



**Australian Pesticides &  
Veterinary Medicines Authority**

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**FINDINGS OF THE RECONSIDERATION OF THE  
REGISTRATION OF PRODUCTS CONTAINING VIRGINIAMYCIN,  
AND THEIR LABELS**

These findings form the basis for regulatory action  
to be taken by the APVMA

**November 2004**

**Australian Pesticides & Veterinary Medicines Authority  
Canberra  
Australia**

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This reconsideration is published by the Australian Pesticides & Veterinary Medicines Authority (APVMA). For further information contact:

Manager, Veterinary Medicines Review  
Australian Pesticides & Veterinary Medicines Authority  
PO Box E240  
KINGSTON ACT 2604  
Australia

Telephone: 61 2 6272 3213  
Facsimile 61 2 6272 3218  
Email: [chemrev@apvma.gov.au](mailto:chemrev@apvma.gov.au)  
APVMA web site: <http://www.apvma.gov.au>

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## FOREWORD

The Australian Pesticides & Veterinary Medicines Authority (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 any 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;
- the products are effective for the purposes claimed; and
- 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 reconsiderations (hereafter referred to as reviews), the APVMA works in close cooperation with advisory agencies including the Department of Health and Ageing, the Department of the Environment and Heritage, the National Occupational Health and Safety Commission, State Departments of Agriculture and other expert advisers, 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 findings is part of that process.

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

These findings outline 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;
- 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 submitted to 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 data which should be submitted to the APVMA to support registration are outlined in various publications which can be obtained by contacting the APVMA.

## 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 and Medical Research Council Expert Advisory Group on Antimicrobial Resistance
FCE	feed conversion efficiency
JETACAR	Joint Expert Technical Advisory Committee on Antibiotic Resistance
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
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
SREf	Streptogramin-resistant <i>Enterococcus faecium</i>
Susceptible humans	Those humans most likely to succumb to an infection caused by a relevant microorganism
SUSDP	Standard for Uniform Scheduling of Drugs and Poisons
VREf	Vancomycin-resistant <i>Enterococcus faecium</i>



## EXECUTIVE SUMMARY

### Introduction

Virginiamycin is one of the streptogramin class of antibiotics. 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, reduction of the risk of acidosis in cattle and sheep and prevention of necrotic enteritis in chickens. Products containing virginiamycin are also used to reduce the risk of laminitis in horses.

Products containing virginiamycin used in food-producing animals are under review due to concerns about the potential to impair the efficacy of other therapeutic antibiotics used for treating human infections, through the development of resistant strains of microorganisms. 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 microorganisms 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 instructions for use of the products and to make objective, scientifically-based recommendations about the future registration of the products. The review 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 that of disease due to infection in susceptible humans by 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 Australian and international data on resistance to virginiamycin in animals and quinupristin-dalfopristin (QD) in humans, and published evidence regarding the risk of transmission of virginiamycin/QD resistance from animals to humans. The assessment is based on 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; and
- the risk.

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

- Use of virginiamycin 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 the dalfopristin component of QD, which is another streptogramin class of antibiotic. Resistance to virginiamycin and QD requires resistance to both streptogramin A and B.
- 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 to be the transfer of streptogramin-resistant *E. faecium* to humans. Based on overseas findings, the following factors were considered:

- Virginiamycin-resistant enterococci which are also resistant to vancomycin have been cultured from food animals, their environment and their meat products.
- Colonisation of humans by animal-derived *E. faecium* and/or transfer of resistance to human strains of *E. faecium* may occur, although transfer of resistance has not yet been observed.
- 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 the volunteers' stools. Intestinal transit did not result in disease.

## Virginiamycin review findings

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

- infection of humans with streptogramin-resistant *E. faecium*;
- infection with vancomycin-resistant *E. faecium*;
- disease due to infection with *E. faecium* resistant to both 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;
- the *vanB* gene complex encodes the more common form of vancomycin-resistant enterococci in Australia. With a single exception, this form of resistance has not been found in animals;
- recent Australian studies have demonstrated no resistance to QD 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 QD, a streptogramin, or the newer antibiotic linezolid;
- the impact of antibiotic failure on relatively minor infections such as wound infections and urinary tract infections is small.

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. Data on the prevalence of virginiamycin-resistant 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:

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

The findings 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, but 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 suggested 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, its continued use in feed as a growth promotant in pigs and poultry is not supported. It is recognised 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 was first registered in Australia.

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 once or twice per week, and as an aid to control lactic acidosis in the initial month of adaptation of feedlot cattle to a high-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 caused 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.

### Findings

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;
- times when the products should be used;
- duration of any treatment using the product; and
- 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
- is 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 are 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 by the following regulatory actions.

- (i) The APVMA proposes to vary conditions of label approval for products containing virginiamycin registered for the prevention of lactic acidosis in sheep, beef and dairy 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 conversion efficiency in pigs, poultry and turkeys; and
  - the addition of more specific instructions and restraint statements pertaining to the products' 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 of product use;
    - the proposed label changes constrain veterinarians' rights to prescribe the antibiotic for periods longer than specified on the label, or to prescribe repeat treatments.
- (ii) The APVMA proposes to affirm the registration of products containing virginiamycin registered for the prevention of lactic acidosis in sheep, beef and dairy cattle and the prevention of necrotic enteritis in chickens.
- (iii) The APVMA proposes to cancel the registration and label approvals of those products that have label claims pertaining to growth promotion and/or improved feed conversion efficiency only. The APVMA is not satisfied that available non-contemporary data demonstrate that these products are effective under Australian farming practices for this purpose or that conditions of registration or approval can be varied to satisfy the APVMA's concerns.



## **SECTION 1: REASONS FOR AND SCOPE OF THE REVIEW**

### **1.1 Current use of virginiamycin in animal health in Australia**

Virginiamycin is one of the streptogramin class of antibiotics. 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. Products containing virginiamycin are used to reduce the risk of laminitis in horses. A product containing virginiamycin is registered for prevention of necrotic enteritis in chickens.

At its meeting in February 2003, the National Drugs and Poisons Schedule Committee (NDPSC) included all uses of virginiamycin in Schedule 4 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP), on the basis of advice from the Expert Advisory Group on Antimicrobial Resistance (EAGAR). This advice stated that continued unrestricted use of virginiamycin posed an unacceptable risk to human health from the development of resistance to the streptogramin class of antibiotics and transfer of resistant organisms from food animals to humans. The scheduling decision was also consistent with the Australian Government response to the September 1999 recommendations of the Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR).

Prior to February 2003, animal feed premixes or animal feed additives containing 2 per cent or less of virginiamycin, or when packed in individual sachets containing 20g or less of virginiamycin, were classified in Schedule 5 of the 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 were included in Schedule 5.

One of the products under review which contains 500g/kg virginiamycin has always been a Schedule 4 product. Schedule 4 is for substances requiring prescription by qualified practitioners (in this case veterinarians).

The Schedule 4 amendment came into effect on 1 September 2003.

### **1.2 Reasons for the reconsideration of registrations and approvals relating to virginiamycin**

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

In 1997 the Minister for Health and Family Services and the Minister for Primary Industries and Energy established the Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR). The committee broadly assessed the use of antibiotics in food-producing animals, the occurrence of antibiotic resistance and its importance in human and veterinary medicine. In its September 1999 report entitled *The use of antibiotics in food-producing animals: antibiotic-resistant bacteria in animals and humans*, JETACAR recommended that the use of virginiamycin in food-producing animals be reviewed by the APVMA. The basis of 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
- the possible emergence of resistant strains of animal bacteria which may cause human disease.

In July 2000 the APVMA decided 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 about whether the continued registration of the products in accordance with the current instructions for use would be likely to have an effect that is harmful to human beings. Additional concerns were whether the products were effective for the purposes claimed and whether the labels contained adequate instructions.

