.

Necrotic enteritis is a common, frequently fatal gastrointestinal disease problem in poultry induced by toxigenic strains of *Clostridium perfringens*. It is particularly prevalent in broiler chickens on wheat-based diets. Virginiamycin is used extensively in the broiler industry for the combined purpose of controlling necrotic enteritis and improving feed conversion/growth promotion. Its use in Australia may have increased following the worldwide withdrawal of avoparcin for this purpose. Chickens fed high grain diets grow more rapidly and reach ideal slaughter weight earlier, increasing production efficiency. However, high wheat and barley grain diets also encourage the gut overgrowth of *Clostridium perfringens*, resulting in a condition of necrotic enteritis, a subacute condition that slows growth and increases mortality. In some cases it may be more acute in onset and associated with high morbidity and mortality. The latter type of necrotic enteritis is rare in Australia. Wheat and barley diets are prominent in Australian broiler production.

Examination of the data presented suggests that it is sufficient to justify a claim of efficacy against necrotic enteritis in poultry.

6.6 Conclusion

The data submitted to support efficacy claims for products containing virginiamycin indicate that the products are effective in reducing the risk of lactic acidosis in sheep fed grain on a weekly or twice weekly basis and help prevent lactic acidosis in the initial month's adaptation of feedlot cattle to a high grain diet. The efficacy assessment found some evidence of improvement in performance parameters of pigs and poultry, however, no recent Australian data is available. The reviewed studies reflected the variation in response to antibiotic administration that is commonly associated with this use pattern and class of drug. Instructions on currently approved labels are considered to be inadequate.

Virginiamycin appears to be effective in controlling necrotic enteritis in chickens, justifying its use by poultry veterinarians under prescription.

SECTION D: PROPOSED REGULATORY APPROACH

7.1 Regulatory options

The basis for a reconsideration of the registration and approvals for a chemical is whether the APVMA is satisfied that the requirements prescribed by the AgVet Codes for continued registration and approval are being met. These requirements are that the use of the product, in accordance with the recommendations for its use:

- would not be an undue hazard to the safety of people exposed to it during its handling or people using anything containing its residues;
- would not be likely to have an effect that is harmful to human beings;
- would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment;
- would not unduly prejudice trade or commerce between Australia and places outside Australia; and
- would be effective for the purposes claimed.

The requirements for product labels are that the label contains adequate instructions. Such instructions include:

- the circumstances in which the product should be used;
- how the product should be used;
- the times when the product should be used;
- the frequency of the use of the product;
- the withholding period after the use of the product;
- the disposal of the product and its container;
- the safe handling of the product.

There are three possible outcomes to the reconsideration of the registration of products containing virginiamycin and their labels. Based on the information reviewed the APVMA may be:

- satisfied that the products and their labels continue to meet the prescribed requirements for registration and approval and therefore confirms the registrations and approvals.
- satisfied that the conditions to which the registration or approval is currently subject can be varied in such a way that the requirements for continued registration and approval will be complied with and therefore varies the conditions of registration or approval.
- not satisfied that the requirements for continued registration and approval continue to be met and suspends or cancels the registration and/or approval.

7.2 Assessment outcomes

The assessments conducted as part of the review process considered the hazard, exposure and potential impact that continued use of products containing virginiamycin would pose on public health. The review process also considered whether the products are effective for the purposes claimed and whether the labels contained adequate instructions.

The public health assessment concluded that the potential risks to susceptible humans are higher than the general population, and although the probability of disease due to infection in susceptible humans due to exposure to streptogramin resistant *E. faecium* of animal origin is low, the severity of impact in susceptible humans being high. The public health assessment noted that the importance of virginiamycin in the selection of enterococci co-resistant to both virginiamycin and vancomycin in food animals is uncertain, and data are limited.

The assessment recognizes that in the case of specified therapeutic uses of products containing virginiamycin for a short, defined periods the probability of resistance development and severity of

impact is unlikely to compromise the treatment of bacterial infections in susceptible humans. It is recommended that such uses should only occur under professional veterinary management.

The efficacy assessment concluded that there is no evidence that the products will provide a consistent, measurable positive effect in growth or feed conversion efficacy. The efficacy assessment found some evidence of improvement in performance parameters of pigs and poultry, however, no recent Australian data is available. The reviewed studies reflected the variation in response to antibiotic administration that is commonly associated with this use pattern and class of drug. With respect to therapeutic uses of products containing virginiamycin the assessment concluded that the products are effective in the management of disease conditions in food producing animals, including necrotic enteritis in chickens and lactic acidosis in cattle and sheep given high grain diets. However instructions on currently approved labels pertaining to the uses in sheep and cattle are considered to be inadequate.

7.3 Recommendations

Based on the information assessed and as outlined, the APVMA cannot be satisfied that currently approved labels carry adequate instructions with regard to:

- the circumstances in which the products should be used;
- the times when the products should be used;
- the duration of any treatment using the product; and
- the frequency of the use of the product.

However, the APVMA can be satisfied that the conditions to which the approval of labels for products for therapeutic uses in sheep, cattle and chickens are currently subject can be varied in such a way that the requirements for continued approval of the labels will be complied with.

The APVMA can be satisfied that the continued use of products containing virginiamycin for prophylactic/therapeutic uses in sheep, cattle and chickens in accordance with varied conditions of label approval:

- would not be likely to have an effect that is harmful to human beings; and
- are effective for the purposes claimed.