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

### 1.3 Overseas regulatory status

#### 1.3.1 Regulatory status in the European Union

In the European Union, virginiamycin was originally authorised as a feed additive for growth promotant purposes in pigs and poultry. In 1998 the Council of the European Union withdrew the authorisation 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 authorised for such uses in food animal species in Europe and does not have an established maximum residue limit (MRL).

The decision to withdraw the growth-promotant use of virginiamycin was made despite advice from the council's 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 the European courts.

However in 2002 the Court of Justice upheld the decision, 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 withdrawal of authorisation for the products was not a disproportionate measure given the need to protect public health.

### **1.3.2 Regulatory status in the United States of America**

In the United States of America, products containing virginiamycin are approved 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 commenced a risk assessment of human health impacts associated with the use of virginiamycin in food-producing animals, particularly in relation to the development of resistant microorganisms. Updates on the progress of that risk assessment have not been published to date.

In February 2002 the United States Senate introduced 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.

In April 2004, the General Accounting Office, an investigating arm of Congress, released a report which concluded that antibiotic resistance in humans resulting from the use of antibiotics in animals was an unacceptable risk. The report recommended that federal agencies focus their efforts to reduce the risk to human health from the transfer of antibiotic resistant bacteria from meat, and further highlighted that trade implications are looming for countries that do not improve their antibiotic-use practices.

### **1.3.3 Regulatory status in New Zealand**

In New Zealand, virginiamycin is approved for use in horses and poultry, and is classified as a prescription animal remedy. There are no approved uses for cattle and sheep. New Zealand has 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 it is essential for the welfare of animals.

## **1.4 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 against which 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. The criteria are that the products are:

- of demonstrable efficacy in livestock production under Australian farming conditions;
- rarely or never used as systemic therapeutic agents in humans or animals, or are not considered critical therapy for human use; and
- 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.

## Virginiamycin review findings

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This review covers the human health aspects as well as the efficacy of products containing virginiamycin. The review scope includes those products that are used in food-producing species, and the 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 review are shown in Table 1.

**Table 1: Registered products and their labels subject to review**

Product number	Product name	Poisons schedule classification on the current label#	Registrant	Label claims
38329	Stafac 20 Feed Premix	Schedule 5	Phibro Animal Health Pty Ltd	For broilers, turkeys and growing pigs to improve growth and feed conversion efficiency.
41286	Virginiamycin 20	Schedule 4	Agribusiness Products Pty Ltd	Improve growth rate and feed conversion efficiency in growing pigs, broilers and turkeys
41476	Virginiamycin 20	Schedule 5	Lienert Australia Pty Ltd	Growth promotion and improved feed conversion efficiency in growing pigs
46049	Eskalin Feed Premix for Cattle	Schedule 4	Phibro Animal Health Pty Ltd	Reduce risk of acidosis in cattle
49111	Eskalin Wetable Powder Spray-on Feed Premix	Schedule 5	Phibro Animal Health Pty Ltd	Reduce risk of acidosis in cattle and sheep
51354	Eskalin 500 Feed Premix	Schedule 4	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

# The current poison schedule classification for virginiamycin is Schedule 4 when the antibiotic is used in food-producing animals. Except for products 41286, 46049 and 51354 the poison scheduling classification on the approved labels for the remaining products is lagging behind the current classification and requires updating.

## SECTION 2: PUBLIC HEALTH RISK ASSESSMENT

### 2.1 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 uses of virginiamycin in Australian livestock.

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

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 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.

### 2.2 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 defined as:

- 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 caused by a relevant microorganism.*

### 2.3 Methodology of assessment

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

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

The risk assessment accessed 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 QD in animals and humans;
- examine the published evidence regarding the risk of transmission of virginiamycin/QD resistance from animals to humans; and
- make recommendations about virginiamycin products taking into account recommendations of the September 1999 JETACAR report.

### 2.4 Information sources

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

## 2.5 Assumptions

The assessment made the following assumptions:

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

## 2.6 Background to the risk assessment

### 2.6.1 Streptogramins and their mode of action

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

Streptogramins are protein synthesis inhibitors, acting at the level of the 50S subunit of the ribosome.

Streptogramin B acts at the same site as macrolides and lincosamides, and can be affected by the same resistance mechanisms.

Streptogramin A, binding at a separate site on the ribosome, has both intrinsic antimicrobial activity and enhances the effect of the streptogramin B component. More detailed information on the mode of action of streptogramins is provided in Appendix A. All currently marketed products containing streptogramins are combinations of A and B components, which act synergistically at the ratios used in the formulations.

### 2.6.2 Spectrum of activity of virginiamycin

Virginiamycin exhibits antibiotic activity against the following microorganisms:

- Gram-positive aerobic and anaerobic bacteria, 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 including most *Haemophilus* species, *Neisseria gonorrhoeae* and *N. meningitidis*; and
- *Lawsonia intracellularis*, *Brachyspira* species and some species of Mycoplasma that are susceptible to virginiamycin.

### 2.6.3 Streptogramin use in humans

QD, brand name Synercid® is one of two streptogramins registered for human use in Australia and is available in an injectable form only. The approved indications for use 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  $\beta$ -lactam, quinolone or glycopeptide intolerant patients.*

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

A relatively new antibiotic, linezolid, has been registered for human use in Australia, and appears to have equivalent efficacy to QD.

### 2.6.4 Other related human agents

Pristinamycin is a minor-use oral antibiotic which has been 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.

### 2.6.5 Streptogramin use in animals in Australia

Virginiamycin is the only streptogramin registered for use in animals.

### 2.6.6 Virginiamycin use patterns including indications and species

Virginiamycin is used in long-term low-dose regimens 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 growth rates and feed conversion efficiency in pigs, broilers and turkeys, and preventing lactic acidosis in cattle and sheep, and laminitis in horses. Products containing virginiamycin are also used for the prevention of necrotic enteritis in chickens.

Detailed assessment of whether the products are effective for the purposes claimed can be found in Section 3: Efficacy assessment.

## 2.7 Hazard characterisation

### 2.7.1 Bacterial resistance mechanisms

Bacterial 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 A.

### 2.7.2 Resistance hazard

The principal resistance hazard is development of *E. faecium* resistance to virginiamycin in animals and subsequent cross-resistance to QD in humans, 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 dalfoprstin component of the QD combination (Werner *et al.*, 2000; Werner *et al.*, 1998; Aarestrup *et al.*, 2000).

### 2.7.3 Secondary resistance hazard

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

### 2.7.4 Resistance breakpoints

Enterococcal resistance to virginiamycin is currently defined as minimum inhibitory concentration (MIC) values of  $\geq 4$ mg/L. This value is based on population distribution of MIC values and is used for Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) resistance surveillance in Denmark (Aarestrup *et al.*, 2000). According to DANMAP 2001, the breakpoint is now  $\geq 8$ mg/L (DANMAP 2001).

QD resistance breakpoints have been established by the National Committee for Clinical Laboratory Standards (NCCLS) for enterococci as  $\leq 1$ mg/L susceptible, 2mg/L intermediate, and  $\geq 4$  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  $\leq 2$ mg/L for susceptible,  $\geq 4$ 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 B. 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 an MIC of 2mg/L are abnormal and harbour resistance determinants such as the *vat(D)* or *vat(E)* genes.

### 2.7.5 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 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).

### 2.7.6 Resistance data in targeted animal bacterial species

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

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

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

### 2.7.7 Resistance data in human bacterial species

Australian studies have demonstrated no acquired resistance to QD in human clinical isolates (following table reproduced from Turnidge & Bell, 1999).

Species (number of isolates)	%QD 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>E. 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 Diameter (mm)		Number QD R** isolates
		50%	90%	Range	Median	Range	
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 one to two-fold higher than the breakpoint MIC of 1mg/L, and only two 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 apparent resistance in *E. faecium* in published studies being due to the inclusion of misidentified *E. faecalis*.

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

### 2.7.8 Cross-resistance data in animal isolates

Data on cross-resistance in animal isolates are available for *E. faecium* and are summarised as follows.

QD-resistant enterococci recovered from animals and raw meat 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) were 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 United States, 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 United States where virginiamycin was in use and 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 (130 days) (Welton *et al.*, 1998).

Turkeys, pigs, treated sewage water, broiler carcasses, and pork were sampled in Germany. Farms that used virginiamycin and farms that did not 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 (two) and pig (six) farms using virginiamycin yielded QD-resistant *E. faecium*. Eleven out of twenty four (46%) poultry carcasses were positive for QD-resistant *E. faecium*. Only one isolate was found to be resistant from 10 samples of raw pork (Werner *et al.*, 2000).

### 2.7.9 QD cross-resistance data in human isolates

Data on QD cross-resistance in human isolates are available for *E. faecium* and are summarised as follows.

In Taiwanese data prior to clinical use of QD (1,287 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 Taiwanese animal production systems. *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 the United States). Fifteen per cent 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 six 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 four QD-resistant strains among 121 strains of *E. faecium* (3.3%). None of these strains were from Australia (Jan Bell, pers. comm.).

### 2.7.10 Data in human isolates re cross-resistance 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 methicillin-resistant *S. aureus* (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.

### 2.7.11 Data on co-selection of resistance to unrelated antibiotics

Published data from Germany and Denmark show 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). Investigators showed a 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 (Aarestrup *et al.*, 2000).

### 2.7.12 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.

## **2.8 Exposure**

### **2.8.1 Volume of streptogramin imports into Australia**

Import volumes of streptogramins into Australia (in kilograms) for the financial years 1992–93 to 1996–97 were published in the JETACAR report. The figures are reproduced in Appendix C and range from 17,000 kg in 1992–93 to 36,005 kg in 1996–97. The preliminary figure for the financial year 1997–98 is 59,172 kg, for 1998–99 nil and for 1999–2000 65,000 kg.

### **2.8.2 Exposure of animal bacteria to virginiamycin**

#### **2.8.2.1 Volume of use in animals**

There are no firm data about end usage in animals in Australia.

#### **2.8.2.2 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.

#### **2.8.2.3 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 relatively likely to select for resistance based on the standard laboratory technique for selecting resistant mutants.

### **2.8.3 Exposure of humans to resistant animal bacteria or transferable genetic elements**

#### **2.8.3.1 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, intermediate and resistant virginiamycin strains have been detected in enterococci which are cultured from minced beef and pork, also resistant to vancomycin (Klein *et al.*, 1998).

#### **2.8.3.2 Viability of animal *E. faecium* strains in human intestine**

A recent Danish study examined the effect on volunteers of a virginiamycin-resistant strain of *E. faecium* (six subjects) as well as a vancomycin-resistant strain (six subjects) (Sorensen *et al.*, 2001). Resistant strains were detected in all subjects until day five after ingestion and in one subject at day 14. The authors concluded that 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 recent study from China demonstrated the transmission of a pathogenic vancomycin-susceptible strain of *E. faecium* from pigs to humans, infection with this pathogen resulting in the deaths of thousands of pigs and 12 humans (Lu *et al.*, 2002). Transmission was confirmed by 16S RNA gene sequencing, and genomic DNA analysis by pulsed-field gel electrophoresis.

### **2.8.3.3 Mitigation procedures likely to reduce bacterial transmission to humans**

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

### **2.8.3.4 Residual concentrations in food**

Virginiamycin is poorly absorbed from the gastrointestinal tract of animals and concentrations in animal tissues for human consumption are undetectable or negligible by standard methods. Maximum residue limits (MRLs) for virginiamycin are published in the APVMA's MRL Standard ([www.apvma.gov.au](http://www.apvma.gov.au)). For some food commodities the MRL is set 'at or about' the limit of analytical quantitation.

## **2.9 Impact**

### **2.9.1 The importance of streptogramin antibiotics in human medicine**

Virginiamycin is not used in human medicine. However, QD is a structurally related streptogramin, and 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 antibiotic, linezolid, has now been registered for human use in Australia, and appears to have equivalent efficacy. Recently published studies from the United States demonstrate that enterococcal resistance to linezolid emerges comparatively rapidly (Gonzales *et al.*, 2001; Jones *et al.*, 2002), and a small hospital outbreak has been described (Herrero *et al.*, 2002). It appears unlikely that linezolid will completely replace QD in the short term to become 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.

### **2.9.2 The importance of enterococcal disease in humans**

Enterococcal infections infrequently cause disease in the general human population. When they do cause disease they are primarily urinary tract infections. Enterococcal infections

occur more frequently and importantly in hospital-acquired infections, affecting urinary tract infections and 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. Cross-resistance is a major problem with these resistant strains of enterococci, resulting from intensive use of cross-selecting antibiotics such as the cephalosporins, glycopeptides and metronidazole. The natural resistance of enterococci to antibiotics, 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 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.).

### 2.9.3 Impact of failure of antibiotic treatment in humans

Septicaemia from *vanA E. faecium* occurs primarily 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.

## **2.10 Assessment of the uncertainty of the data used in risk assessment**

### **2.10.1 Uncertainty due to inherent variability and measurement error**

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

### **2.10.2 Uncertainty due to lack of information**

As stated previously, accurate data on virginiamycin-resistant 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.

## 2.11 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
<b>Hazard</b>	
Selection of resistance genes to virginiamycin in <i>E. faecium</i> of animals	<ul style="list-style-type: none"> <li>• Virginiamycin use in food-producing animals can select for <i>E. faecium</i> possessing either the <i>vat</i>(D) or <i>vat</i>(E) genes, which encode for production of a streptogramin A acetyltransferase (an inactivating enzyme), resulting in virginiamycin-resistant <i>E. faecium</i> (VirREF).</li> <li>• Production of streptogramin A acetyltransferases confers resistance to the dalfopristin component of another streptogramin (QD). Resistance to QD requires resistance to both streptogramin A and B.</li> <li>• VirREF found in food-producing animals and their commercial products can be co-resistant to other antimicrobials, including vancomycin.</li> </ul>
<b>Exposure</b>	
Consumption of streptogramin-resistant <i>E. faecium</i> by humans	<ul style="list-style-type: none"> <li>• Virginiamycin-resistant strains have been detected in enterococci also resistant to vancomycin cultured from food animals, their environment and their meat products.</li> </ul>
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"> <li>• VirREF of animal origin can survive gastric passage, multiply, and be isolated for up to 14 days from human volunteers.</li> </ul>

Factors Considered	Findings
<b>Impact</b>	
Infection of humans with animal-derived streptogramin-resistant <i>E. faecium</i>	<ul style="list-style-type: none"> <li>• Conclusive evidence of human infection with animal-derived streptogramin-resistant <i>E. faecium</i> is lacking.</li> </ul>
Infection of humans with <i>E. faecium</i> resistant to vancomycin	<ul style="list-style-type: none"> <li>• Vancomycin-resistant enterococci have a high propensity to cause outbreaks in hospitals.</li> <li>• 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.</li> </ul>
Disease in humans due to infection with <i>E. faecium</i> resistant to vancomycin and streptogramins	<ul style="list-style-type: none"> <li>• Recent Australian studies have demonstrated no resistance to QD in human clinical isolates.</li> </ul>
Treatment failure attributable to acquisition of streptogramin-resistant <i>E. faecium</i> from animals	<ul style="list-style-type: none"> <li>• 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 – QD, or the newer antibiotic linezolid.</li> </ul>
<b>Assessment of data uncertainty</b>	
<p>Uncertainty due to inherent variability and measurement error</p> <p>Uncertainty due to lack of information or understanding</p>	<ul style="list-style-type: none"> <li>• It is not clear whether strains of <i>E. faecium</i> with so-called intermediate resistance (MIC &gt;2mg/L) to virginiamycin are truly resistant and possess relevant resistance genes.</li> <li>• Data on virginiamycin-resistant and vancomycin-resistant <i>E. faecium</i> prevalence in food animals and incidence of human infections in Australia are lacking.</li> <li>• There are limited data on the importance of virginiamycin in the selection of enterococci co-resistant to both virginiamycin and vancomycin in food animals.</li> </ul>