The APVMA cannot be satisfied that the conditions of product registration and label approval can be varied for products that are registered for improvement of growth rate and feed conversion efficiency in pigs, poultry and turkeys. There is insufficient data to qualify the circumstances in which the products should be used, the times when the product should be used or the duration of the treatment.

The APVMA proposes to address the potential risks identified in this report using the following regulatory actions.

(i) Vary conditions of label approval for products containing virginiamycin registered for the prevention of lactic acidosis in sheep and cattle and the prevention of necrotic enteritis in chickens by:

- the deletion of all label claims, indications and use patterns that pertain to growth promotion, and/or improved feed efficiency in pigs, poultry and turkeys; and
- the addition of more specific instructions and restraints statements pertaining to their use for the prevention of lactic acidosis in sheep and cattle and necrotic enteritis in chickens. The proposed label instructions relate to the circumstances in which the product should be used, how the product should be used, the times when the product

should be used, the duration of any treatment using the product and the frequency product use. The proposed label restraints constrain veterinarian rights to prescribe the antibiotic for periods longer than specified on the label or to prescribe repeat treatments. Should situations arise where it is deemed to be necessary to prescribe the products for a period longer than specified on the label or to prescribe a repeat treatment (for example, for animal welfare reasons) the APVMA may issue a permit to allow a person or organisation to use a product containing virginiamycin in a way not authorised by the approved label.

(ii) Affirm registration of products containing virginiamycin registered for the prevention of lactic acidosis in sheep and cattle and the prevention of necrotic enteritis in chickens.

(ii) Cancel the registration and label approvals of those products that have label claims pertaining to growth promotion and/or improved feed efficiency only. The APVMA is not satisfied that these products are effective for this purpose or that conditions of registration or approval can be varied to satisfy the APVMA's concerns.

Details of the proposed regulatory actions including proposed new recommendations for use and label instructions are provided in the Table 2 Regulatory Decisions Table.

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Table 2. Regulatory Decision Table

Product , Active Ingredient and Poison Schedule classification	Registrant	Claims on APVMA-approved label	Recommendations	Proposed label amendments	Regulatory decision
51354 Eskalin 500 Feed Premix– <i>virginiamycin</i> 500g/kg Schedule 4	Phibro Animal Health	For use in complete feeds (dry mash or pellets) for <u>cattle</u> to reduce the risk of acidosis caused by high levels of grain in the feeds. For prevention of necrotic enteritis caused by <i>Clostridium perfringens</i> susceptible to virginiamycin. Administer medicated feed to birds at risk for a period up to 21 days. Not to be fed to laying birds.	Label changes required	<u>Feedlot and dairy cattle</u> : For use in cattle diets at times of increased risk of acidosis during adaptation to high grain diets. <u>Drought fed cattle</u> : For use to reduce the risk of acidosis in cattle fed grain on a weekly or twice weekly basis. <u>Chickens</u> : For prevention of necrotic enteritis caused by <i>Clostridium perfringens</i> susceptible to virginiamycin. Administer medicated feed to birds at risk for a period up to 21 days. Not to be fed to laying birds. Addition of restraint statements: <u>Feedlot Cattle</u> : DO NOT USE continuously for a period of more than one month. DO NOT repeat treatment. <u>Dairy Cattle</u> : DO NOT USE continuously for a period of more than one month. DO NOT repeat treatment in same lactation period in dairy cattle. <u>Chickens</u> : DO NOT USE for a period of more than 21 days. DO NOT repeat treatment	Vary conditions of label approval Affirm registrations.
49111 Eskalin Wettable Powder Spray-On Feed Premix– <i>virginiamycin</i> 400g/kg (individual sachets of 20g) Schedule 5	Phibro Animal Health	For use in <u>cattle</u> and <u>sheep</u> rations to reduce the risk of acidosis (grain poisoning) when feeding grain	Label changes required. Schedule currently under consideration by NDPSC.	<u>Feedlot and dairy cattle</u> : For use in cattle diets at times of increased risk of acidosis during adaptation to high grain diets. <u>Drought fed sheep and cattle</u> : For use to reduce the risk of acidosis in sheep and cattle fed grain on a weekly or twice weekly basis. <u>Feedlot Cattle</u> : DO NOT USE continuously for a period of more than one month. DO NOT repeat treatment. <u>Dairy Cattle</u> : DO NOT USE continuously for a period of more than one month. DO NOT repeat treatment in same lactation period in dairy cattle.	Vary conditions of label approval Affirm registrations.
46049 Eskalin Feed Premix for Cattle– <i>virginiamycin</i> 20g/kg Schedule 5	Phibro Animal Health	For use in complete rations for <u>cattle</u> to reduce acidosis due to high grain diets	Label changes required. Schedule currently under consideration by NDPSC.	<u>Feedlot and dairy cattle</u> : For use in cattle diets at times of increased risk of acidosis during adaptation to high grain diets. <u>Drought fed cattle</u> : For use to reduce the risk of acidosis in cattle fed grain on a weekly or twice weekly basis. <u>Feedlot Cattle</u> : DO NOT USE continuously for a period of more than one month. DO NOT repeat treatment. <u>Dairy Cattle</u> : DO NOT USE continuously for a	Vary conditions of label approval Affirm registrations.