Factors Considered	Findings
<b>Preventative/therapeutic uses</b>	
Use in food-producing animals	<ul style="list-style-type: none"> <li>• Virginiamycin plays a role in the management of the following diseases in food-producing animals:               <ul style="list-style-type: none"> <li>○ lactic acidosis in cattle and sheep given high grain diets</li> <li>○ necrotic (clostridial) enteritis in chickens.</li> </ul> </li> </ul>
<b>Level of acceptable risk</b>	
Use in food-producing animals	<ul style="list-style-type: none"> <li>• An acceptable level of risk is that level of probability <b>and</b> 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.</li> </ul>
<b>Risk characterisation</b>	
Risk in susceptible humans	<ul style="list-style-type: none"> <li>• 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, <b>and</b> the severity of impact in susceptible humans is high.</li> </ul>
Risk in general population	<ul style="list-style-type: none"> <li>• 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, <b>and</b> the severity of impact in humans in the general population is low.</li> </ul>

## 2.12 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 (see Section 3) 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 advises that all uses of virginiamycin in animals should be classified in Schedule 4 as was done by NDPSC in February 2003.

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 suggests that label

## Virginiamycin review findings

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instructions be modified so as to permit use of a 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 6: Review findings and reasons.

## SECTION 3: EFFICACY ASSESSMENT

### 3.1 Introduction

The first recommendation of the JETACAR report stated, *inter alia*, that use of in-feed antibiotics 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 practised unless the antibiotics 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.

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.

### 3.2 Terminology

**Efficacy** is defined as: *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** is defined as: *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** is defined as: *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** is defined as: *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.*

### 3.3 Methodology of assessment

The data assessed are 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.

### **3.4 Information sources**

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

### **3.5 Summary of studies submitted in support of label claims**

Data were 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 were provided in support of virginiamycin to prevent 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 D.

#### **3.5.1 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 assessment. Some overseas studies demonstrated that treatment with virginiamycin at suggested dosages had a measurable effect on weight gain and/or on feed conversion efficiency. However, more recent studies do not show any significant growth promotion effect due to use of virginiamycin. 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.

#### **3.5.2 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 may result in ruminal acidosis with subsequent stasis and devitalisation of the ruminal wall. Systemic acidosis 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. 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 that they help prevent lactic acidosis in the initial month's adaptation of feedlot cattle to a high grain diet.

### **3.5.3 Use of virginiamycin to prevent necrotic enteritis in chickens**

Necrotic enteritis is a common disease of poultry induced by toxigenic strains of *C. perfringens*, which may express itself as a subacute condition which slows growth, or as a fatal gastrointestinal disease. It is particularly prevalent in broiler chickens on wheat-based diets. Wheat and barley diets are prominent in Australian broiler production.

Virginiamycin is used extensively in the broiler industry for the combined purposes of controlling necrotic enteritis and improving feed conversion efficiency/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 *C. perfringens*, resulting in necrotic enteritis.

Examination of the data presented suggests that the data are sufficient to justify a claim of efficacy for virginiamycin against necrotic enteritis in poultry.

## **3.6 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 that they 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 are available. The assessed studies reflected the variation in response to antibiotic administration that is commonly associated with this use pattern and class of drug.

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



## SECTION 4: PUBLIC CONSULTATION

### 4.1 Scale of submissions

On 1 April 2003, the APVMA published a call for public comments on the draft review report, to be submitted by 23 May 2003. The deadline for written submissions was subsequently extended to 31 July 2003. A total of 39 submissions were received from private individuals, product manufacturers, academia, State Departments of Agriculture and Health, medical and veterinary practitioners, and public interest groups and associations, including the Australian Consumers' Association.

### 4.2 Summary of issues raised in the submissions

Issues raised in the submissions are summarised as follows:

#### *Quantitative risk assessment*

The use of a quantitative risk assessment as part of the evaluation of applications for registration of antibiotics is canvassed in *Part 10: Special Data Requirements*, of the APVMA's *Guidelines for Registering Veterinary Chemicals*.

One submission contained a quantitative risk assessment which estimated the upper-bound annual statistical risk of death to an individual Australian caused by streptogramin-resistant *E. faecium* (SREf), directly attributable to the use of virginiamycin in animals, at  $1:3 \times 10^{-12}$ . This submission contends that the statistical risk equates to less than one Australian fatality every thousand years. A detailed analysis of the quantitative risk assessment is in paragraph 5.5, where this conclusion is questioned on a number of bases.

#### *Qualitative risk assessment*

Comments presented in response to the APVMA qualitative risk assessment in the draft review report included claims that:

- the public health risk assessment is hazard characterisation since it does not link the potential for harm to the likelihood of harm;
- the risk to human health is not identified;
- the assumption that the hazards, exposure, impact and risk characterisation are essentially similar between the animal species is invalid;
- QD therapy failure is very low or rare, and failure may be due to factors other than resistance; and
- there is no scientific justification for further regulation of virginiamycin.

### *Efficacy, duration and frequency of treatment*

Some submissions questioned the effect of virginiamycin on milk production and its efficacy in treating and preventing disease in pigs and poultry. Clarification on the dose of virginiamycin required to prevent acidosis in cattle was given, while it was purported that mortality rates in chickens increased despite virginiamycin treatment.

In several submissions there were arguments against the prophylactic use of virginiamycin in poultry and in all animals.

Arguments were presented in favour of a 14-day treatment period in all species as well as arguments against a 14-day treatment period for chickens, dairy cattle, beef cattle and lambs. In some instances respondents interpreted the term 're-treatment' differently and presented views either for or against continued approval of re-treatment instructions.

### *Antimicrobial resistance and prophylaxis*

A number of respondents were concerned with the development of antimicrobial resistance. Their arguments were:

- there should be no animal use of antibiotics as growth promotants and for prophylactic use, because of the risk of induction of antimicrobial resistance and transfer of that resistance to human pathogens;
- where antibiotics are 'critical use' antibiotics in human medicine (as defined in the JETACAR report), there should be no use at all of these antibiotics in animals, or of other antibiotics from the same class. The human antibiotic QD is such a 'critical use' antibiotic and virginiamycin is a member of the same streptogramin class of antibiotics. There is a specific risk of SREf of animal origin causing harm to human health.

### *Need for virginiamycin in animal production*

A number of submissions highlighted the need for virginiamycin in current cattle, sheep and poultry production systems in Australia. The antibiotic is important for preventing and treating lactic acidosis in ruminants, and necrotic enteritis in chickens. Restrictions in its use (such as a restraint statement limiting the period of use) will have an adverse effect on animal welfare and will lead to increased use of other antibiotics.

### *Regulatory concerns*

In February 2003, the NDPSC rescheduled virginiamycin to Schedule 4 (Prescription Animal Remedy) and in June 2003 that decision was re-affirmed. There were few comments at the time against the Schedule 4 decision.

Substitute label statements were suggested as replacements for the restraints proposed in the APVMA draft review report. In addition, arguments were presented that Schedule 4 scheduling and removal of growth promotion uses of virginiamycin are sufficient to restrict access to the antibiotic.

### *Veterinary prescribing rights*

In response to the restraints on duration and frequency of treatments proposed in the APVMA draft review report, several respondents were of the opinion that controls on the use of virginiamycin should be via the development of veterinary codes of practice and guidelines rather than by imposing label restraint statements. These respondents contend that mandatory re-treatment restraint statements on Schedule 4 drugs are an unnecessary restraint on the veterinarian's right to prescribe.

### *Use of permits for off-label use*

The use of permits for off-label use was generally not supported on the grounds of cost, time delays, possible litigation for damages, potential operational issues related to how permits would work, and a range of legal and welfare consequences of the death of any animal treated under permit.