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				period of more than one month. DO NOT repeat treatment in same lactation period in dairy cattle.	
38329 Stafac 20 Feed Premix– <i>virginiamycin</i> 20g/kg Schedule 5	Phibro Animal Health	For use in rations of <u>growing pigs, broilers</u> and <u>turkeys</u> to improve growth rate and feed conversion efficiency. Not to be fed to laying birds.	All label claims to be deleted. Continued registration not supported	The deletion of all label claims, indications and use patterns that pertain to growth promotion, and/or improved feed efficiency in pigs, poultry, sheep and cattle	Cancel registration and label approvals
41286 Virginiamycin 20– <i>virginiamycin</i> 20g/kg Schedule 5	Agribusiness Products Pty Ltd	For use in rations of <u>growing pigs, broilers</u> and <u>turkeys</u> to improve growth rate and feed conversion efficiency	All label claims to be deleted. Continued registration not supported	The deletion of all label claims, indications and use patterns that pertain to growth promotion, and/or improved feed efficiency in pigs, poultry, sheep and cattle	Cancel registration and label approvals
41476 Virginiamycin 20– <i>virginiamycin</i> 20g/kg Schedule 5	Lienert Australia Pty Ltd	Growth promotion and improved feed conversion efficiency in growing pigs	All label claims to be deleted. Continued registration not supported	The deletion of all label claims, indications and use patterns that pertain to growth promotion, and/or improved feed efficiency in pigs, poultry, sheep and cattle	Cancel registration and label approvals

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APPENDIX 1 STREPTOGRAMIN ACTION AND RESISTANCE

Streptogramins

In human medicine there are two streptogramin products: *pristinamycin* and *quinupristin-dalfopristin* (QD). Pristinamycin is similar to virginiamycin in that it is a relatively crude combination of streptogramins A and B (pristinamycins II_A and II_B, and pristinamycins I_A, I_B and I_C). Pristinamycin II is identical to virginiamycin M₁. This drug has been available in France (only) for many years as an oral preparation for the treatment of gram-positive infections. Because gastrointestinal tolerance of the drug is poor in many patients, its use has been limited. If it were not for these reasons, it might well have become popular for the treatment of multi-resistant *Staphylococcus aureus* infections in France.

QD was developed from pristinamycin. It is composed of chemical modifications of the two pristinamycin components, dalfopristin (a streptogramin A) and quinupristin (a streptogramin B). It is active against multi-resistant strains of *Staphylococcus* species and *Streptococcus pneumoniae*.

Table 1

Component	Pristinamycin	Virginiamycin	Quinupristin-dalfopristin
Streptogramin A	pristinamycins II _A and II _B	virginiamycin M ₁	dalfopristin
Streptogramin B	pristinamycins I _A , I _B and I _C	virginiamycin S ₁	quinupristin

Streptogramin mechanism of action

There are two structurally different groups of streptogramins (Group A & group B). They both bind to bacterial ribosomes and inhibit the translation of messenger RNA at the elongation step. However, they act at different sites.

- Group A streptogramins inactivate the donor and acceptor sites of peptidyltransferase, thus interfering with the function of this enzyme. They block two of the peptide chain elongation steps. This action is partly due to the presence of the antibacterial on the ribosome and partly due to the conformational alterations consecutive to this binding.
- Group B streptogramins interfere with the correct positioning of peptidyl-tRNA at the P site – inhibiting peptide bond formation, resulting in the release of incomplete peptide chains. This is also the site of action of the macrolides (and the new related ketolides) and lincosamides. The group of agents active at this site are therefore referred to as the MLS_B group

Typically, the in vitro antibacterial activity of the combination of Group A and Group B streptogramins is at least 10 fold greater than the sum of the activity of the individual agents. This synergy is due to a conformational change caused by streptogramin A that results in increased ribosome affinity for type B streptogramins.

Streptogramin resistance and cross resistance

A summary of the known resistance genes and patterns for macrolides (and the related ketolides), lincosamides and streptogramins is provided in Tables 1 and 2. The area is somewhat complex, and is further complicated by the dual components of streptogramin therapeutic agents.

Resistance to streptogramins B can be selected for by macrolides and lincosamides in addition to streptogramins. 16-membered macrolides will select for resistance to all macrolides, lincosamides and streptogramins B. As streptogramins A have a different site of action, only streptogramins A or their combination with streptogramins B can select for streptogramin A resistance.

Streptogramin use itself will select for resistance to streptogramins A and B and this will result in cross-resistance to all types of macrolides (14-, 15- and 16-membered) and to lincosamides.

Resistance to streptogramins A is described, and confers reduced susceptibility to streptogramins B and the combination.