One submission was highly critical of the permit system as a regulatory option. This submission objected to the implementation of the permit system on grounds that it is contrary to the principles of good regulatory practice, whilst contending that the permit proposal is against the Council of Australian Governments principles (1995) set out in *Principles and Guidelines for National Standard Setting and Regulatory Action by Ministerial Councils and Regulatory Bodies*. Apart from querying how the permits will work and be policed, rules and procedures for granting and denying the permits, and the method for resolving appeals against decisions, the submission remarked that it is better to rely on the professional judgement and integrity of veterinarians rather than imposing a remote and arbitrary system that is costly, impractical and less effective than the alternative approach of relying on veterinarians.

### *Other comments received*

Other comments included:

- a suggestion for the use of probiotics as an alternative means of disease prevention;
- concerns over the preclusion of treatment of small ruminants who also suffer from acidosis under the same husbandry conditions; and
- comments on the proposed label amendments indicating that readers had different definitions and interpretations of drought and feedlot feeding, and feeding weekly or twice weekly, as they apply to sheep, lambs, dairy and beef cattle.
- there should be precise record keeping relevant to virginiamycin use, periodic review of records, and imposition of substantial penalties for breaches of the label instructions. There should be a program to monitor development of resistance in enteric pathogens in animals. The impracticality of compliance monitoring was highlighted as there are no residues to measure thus making non-compliance difficult to prove.

#### 4.2.2 Summary of opinions

The range of opinions expressed in the submissions was very broad but tended to cluster around two opposing positions:

1 *The regulatory proposals do not adequately restrict the use of virginiamycin in animals*

Supporters of this position expressed a view that:

- a link exists between the use of virginiamycin in animal production and antibiotic resistance in human pathogens; and
- it is not good policy to allow the widespread use of antibiotics in food-producing animals, where those antibiotics are also important in human antibiotic therapy.

2 *The regulatory proposals are too restrictive*

Supporters of this position expressed the view that:

- virginiamycin is a very useful antibiotic in cattle, sheep and broiler production;
- the risk to human health is unsubstantiated and may be non-existent;
- the 2003 rescheduling of virginiamycin products to Schedule 4 has placed appropriate levels of veterinary control over the use of virginiamycin;
- any further restraints on the use of virginiamycin, such as label restraints on duration of use, would not be appropriate and would limit a veterinarian's professional right to prescribe; and
- restrictions on virginiamycin will have negative implications for animal health and welfare.

#### 4.2.3 Meeting to discuss the issues

On 30 January 2004 the APVMA convened a meeting of representatives of major interest groups to discuss the issues raised by the draft review report and the public submissions.

At the meeting some stakeholders were opposed to label restraint statements that would limit the duration of use of virginiamycin and limit the capacity for re-treatment. Other stakeholders remained opposed to continued use of virginiamycin unless there were restraints on its duration of use and restraints on re-treatment.

It was stated at the meeting that consumers take the issue of antimicrobial resistance seriously and generally were opposed to the use of antibiotics for growth promotion and long-term prophylaxis.

By the end of the meeting, the debate had crystallised into two linked (and unresolved) questions: should there be restraints on the duration of use of virginiamycin, and should there be restraints on re-treatment?

## **SECTION 5: REVIEW CONSIDERATIONS RELEVANT TO THE PUBLIC CONSULTATION: ASSESSMENT OF INFORMATION PROVIDED TO THE REVIEW**

### **5.1 The JETACAR report**

The JETACAR report was delivered in September 1999 and subsequently accepted by the Australian Government. The committee's principal terms of reference were to review the scientific evidence on the link between the use of antibiotics in food-producing animals; the emergence and selection of antibiotic-resistant bacteria and the spread of these resistant organisms to humans; and the transfer of resistance genes from bacteria of animal origin to bacteria of human origin.

The JETACAR report agreed that there 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 JETACAR position is supported by the outcomes of the Joint Food and Agriculture Organization/World Organisation for Animal Health/World Health Organization Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment, conducted in Geneva in December 2003, which concluded that:

...there is clear evidence of adverse human health consequences due to resistant organisms resulting from non-human usage of antimicrobials. These consequences include infections that would not have otherwise occurred, increased frequency of treatment failures (in some cases death) and increased severity of infections, as documented for instance by fluoroquinolone-resistant *Salmonella* infections. Evidence shows that the amount and pattern of non-human usage of antimicrobials affect the occurrence of resistant bacteria in animals and on food commodities and thereby human exposure to these resistant bacteria. The food-borne route is the major transmission pathway for resistant bacteria and resistance genes from food animals to humans, but other routes of transmission exist.

It should be noted that fluoroquinolones have not been registered for use in food-producing animals in Australia, therefore the risk of fluoroquinolone-resistant *Salmonella* infections in Australia is minimal. The fluoroquinolone example is relevant because it provides evidence that there is a link between antibiotic usage in food-producing animals and the emergence of bacterial resistance in human pathogens. Concerns are thus valid that there could be a link between adverse human health consequences due to streptogramin-resistant enterococci and the usage of virginiamycin in food-producing animals.

JETACAR undertook a literature review, which concluded that:

...with regard to enterococci, non-typhoid salmonellae, *E. coli* and campylobacter, the selective pressure provided by non-human use of antibiotics has been sufficient to cause development of multiple resistance:

- there is qualitative evidence that feeding antibiotics to animals leads to resistant bacteria and these bacteria or their resistance genes are passed on to humans, principally via the food chain;
- there exists strong evidence (for salmonella, campylobacter and enterococci) that human disease is caused by resistant bacteria or bacteria containing resistance determinants that are present in animals;
- the Australian situation in this area has not been well studied. However, demonstrated emergence of transfer of resistance overseas provides an important model that should inform development of Australian policy.

However, the JETACAR report also stated:

Based on present inadequate survey information from animals in Australia, only a proportion of human infection can so far be suspected to result from resistance transfer to human enterococci.

The JETACAR report made a total of 22 recommendations, the first eight of which were framed on the basis that for those antibiotics within groups of antibiotics critical to human health, growth promotant and oral prophylactic uses in food-producing animals should be reviewed and phased out if they fail to meet three key criteria that are stated in paragraph 1.4 of this report.

The JETACAR report classified the streptogramin class of antibiotics as Category A, that is, essential antibiotics for treatment of human infections where there are few or no alternatives for the treatment of many infections.

## 5.2 Public health assessment

The public health assessment of this review 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. Nevertheless, the public health assessment concluded that the potential risks to susceptible humans due to exposure to SREf are higher than those for the general population. Although the probability of disease in susceptible humans due to exposure to SREf of animal origin is low, the severity of impact in susceptible humans is high.

The assessment recognised that in the case of specified animal therapeutic uses of products containing virginiamycin for a short, defined period, the probability of resistance development and severity of impact is unlikely to compromise the treatment of bacterial infections in susceptible humans.

In response to a second submission from the veterinary profession, the APVMA's health advisors re-emphasised concerns of the possibility of cross-resistance to QD emerging as a result of the use of virginiamycin. The health advisors repeated their original advice for limiting re-treatment and the duration of use of virginiamycin.

## 5.3 Efficacy assessment

The efficacy assessment of this review concluded that there is not consistent evidence that products containing virginiamycin will produce a measurable positive effect in growth or feed conversion efficiency. The efficacy assessment found some evidence of improvement in

performance parameters of pigs and poultry. However, no recent Australian data are available. The assessed studies reflected the variation in response to administration of antibiotics as growth promotants 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 certain disease conditions in food-producing animals, including necrotic enteritis in chickens and lactic acidosis in cattle and sheep given high-grain diets. However, the review noted that existing instructions on currently approved labels pertaining to the uses of virginiamycin in sheep and cattle were considered to be inadequate.

### 5.4 Literature review of the public health significance of enterococci in cattle

A literature review provided to the APVMA identified the following main points:

- There is a lack of Australian studies on bovine enterococci and their antimicrobial resistance patterns.
- *E. faecium* and *E. faecalis* are present in very low numbers in adult cattle.
- Meat and meat products from cattle, pigs, poultry and turkeys are possible sources of resistant enterococcal strains, but the review was inconclusive in establishing whether the strains isolated from humans were of animal origin or whether resistance determinants had been transmitted from animal bacterial strains to human strains.
- There is a close association between the withdrawal of authorisations in Europe for the use of antimicrobials for growth promotion and a decrease in prevalence of resistant enterococci in food animals, food and people.
- Cross-resistance exists between virginiamycin and either QD or the macrolides or lincosamides.

On the weight of the available evidence, the literature assessment concluded that the overall risk of antimicrobial use in cattle contributing to the burden of multi-drug resistant *E. faecium* and *E. faecalis* in humans is small.

### 5.5 Quantitative risk assessment

The major registrant of virginiamycin products submitted a quantitative risk assessment that estimated the upper-bound annual statistical risk of death to an individual Australian caused by SREf, directly attributable to the use of virginiamycin in animals, at  $1.3 \times 10^{-12}$ . This represents less than one statistical fatality over the next 1,000 years for the entire Australian population.

Approaches to risk assessment of antibiotic resistance may span a continuum from qualitative to quantitative. Data on the link between use of antibiotics in animals and the emergence of antibiotic resistance in human pathogens are often incomplete.

An inherent problem of quantitative risk assessment relates to reliability when fundamental data to support key parameters are sparse. In that case, the outcome of a quantitative risk assessment may result from a chained series of speculations. The statistical assumptions in the

quantitative risk assessment rely on the multiplication of probabilities, assuming events are statistically independent.

As the issue of health risks arising from the use of virginiamycin in food animals is controversial, a quantitative risk assessment is vulnerable to biases introduced intentionally or unintentionally. Biases could include understating sources of uncertainty, excluding the unknown factors about antimicrobial resistance which could affect the predictions of risks, and failing to quantify the importance of critical assumptions such as the proportion of cases of VREf that are attributable to ingestion of food derived from animals.

The quantitative risk assessment does not seem to be consistent with the multi-causal nature of enterococcal disease in humans and does not account for the many ways by which humans could be exposed to enterococci and subsequently develop disease. A number of pathways and biological phenomena which impact on the emergence and spread of resistant bacteria have been excluded from consideration in the quantitative risk assessment. Pathways of exposure leading to disease that are not accounted for include:

- the effects of secondary amplification in humans following acquisition of VREf;
- interchange of genes between different bacterial species; and
- clonal spread.

Moreover, the quantitative risk assessment does not take into account secondary amplification or horizontal transfer of antimicrobial resistance through the use of antibiotics in the community or hospitals as is manifested by normally low isolation rates but occasional large outbreaks of VREf infection in Australia. These omissions have the effect of underestimating the risk.

Feedback loops that increase or decrease risk may become important with time; therefore, in the modelling assumption on the treatment of time, assumptions that are valid now may not be valid with the passage of time.

Amplifying resistance to human antibiotics in food animals is inherently problematic. Even if transmission to humans is infrequent, amplifying the resistance reservoir will make transmission via food more likely.

The quantitative risk analysis submitted to the review is a useful tool to help structured thinking, particularly the health consequences approach. However, its complex presentation detracts from its transparency and hence the capacity for findings to be incorporated in risk management.

VREf can co-select for resistance to other antimicrobials including QD and vancomycin. In overseas studies, acquired resistance to QD has been observed in isolates of VREf.

The major registrant of virginiamycin products gave a response to each of the above comments on the quantitative risk assessment. The responses seek to justify assumptions made; reinforce that the original conclusion is robust; and clarify that apparent omissions are accounted for in the total number of human cases per year. Nevertheless, low levels of recorded human morbidity and mortality cannot be used as an indicator that antibiotic resistance in the human population is not associated with the use of virginiamycin in food-producing animals.

## 5.6 Therapy, prophylaxis and growth promotion

It is a general principle of good veterinary practice that routine prophylactic use of antimicrobials should not be a substitute for good animal health management.

However, in veterinary practice there is sometimes not such a clear-cut distinction between therapy and prophylaxis as there is in human practice, particularly when the veterinarian is dealing with herds of animals. When a certain proportion of animals in a herd is clinically affected by a disease process, or when there is a high probability that the herd will become affected, it is common practice to medicate all the 'at risk' animals. This represents a blurring of the academic medical distinction between therapy and prophylaxis. This process of group-medication is sometimes referred to as metaphylaxis, in which sick animals are treated at one dose level, whilst others in the group are medicated at a different but lower dose level that is sufficient to prevent the development of clinical signs of the disease.

Growth promotants are usually administered in the feed for long periods at relatively low concentrations (<200mg/kg) which would normally be sub-therapeutic.

## 5.7 Animal welfare

Animal welfare is not within the formal scope of the review, but it was an issue raised by a number of submissions to the review.

Arguments centred on animal welfare suggest that if the use of virginiamycin is restricted there could be increased morbidity and mortality due to lactic acidosis in cattle and sheep and increased necrotic enteritis in poultry, which it is suggested would be an abuse of animal welfare.

The APVMA does not accept this argument, because antibiotics such as virginiamycin should not be used as a substitute for good feeding management. If animal health (and welfare) suffer because of recurrent lactic acidosis after an initial introductory period, the remedy should lie in husbandry and dietary management, rather than on reliance on prolonged administration of virginiamycin.

Based on the European Union experience subsequent to the withdrawal of authorisations for the use of antibiotics as growth promotants (bacitracin, spiramycin, tylosin and virginiamycin), there is some evidence that withdrawal was associated with an increased incidence of diarrhoea in early post-weaning pigs, and clostridial necrotic enteritis in broilers (Casewell *et al.*, 2003). While the article reports an increase in usage of therapeutic antibiotics in food animals, it also notes a reduction in acquired resistance in enterococci isolated from human faecal carriers. The article does not apportion any effects specifically to the ban on virginiamycin.



## **SECTION 6: REVIEW REGULATORY FINDINGS AND REASONS**

### **6.1 Background**

Section 34 of the Agvet Code provides that the basis for a reconsideration of the registration and approvals for a chemical product is whether the APVMA is satisfied that the requirements prescribed by the Agvet Code for continued registration and label approval are being met. These requirements are that the use of the product in accordance with the instructions for its use:

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

In reconsidering the registration of products containing virginiamycin and their labels, there are three possible outcomes. The APVMA may be:

- i) satisfied that the products and their labels continue to meet the prescribed requirements for registration and approval, and therefore confirm the registrations and approvals;
- ii) 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 vary the conditions of registration or approval; or
- iii) not satisfied that the requirements for continued registration and approval continue to be met and suspend or cancel the registration and/or approval.

## 6.2 Regulatory findings

The APVMA findings of its review of virginiamycin relate to human safety and efficacy of use in animals.

### 6.2.1 Would not be likely to have an effect that is harmful to human beings (Section 34 (1)(a)(ii) of the Agvet Code)

#### 6.2.1.1 Growth promotion and long-term prophylaxis

The APVMA finds that without limits to re-treatment and duration of use, it is not satisfied that the continued registration of virginiamycin as:

- i) a growth promotant in growing pigs, broilers and turkeys;
- ii) a prophylactic treatment for lactic acidosis in cattle and sheep and necrotic enteritis in chickens;  
- would not be likely to have an effect that is harmful to human beings.

#### **Reasons for the finding**

There is an unacceptable risk that the use of virginiamycin for undefined periods of time will induce antimicrobial resistance in *E. faecium* in animals and poultry. Such resistant bacteria may colonise humans directly, or transfer genetic determinants of resistance to human pathogens, which may cause subsequent disease in humans.

The 1999 JETACAR report concluded that:

- there is evidence for transfer of resistance determinants from animal to human bacteria;
- selective pressure provided by non-human use of antibiotics has been sufficient to cause the development of multiple resistance;
- there is qualitative evidence that feeding antibiotics to animals leads to resistant bacteria and these bacteria or their resistance genes are passed on to humans, principally via the food chain;
- there exists strong evidence (for salmonella, campylobacter and enterococci) that human disease is caused by resistant bacteria or bacteria containing resistance determinants that are present in animals.