Table 2Common resistances and cross-resistances to the MLS_B antimicrobials in humans

Mechanism of resistance	Main species	Macrolides		Keto- lides	Lincos- amides	Streptogramins		
		14 & 15	16			A	B	A+B
Intrinsic	Enterobacteriaceae	R	R	R	R	R	R	R
	<i>Enterococcus faecalis</i>	R	R	R	R	R	R	R
	<i>Enterococcus faecium</i>	R	R	R	R	S	R	S
Ribosomal RNA Site methylation								
<i>erm</i> -inducible	Staphylococci	R	S	R	Si	S	S	S
<i>erm</i> -constitutive	Staphylococci	R	R	R	R	S	R	r
<i>erm</i> -inducible	Streptococci	R	S	r	Si	S	S	S
<i>erm</i> -constitutive	Streptococci	R	R	r	R	S	R	r
Efflux								
<i>mef</i>	Streptococci	R	S	S	S	S	S	S

S = susceptible, R = resistant, r = reduced susceptibility (tests as susceptible), Si = resistance inducible by erythromycin (tests as susceptible in absence of erythromycin)

Table 3
Rare resistances and cross-resistances to the MLS_B antimicrobials in humans

Mechanism of resistance	of	Macrolides		Keto- lides	Lincos- amides	Streptogramins		
		14 & 15	16			A	B	A+B
Efflux								
<i>msr</i>	Staphylococci, Enterococci	R	S	?	S	S	Si	S
<i>vga*</i>	Staphylococci	S	S	?	?	R	S	R
Esterases								
<i>ere</i>	Enterobacteria	high level R						
Phosphorylases								
<i>mph</i>	Enterobacteria	high level R						
Hydrolases								
<i>vgb</i>	Enterococci, Staphylococci						R	
Transferases								
<i>vat*</i>	Staphylococci, Enterococci	S	S	S	S	R	S	R
<i>lnu (lin)</i>	Staphylococci	S	S	?	R or r	S	S	S

S = susceptible, R = resistant, r = reduced susceptibility (tests as susceptible), Si = inducible by erythromycin (tests as susceptible in absence of erythromycin)

* Found combined in staphylococci

Staphylococcal resistance genes (Lina *et al* 1999):

erm(A), *erm(B)*, *erm(C)* confer resistance by target site alteration of the ribosome. (Affects only Streptogramin type B)

msr(A) resistance to Streptogramin B by efflux after induction by erythromycin

vgb(A), *vgb(B)* encode a hydrolase that inactivates type B streptogramins

vat(A), *vat(B)*, *vat(C)* encode acetyltransferases which inactivate the streptogramin A

vga(A), *vga(B)* encode related ATP binding proteins involved in active efflux of the Streptogramin A compounds

Enterococci (Roberts *et al* 1999):

erm(B) target site alteration – streptogramin B

vat(D), *vat(E)* encode acetyltransferases – streptogramin A

vgb(A) hydrolase streptogramin B

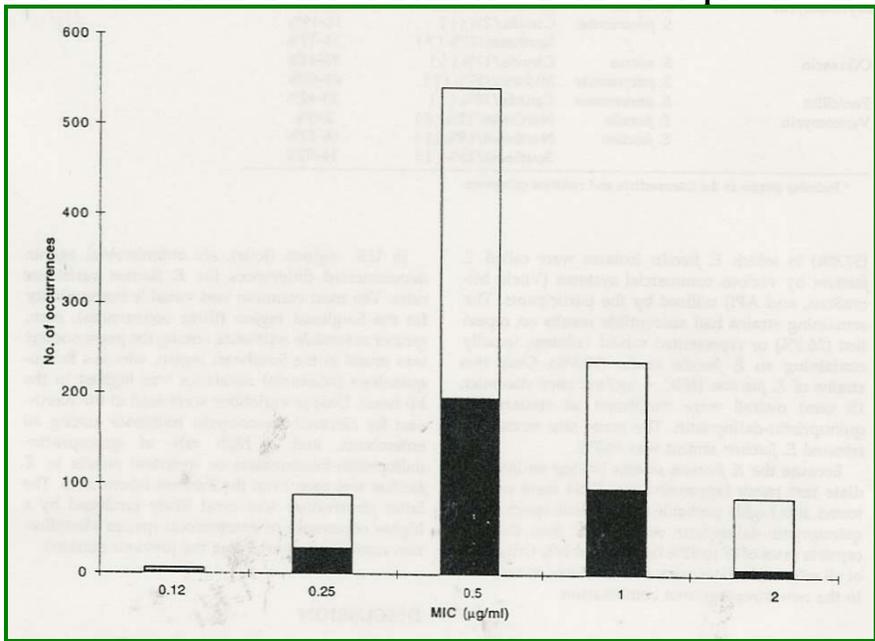
Resistance of *E. faecium* to QD requires the presence of resistance to both dalbopristin and quinupristin. By constructing recombinant plasmids the *erm* gene did not alter the MIC, the *vgb* gene lead to a fourfold increase in MIC (although still in the susceptible range), *vatD* – a one dilution difference, the combination of *ermB* and *vgb* genes– no change, combination of *ermB* and *vatD* – one-two fold increase with a shift to the intermediate range. Only the combination of *vatD* and *vgb* genes conferred resistance. However there was another uncharacterised resistance determinant present in organisms with a lincosamide, streptogramin A resistance phenotype that conferred resistance in combination with any of the other resistance genes (Bozdogan and Leclercq, 1999).

APPENDIX 2 – *E. FAECIUM* MIC DISTRIBUTIONS

Agent	Ref	Method	MIC (mg/L)										
			≤0.25	0.5	1	2	4	8	16	32	64	128	>128
Virginiamycin													
n													
Total	Aarestru <i>p et al;</i> 2000	AD-MH	29	32	101	64	15	30	61	59	16	1	1
Denmark	“		1	9	42	40	15	29	57	56	14	1	1
Finland	“		17	14	25	19		1	4	3	2		
Norway	“		11	9	32	5							
Virginiamycin M													
Resistant strains	Werner <i>et al;</i> 2000	BMD-ISO									2	45	65
Quinupristin- dalfopristin													
North America	Jones <i>et al;</i> 1998	BMD-MH	see graph below										
Resistant	Werner <i>et al;</i> 2000	BMD-ISO					1	8	56	6	1		
Australia	*	BMD-MH	1	18	8	4							
Japan	*		3	25	8	7	2	1					
Others	*		4	23	7	7	1						
AUS vanR	Turnidge & Bell; 1999	BMD-MH	8	14	4	2							
AUS vanS	“		20	42	5	12	1						
Thailand	“		1	16	1	4							
AUS	Bell & Turnidge; 2001	BMD-MH	7	26	2	6							
ICU	Hallgren <i>et al;</i> 2001	Etest-PDM		36	22	15	1						

* SENTRY data from Western Pacific, 1998-2000 – Jan Bell, personal communication

Graph from Jones *et al* ; 1998 MIC distributions for quinupristin-dalfopristin and *E. faecium* isolated from Humans in North America. Dark bars represent vancomycin-resistant strains



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APPENDIX 3 – STREPTOGRAMIN USE IN AUSTRALIA*

Streptogramin	Import amounts in kilograms																			
	1992-3				1993-4				1994-5				1995-6				1996-7			
	Human	Stock	Vet	Total	Human	Stock	Vet	Total	Human	Stock	Vet	Total	Human	Stock	Vet	Total	Human	Stock	Vet	Total
Quinupristin-dalfopristin													5			5				
Pristinamycin									1			1	1			1	5			5
Virginiamycin		1700 0		1700 0		9500		9500		2150 0		2150 0		3185 0		3185 0		3600 0		3600 0

*Data from JETACAR report (1999)

APPENDIX 4 - DETAILED EFFICACY DATA

Efficacy in Chickens

Growth Promotant

A number of reviews of use of virginiamycin and other antibiotics as growth promotants were included in the submission (Groves, 2000; Hays Report, 2000; Zimmerman, 1986). However these reviews did not contain data that could be assessed as evidence of efficacy. In the Hays report and the Zimmerman review pooled summaries of studies were presented, but it is impossible from this pooled data to establish whether the size of the pooled effect is predominantly a result of an effect in a restricted number of flocks as there is no measurement of data variability. Such pooled studies also do not allow a full assessment of the suitability of the controls used in such studies. It is possible that apparent positive effects on growth promotion were as a result of therapeutic/preventative effects in a limited number of flocks with necrotic enteritis. The Groves report summarises current usage, but does not provide data to support efficacy.

Early studies in chickens and turkey poults (Yates & Schaible, 1962) showed a significant, dose dependent response to the inclusion of virginiamycin from 4 to 100 g/ton of food in terms of weight gain, and an improvement in feed conversion efficiency (FCE) which was best at 9 g/ton. There was also a reduction in mortality in some trials although this was not dose dependent.

Further studies (Combs & Bossard, 1963) showed a significant improvement in weight gain in chickens fed between 4.4 and 17.6 ppm virginiamycin in feed, although the effect was inconsistent, with no improvement in weight gain in chickens on clean, rather than reused, litter.

The original registration documentation for Eskalin claimed use at 5 – 10 g/ton of feed for growth promotant use in broilers and turkeys to improve weight gains and food conversion efficiency (Pfizer, 2000).

Studies reported in the original registration dossier suggest that 5 g/ton of virginiamycin in feed offers no significant advantage in weight gain if included in feed throughout production, or included in the first half of the production period or the second half of the production period. There was no evidence of a significant difference in chickens fed virginiamycin at 5 or 10 g/ton. In these trials feed conversion efficiency (FCE) was significantly greater in chickens fed virginiamycin under some regimens, but the data do not indicate any differences between dosages at 5 g/ton and 10 g/ton or between feeding for the full production cycle or only half of the cycle (Pfizer, 2000).

In field trials reported in the dossier significant improvements in weight gain and FCE were seen in chickens fed 5 g/ton and 10 g/ton for the full 9 week production cycle with no significant differences between the two doses (Pfizer, 2000).

Other studies in the original dossier only compare performance of chickens fed virginiamycin with that of chickens fed either penicillin or oxytetracycline (Pfizer, 2000).

Local efficacy studies in chickens found no difference in efficacy between 5 g/ton and 10 g/ton but found that at both levels both final weight and FCE were significantly improved (Pfizer, 2000).

The dossier presented an economic analysis of the use of virginiamycin in Australia in 1969.

No local data was provided to support use in turkeys.

There are a number of studies on growth promotion summarised in the virginiamycin technical manual but there is insufficient detail to allow full assessment. Similarly a number of summaries of studies are included in the Stafac Product file manual but there is also insufficient description of the studies to allow an assessment (Pfizer, 2000).

In more recent studies, doses of 11 and 22 ppm were assessed in Cobb broiler chickens, on reused litter, with inclusion throughout production (42 days). At 21 days both treatment levels had improved weight gain and FCE. However, at 42 days a significant effect on weight gain was only apparent at 11 ppm and a significant effect on FCE was only apparent at 22 ppm. The carcass and breast weights were significantly greater in chickens fed 11 ppm, but not in chickens fed 22 ppm. There was no effect on mortality rates (Pfizer, 2000).