A WHO report (November 2002) on the impacts in Denmark of termination of the use of antimicrobial growth promotants concluded that there is some indication that the ban on the use of antibiotics as growth promotants may be associated with a decline in the prevalence of streptogramin resistance among *E. faecium* from humans.

A joint Food and Agriculture Organization/World Organisation for Animal Health/World Health Organization expert workshop (December 2003) concluded that:

- there is clear evidence of adverse human health consequences due to resistant organisms resulting from non-human usage of antimicrobials;
- evidence shows that the amount and pattern of non-human usage of antimicrobials impact on the occurrence of resistant bacteria in animals and on food commodities, and thereby human exposure to these resistant bacteria.

The assessment of available literature on the public health significance of enterococci in cattle found that there are suggestions that meat and meat products from cattle, pigs, poultry and turkeys are possible sources of resistant enterococcal strains, although the literature was inconclusive in establishing whether the strains isolated from humans were of animal origin and whether transmission had occurred.

Evidence for transfer of resistance determinants from animal to human bacteria has been supported by instances where resistances have emerged in humans when the selective pressure is exerted only in animals. For example, when virginiamycin was used for growth promotion in the United States, QD resistant *E. faecium* were isolated from people prior to QD approval for human use. In support of this evidence is an observation that the reduction in use of antibiotics, including for growth promotion, has decreased the occurrence of resistant enterococci in livestock and humans.

In April 2004, an investigating arm of the United States Congress, the General Accounting Office, who investigated the link between antibiotic use in animals and human antibiotic resistance, reported that antibiotic-resistant bacteria have been transferred from animals to humans. Although researchers disagree about the extent of the human health risk caused by this transference, many of the studies that were reviewed found that the use of antibiotics in animals and the transference of antibiotic-resistant bacteria pose significant risks for human health.

### **6.2.1.2 Therapeutic use and limited-term prophylaxis**

The APVMA finds that with revised instructions for use which limit duration of use and re-treatment, it is satisfied that the continued registration of virginiamycin to prevent necrotic enteritis in chickens and to treat and prevent lactic acidosis in cattle and sheep would not pose an undue risk to human health.

#### **Reasons for the finding**

Scheduling of virginiamycin to Schedule 4 (Prescription Animal Remedy) has placed additional responsibility on veterinarians. However, the veterinary profession is not helped in this role by existing label instructions, as they provide no information on duration and frequency of treatments. The APVMA therefore welcomes the initiative by the veterinary profession in establishing a reference advisory group which field veterinarians may approach for information and guidance on preventing and managing lactic acidosis in cattle and sheep.

Notwithstanding the additional information that members of the veterinary profession provided to the APVMA, the APVMA is not satisfied that scheduling virginiamycin to Schedule 4 would by itself be sufficient to reduce the risk of adverse health consequences in susceptible humans exposed to streptogramin-resistant enterococci.

There are currently insufficient real-time data to support critical parameters that are necessary to develop a sound probabilistic assessment. Evidence exists that transfer of antibiotic resistance in animal strains to human strains could possibly occur in Australia, given the intensive animal production systems and use patterns of virginiamycin. Therefore, limiting virginiamycin use in animals to single, defined-period treatments would lower the risk of resistance transfer to humans. This measure will be supported by EAGAR's continuing cooperation with regulatory agencies in monitoring the usage of antibiotics and the development of resistance. EAGAR has also offered to assist veterinarians, human health specialists and their associations with education in the proper use of antibiotics.

The review recognises that label restraint statements will restrict a veterinarian’s ability to prescribe virginiamycin. However, the Department of Health and Ageing has confirmed to the APVMA its view that antimicrobial agents such as virginiamycin should be subjected to use restraints over and above those conferred by scheduling. Such controls could be expected to delay the emergence of antimicrobial resistance to important antibiotics.

**6.2.2 Would be effective for the purposes claimed (Section 34(1)(b) of the Agvet Code)**

The APVMA finds the following with respect to efficacy for the purposes claimed:

<b>Label claim</b>	<b>Finding</b>
<i>Prevention of necrotic enteritis in broilers</i>	Virginiamycin is likely to be effective.
<i>Growth promotion in growing pigs, broilers and turkeys</i>	Evidence of the efficacy of virginiamycin is equivocal, and virginiamycin may or may not be effective.
<i>For use in <u>cattle</u> and <u>sheep</u> rations to reduce the risk of acidosis (grain poisoning) when feeding grain</i>	Virginiamycin is likely to be effective.

**Reasons for the finding**

The efficacy assessment found that there is variation in the response to treatment with virginiamycin for improving growth rates and feed conversion efficiency in pigs and poultry, suggesting that users cannot be certain of a predictable growth promotion benefit when using virginiamycin for this purpose.

There is only equivocal evidence that virginiamycin is an effective growth promoting agent as claimed. In addition, the available evidence suggests that the growth promoting usage has an effect that will pose an undue risk to human health. Therefore, the APVMA does not support the continued registration and label approval of virginiamycin as a growth promotant in pigs, broilers and turkeys.

### 6.3 Regulatory actions

The Agvet Code requirements for **product labels** are that the labels 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.

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

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

However, the APVMA is 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 proposes to address the potential risks identified in this report in line with its legislative powers by using the following regulatory actions:

- 1 Affirm the active constituent approval for virginiamycin, and registration of products containing virginiamycin registered for the prevention of lactic acidosis in sheep, beef and dairy cattle, and the prevention of necrotic enteritis in chickens.
- 2 Vary conditions of label approval for products containing virginiamycin registered for the prevention of lactic acidosis in sheep, beef and dairy cattle, the prevention of necrotic enteritis in chickens, and growth promotion by:
  - the deletion of all label claims, indications and use patterns that pertain to growth promotion, and/or improved feed conversion 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 of product use;

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- the proposed label restraints would not allow veterinarians to prescribe the antibiotic for periods longer than specified on the label or to prescribe repeat treatments within a period of twelve months.
- 3 Cancel the registration and label approvals of those products that have label claims pertaining to growth promotion and/or improved feed conversion efficiency only.

Details of the proposed regulatory actions including proposed new recommendations for use and label instructions are provided in Table 2: Regulatory Action Table (see below).

This review will be concluded when the APVMA gazettes the final regulatory decisions.



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Product, active constituent and poison schedule on the approved label	Registrant	Claims on APVMA-approved label	Regulatory action	Proposed label amendments
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	Affirm registration  Vary conditions of label approval	Cattle and sheep: For reducing the risk of acidosis when used in stock feeds at times of increased risk of acidosis or during adaptation to high grain diets.  Addition of restraint statements: DO NOT USE this product to treat any animal species not indicated on the label.  Cattle and sheep: DO NOT administer medicated feed to a group of animals over a period of more than 28 days.  DO NOT repeat a course of treatment in a twelve-month period.
46049 Eskalin Feed Premix for Cattle– <i>virginiamycin</i> 20g/kg Schedule 4	Phibro Animal Health	For use in complete rations for <u>cattle</u> to reduce acidosis due to high grain diets	Affirm registration  Vary conditions of label approval	Cattle: For reducing the risk of acidosis when used in stock feeds at times of increased risk of acidosis or during adaptation to high grain diets.  Addition of restraint statements: DO NOT USE this product to treat any animal species not indicated on the label.  Cattle: DO NOT administer medicated feed to a group of animals over a period of more than 28 days.  DO NOT repeat a course of treatment in a twelve-month period.
38329 Stafac 20 Feed Premix– <i>virginiamycin</i> 20g/kg Schedule 5	Phibro Animal Health	For use in rations of <u>growing pigs</u> , <u>broilers</u> and <u>turkeys</u> to improve growth rate and feed conversion efficiency. Not to be fed to laying birds.	Cancel registration and label approvals	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