Another recent study examined the effect of 10 ppm on Ross broiler chickens with inclusion throughout production. At 14 days the treated group had gained significantly less weight, although by day 42 there was no difference. There was no difference in FCE between treated and untreated groups. There was no difference in carcass weight or breast weight. Mortalities were higher in treated groups between 0 and 14 days, but over the full 42 days of production there was no difference (Pfizer, 2000).

A third study on Ross 308 chickens examined the effect of 10 ppm throughout production and found a significant effect on weight gain and FCE, but not on carcass or breast weight. There was no difference in mortality (Pfizer, 2000).

A fourth study on Cobb 500 chickens examined the effect of 20 ppm of virginiamycin at different dietary energy levels, and different energy to protein ratios. There was no significant effect of treatment on weight gain, FCE, carcass weight or breast weight on any diet. There were no differences in mortality rates (Pfizer, 2000).

A fifth study on Ross x Arbor Acres chickens examined the effect of 15 ppm or 10 ppm throughout the production period or of 15 ppm in the starter ration and 10 ppm in the grower ration. The study was performed on reused litter. All treatments resulted in significant improvements in weight gain, and in FCE in males but not females. Breast weight was also increased in treated birds (Pfizer, 2000).

A sixth study examined the effect of 15 g/ton on Ross x Arbor Acres chickens throughout production. These birds were also being treated with roxarsone and were reared on used litter. Virginiamycin treated birds had significantly greater weight gains (1%) and FCE (0.8%). However, virginiamycin treated chickens had significantly higher mortality rates (2.6%) (Pfizer, 2000).

In conclusion there is no assessable evidence that demonstrates that the situations in which virginiamycin will deliver a measurable positive effect in growth or feed conversion efficiency that can be predicted.

Prevention of Necrotic Enteritis in Chickens

Study VM – 5050 – 82 (Pfizer, 2000)

In this study chickens were placed on feed containing 0, 11, 22 or 33 g virginiamycin per tonne from 1 – 13 days of age.

Challenge was by incorporation of *Clostridium perfringens* type A cultures in feed from day 15 to 19. From days 20 – 35 birds were placed on diets without *C. perfringens*. The results suggest that all dose rates prevented lesions of necrotic enteritis but mortalities did not differ between groups. There was a dose dependent weight gain and FCE response. The effect virginiamycin had on the challenge inoculum while in the feed, thereby reducing the effective challenge dose, may have confounded the interpretation of this trial.

Study VM – 5089 – 83 (Pfizer, 2000)

In this study chickens were placed on a starter ration with added fish meal (which exacerbates necrotic enteritis) from 4 days of age to 19 days of age. From day 21 to 26 they were fed medicated feed containing a *C. perfringens* culture. From day 27 to 41 the medicated feed was provided. The dose rates were 0, 10, 20, or 30 g/ton. As mentioned above, the medication of feed may have reduced the challenge dose. There appears to have been little effect of treatment on mortality or FCE, although the overall FCE for the entire trial was not summarised or analysed. There was some reduction in aggregate lesion scores but these were not suitable for statistical analysis. Only 30 g/ton improved weight gain.

The applicant has combined both studies for some analysis, but there were significant differences between the studies that suggest this is questionable. The two studies suggest a dose of 20 g/ton is able to prevent the effects of experimentally induced necrotic enteritis.

Study VM – 5084 – 83 (Pfizer, 2000)

This study examined the effect of 10, 20 or 30 g/ton on broilers placed on contaminated litter at 1 day old. Medication of feed commenced on the day of placement (1 day old). All dose rates had a significant effect on weight gain, FCE, the proportion of chickens with lesions of necrotic enteritis at 15 days of age, and mortalities due to necrotic enteritis. The study indicates that 20 g/ton will prevent necrotic enteritis.

Study VM – 5187 – 86 (Pfizer, 2000)

This study was not presented in sufficient detail to allow a full evaluation, but the statistical analysis presented supports the observation that 20 g/ton can reduce mortalities and the proportion of birds with lesions as well as the severity of lesions.

Study VM – 5163 – 85 (Pfizer, 2000)

This study was insufficiently detailed to allow full evaluation, but statistical analysis presented indicates that virginiamycin at 20 g/ton had no significant effect on weight gain, FCE, mortalities or severity of lesions, but did reduce the number of birds with lesions.

Study VM – 5138 – 85 (Pfizer, 2000)

This study too had insufficient detail to allow for a full evaluation, the statistical analysis presented suggests that virginiamycin at 20 g/ton resulted in an increased body weight, but did not have an effect on lesions or the severity of lesions.

Efficacy in Turkeys

The study included (Fagerberg *et al*, 1984) is not described in sufficient detail to fully assess efficacy. Weight gain and FCE cannot be assessed. From the data included, trial 1 does not establish that 20 g/ton reduced mortalities. Trial 2 showed a significantly lower mortality rate in poult fed 30 g/ton, but not in those fed 20 or 40 g/ton. These data require replication for efficacy to be established.

Other reports included in the dossier have insufficient detail to support any claims of efficacy in turkeys.

Efficacy in Pigs

A number of reviews of use of virginiamycin and other antibiotics as growth promotants were included in the submission (Hays Report, 2000; Zimmerman, 1986). However these reviews did not contain data that could be assessed as evidence of efficacy. As mentioned earlier, the Hays report and the Zimmerman review pooled summaries of studies, and it was impossible from this pooled data to establish whether the size of the pooled effect is predominantly a result of an effect in a restricted number of herds as there is no measurement included of variability in the data. Such pooled studies do not allow a full assessment of the suitability of the controls used in such studies.

The original registration dossier includes experimental trials that indicate that 40 g/ton in feed during the period from weaning to around 100 lb in weight (about 70 days) has a significant effect on weight gain, although not consistently on FCE, while use at 20 g/ton does not offer a consistent advantage. Use during the period of growth from 110 lb to 200 lb offered no significant advantage in weight gain or FCE (Pfizer, 2000).

Further studies suggested a weight gain advantage between the growth stages of 50 – 110 lb with 40 g/ton, but not 20 g/ton, included in feed and not when performance throughout production was considered (Pfizer, 2000).

Several studies summarised in the dossier suggest a feed inclusion rate of 20 g/ton of virginiamycin improved average daily weight gain from weaning to 125 lb, although higher and lower levels (10 g/ton and 40 g/ton) did not. The significance of differences in FCE was inconsistent, although in all studies FCE was better in pigs fed virginiamycin (Pfizer, 2000).

Local trials included in the dossier examined the effect of virginiamycin at 20 g/ton in minimal disease pigs. In one trial pigs were fed virginiamycin from weaning at 32 days till they were 60 days of age. There was a significantly greater weight gain in those pigs fed virginiamycin. FCE could not be evaluated statistically. In a second trial, boars were fed virginiamycin at 20 g/ton from 12 to 20 weeks of age. In this trial there was no demonstrable advantage to inclusion of virginiamycin in feed. A series of U.S. trials were summarised but no statistical analyses of these studies were included. These studies seem to suggest that the effects of virginiamycin on weight gain and on FCE are inconsistent (Pfizer, 2000).

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Many growth promotion studies are summarised in the virginiamycin technical manual but there are insufficient data to allow a full assessment of these studies. Similarly studies on prevention of swine dysentery are summarised but the data are insufficient for assessment.

A recent study in pigs (Vol 15, page 00 460) examined growth promotion at 40 mg/kg from approximately 5 weeks old to 11 weeks of age (39 kg). There was no significant difference in weight gain between treated and untreated piglets, but the FCE was significantly better in treated piglets. However this advantage was predominantly conferred during the first 2 weeks of treatment. In a second study examining just the first 2 weeks of treatment the improvement in FCE was not seen (Pfizer, 2000).

A trial examining the effect of inclusion of 25 g/ton from age 70 – 80 days to 97 – 107 days, 10 g/ton till age 154 – 164 days then 5 g/ton until slaughter at 192 to 202 days found no overall improvement in weight gain, FCE, disease status, mortalities or carcass characteristics (Pfizer, 2000).

Some studies were included in the dossier that examined the use of virginiamycin as an adjunct to control of clostridial enterotoxaemia in piglets, but the studies do not appear sufficient to establish efficacy (Pfizer, 2000).

One study showed that under experimental conditions virginiamycin may reduce carbohydrate usage by pigs (Vervaeke *et al*, 1979).

Efficacy in Sheep

Efficacy data was included in the dossier to support claims of efficacy in sheep fed concentrate rich rations under a range of different specific Australian conditions. The following review refers only to studies that were presented in sufficient detail to allow a proper assessment of data. Other studies were included in the dossier, but were only presented in summary form, and there was insufficient description of the experimental detail and/or the data used to draw conclusions to allow the reviewer to assess the validity of the studies. For this reason these studies were not used to assess the efficacy of the product.

Hungry sheep introduced to grain

Study 9401 WAO established that drenching hungry sheep with 80mg (2.7 mg/kg) virginiamycin reduced the severity of lactic acidosis when the sheep were given whole wheat *ad libitum* immediately after drenching. However neither 80mg nor 160mg of virginiamycin prevented a break in wool or inappetence (although the data provided are not complete) (Pfizer, 2000).

Study 9205 WAO established that mixing virginiamycin at 40 ppm with wheat or barley reduced the severity of lactic acidosis when it was fed *ad libitum* to sheep. However it did not have a significant effect on sheep fed oats (Godfrey *et al*, 1993).

Study 9207 WAO found that 4 daily drenches with 30mg virginiamycin was as effective as gradual introduction of barley to the diet in preventing decreased rumen pH and increased caecal L-lactate and total volatile fatty acids in sheep fed whole barley (Godfrey *et al*, 1993a).

Grain supplementary to roughage

Study 8902 WAO showed that barley coated with virginiamycin at 40g / tonne could be fed to sheep twice weekly or weekly and resulted in significantly greater weight gains for the first 8 weeks of supplementary feeding than when barley was fed without virginiamycin. There was no difference if barley was fed daily. The virginiamycin treatment also reduced the proportion of sheep with diarrhoea immediately after feeding, but did not result in greater wool growth (Godfrey *et al*, 1993a; Godfrey *et al* 1990)

Grain – based pelleted diets

Study 8701 WAO showed that inclusion of virginiamycin in a pellet ration at 20 ppm or greater resulted in decreased feed intake and reduced weight gain. There was no advantage to inclusion of virginiamycin, even at 10 ppm, on wool production (Pfizer, 2000; Murray *et al*, 1992).

Study 8702 WAO did not establish any clear advantage to including virginiamycin in pelleted feed under conditions simulating live export. There was no significant difference in the weight loss of any group of sheep over the full course of the trial, whether treated with virginiamycin in the assembly and/or shipping phase or untreated. The only significant effect over the whole trial was decreased feed intake in the assembly phase in sheep fed virginiamycin (Pfizer, 2000).

Study 9101 WAO shows that virginiamycin at 40 ppm over 8 days has a positive effect in increasing feeding by sheep introduced to a pelleted diet containing 30% barley. This effect was similar to that provided by replacing half the barley with lupin seed. The provision of hay with the pellets had no positive effect. (McDonald *et al*, 1994).

Study 9102 WAO shows that virginiamycin at over 20 ppm in a pelleted diet containing 15% barley reduced feed intake and weight gain for the first five weeks. There was no effect on wool production or fibre diameter (Murray *et al*, 1992a).

In sheep on a pelleted diet with only lupin grain and 20 ppm virginiamycin over nine weeks there was no effect on feed intake or weight gain, but virginiamycin treatment did reduce wool growth in the first four weeks in both weaners and adults.

Study 9417 NSW shows that on a pelleted diet containing 56% oats, 55% wheat, 40% barley or 85% oats, virginiamycin at 20 ppm had no effect on feed intake or weight gain (Pfizer, 2000).

There is little evidence in these studies that virginiamycin has any health or production value in sheep on pelleted rations. The only positive effect of virginiamycin was to increase the number of sheep feeding in one study (WAO 9101). However in the other studies there was no effect on feed intake or weight gain.

Lactic Acidosis and Wool Strength

Study 9104 found that in experimentally induced lactic acidosis (using intraruminal ground wheat slurry), sheep treated intraruminally with virginiamycin at 80mg/day (equivalent to about 25 ppm) had a lesser loss of wool strength if they had developed lactic acidosis. However over the whole group of sheep virginiamycin treatment offered no positive effect. (Murray *et al*, 1992a).

Field Studies

Study 9515 NSW found no advantage in use of virginiamycin in sheep introduced suddenly to wheat at rate of 200g/day fed twice weekly (Pfizer, 2000).

Study 9516 VICO found that 14 month old hoggets gained weight more rapidly when introduced gradually to twice weekly feeding of barley than when introduced suddenly to barley treated with 40 ppm virginiamycin over 4 weeks (Pfizer, 2000).

Efficacy in Cattle

Efficacy data was included in the dossier to support claims of efficacy in cattle fed concentrate rich rations under a range of different specific Australian conditions. The following summaries refer only to studies that were presented in sufficient detail to allow a proper assessment of data. Other studies were included in the dossier, but were only presented in summary form.

Studies 9201 WAB, 9208 WA and 9505 VICB established that use of virginiamycin at 40 ppm on barley or triticale allows it to be fed weekly to cattle at different rates up to *ad libitum* as a supplement to hay or pasture with similar growth rates to those achieved with daily feeding (Pfizer, 2000; Zorilla-Rios *et al*, 1994).

Study 9505 VICB does suggest alternatives to use of virginiamycin, including a limit lick grain feeder and a “waste not” system, although the information provided does not allow a full comparison. Fuller statistical detail on 9201 WAB and 9208 WAB are needed for a full assessment (Pfizer, 2000).

Field Studies

Cattle fed grain supplementary to roughage at pasture

A series of trials suggest that use of grain treated with virginiamycin is a cost effective alternative to other supplementary feeding regimens.

Study 9406 found that use of virginiamycin at 40 ppm for 1 week then at 20 ppm continuously in cattle for 8 weeks had little advantage over use for only 4 weeks, but that virginiamycin treatment resulted in better weight gain than feeding the grain alone (Pfizer, 2000).

Efficacy under feedlot conditions

Neither study SKB – 1 or SKB – 2 demonstrated any positive effect from virginiamycin at 20 or 40 ppm in cattle introduced to wheat as an increasing proportion of diet with increases from 30%, increasing by 20% increments every 4 days to 90% of the diet. Cattle introduced immediately to a 90% wheat diet treated with virginiamycin did not suffer lactic acidosis. All virginiamycin treated cattle had reduced feed intake and growth rate (Pfizer, 2000).

A combination analysis of four North American studies (VM – 5196 – 86, VM – 5208 – 87, VM – 5226 – 88 and VM – 5236 – 88) (Pfizer, 2000) found that between 17.5 and 25 ppm of virginiamycin in feed resulted in improved weight gain and feed conversion efficiency in feedlot cattle. This level of virginiamycin also reduced the severity of liver abscessation in the cattle. The diets of these cattle were 80% cracked corn (VM – 5196 – 86), 82.5% dry rolled barley (VM – 5208 – 87), 82% corn (VM – 5226 – 88) and 75% steam flaked sorghum (VM – 5234 – 88). In all these studies cattle were gradually introduced to the high concentrate ration.

Efficacy in Dairy Cattle

A study authored by Clayton, Lean, Rowe and Cox examined the effect of virginiamycin at 30 mg/kg on milk production in cattle fed high grain pellets. There was no significant effect on milk production or on milk composition (Pfizer, 2000).

Methane Production by Cows

One study was included that showed that virginiamycin reduced methane production by cattle placed onto a high starch diet. The same study showed that virginiamycin did not affect the methane production of cattle on a high roughage diet (Clayton *et al* ,1996).