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Product, active constituent and poison schedule on the approved label	Registrant	Claims on APVMA-approved label	Regulatory action	Proposed label amendments
41286 Virginiamycin 20– <i>virginiamycin</i> 20g/kg Schedule 4	Agribusiness Products Pty Ltd	For use in rations of <u>growing pigs</u> , <u>broilers</u> and <u>turkeys</u> to improve growth rate and feed conversion efficiency	Cancel registration and label approvals	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
41476 Virginiamycin 20– <i>virginiamycin</i> 20g/kg Schedule 5	Lienert Australia Pty Ltd	Growth promotion and improved feed conversion efficiency in growing pigs	Cancel registration and label approvals	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



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## Appendix A: Streptogramin action and resistance

### Streptogramin types

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<sub>A</sub> and II<sub>B</sub>, and pristinamycins I<sub>A</sub>, I<sub>B</sub> and I<sub>C</sub>). Pristinamycin II is identical to virginiamycin M<sub>1</sub>. This drug has been available in France (only) for many years as an oral preparation for the treatment of gram-positive infections. Because gastro-intestinal 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 A1: Relative composition of streptogramin antibiotics**

Component	Pristinamycin	Virginiamycin	Quinupristin-dalfopristin
Streptogramin A	pristinamycins II <sub>A</sub> and II <sub>B</sub>	virginiamycin M <sub>1</sub>	dalfopristin
Streptogramin B	pristinamycins I <sub>A</sub> , I <sub>B</sub> and I <sub>C</sub>	virginiamycin S <sub>1</sub>	quinupristin

### Streptogramin mechanism of action

There are two structurally different groups of streptogramins (Group A and 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<sub>B</sub> 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 A2 and A3. 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. Sixteen-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 A2: Common resistances and cross-resistances to the MLS<sub>B</sub> antimicrobials in humans**

Mechanism of resistance	Main species	Macrolides		Ketolides	Lincosamides	Streptogramins		
		14 & 15	16			A	B	A+B
<i>Intrinsic</i>	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
<i>Ribosomal RNA site methylation</i>								
<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
<i>Efflux</i>								
<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)

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**Table A3: Rare resistances and cross-resistances to the MLS<sub>B</sub> antimicrobials in humans**

Mechanism of resistance	Main species	Macrolides		Ketolides	Lincosamides	Streptogramins		
		14 & 15	16			A	B	A+B
<i><b>Efflux</b></i>								
<i>msr</i>	Staphylococci, Enterococci	R	S	?	S	S	Si	S
<i>vga*</i>	Staphylococci	S	S	?	?	R	S	R
<i><b>Esterases</b></i>								
<i>ere</i>	Enterobacteria	high level R						
<i><b>Phosphorylases</b></i>								
<i>mph</i>	Enterobacteria	high level R						
<i><b>Hydrolases</b></i>								
<i>vgb</i>	Enterococci, Staphylococci						R	
<i><b>Transferases</b></i>								
<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)* confer 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 dalbapristin and quinupristin. By constructing recombinant plasmids the *erm* gene did not alter the MIC.

The *vgb* gene leads 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 on organisms with a lincosamide, streptogramin A resistance phenotype that conferred resistance in combination with any of the other resistance genes (Bozdogan and Leclercq, 1999).



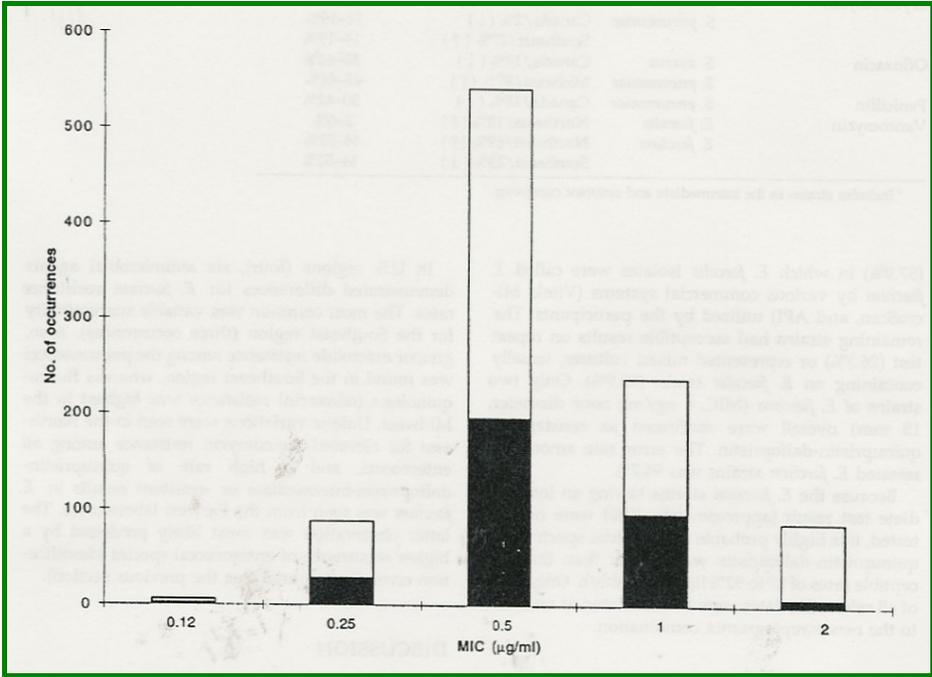
## Appendix B: *E. faecium* MIC distributions

Agent	Ref	Method	MIC (mg/L)										
			≤0.25	0.5	1	2	4	8	16	32	64	128	>128
<b>Virginiamycin</b>													
Total	Aarestrup <i>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							
<b>Virginiamycin M</b>													
Resistant strains	Werner <i>et al</i> ; 2000	BMD-ISO									2	45	65
<b>Quinupristin- dalfopristin</b>													
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	Hällgren <i>et al</i> ; 2001	Etest-PDM		36	22	15	1						

\* SENTRY data from Western Pacific, 1998–2000 (Jan Bell, pers. comm.)

**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.



## Appendix C: Streptogramin use in Australia

Streptogramin	Import amounts in kilograms														
	1992-93			1993-94			1994-95			1995-96			1996-97		
	Human	Stock	Total	Human	Stock	Total	Human	Stock	Total	Human	Stock	Total	Human	Stock	Total
Quinupristin-dalfopristin										5		5			
Pristinamycin							1		1	1		1	5		5
Virginiamycin		17 000	17 000		9500	9500		21 500	21 500		31 850	31 850		36 000	36 000

\*Data from JETACAR report (1999)



## Appendix D: Detailed efficacy data

### Efficacy in chickens

#### Growth promotion

A number of evaluations of the use of virginiamycin and other antibiotics as growth promotants were included in the submission (Groves, 2000; Hays Report, 2000; Zimmerman, 1986). However these evaluations did not contain data that could be assessed as evidence of efficacy.

In the Hays report and the Zimmerman evaluation, 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/tonne of food in terms of weight gain, and an improvement in feed conversion efficiency (FCE) which was best at 9 g/tonne. 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 to 10 g/tonne 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/tonne 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/tonne. In these trials FCE was significantly greater in chickens fed virginiamycin under some regimens, but the data do not indicate any differences between dosages at 5 g/tonne and 10 g/tonne 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/tonne and 10 g/tonne for the full nine-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).

## Virginiamycin review findings

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Local efficacy studies in chickens found no difference in efficacy between 5 g/tonne and 10 g/tonne 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 were 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/tonne 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).

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## 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 one to 13 days of age.

Challenge was by incorporation of *C. perfringens* type A cultures in feed from day 15 to 19. From days 20 to 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 four 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/tonne. 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/tonne 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/tonne 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/tonne on broilers placed on contaminated litter at one day old. Medication of feed commenced on the day of placement (one 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/tonne 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/tonne 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/tonne 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)**

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This study had insufficient detail to allow for a full evaluation. The statistical analysis presented suggests that virginiamycin at 20 g/tonne 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/tonne reduced mortalities. Trial 2 showed a significantly lower mortality rate in poults fed 30 g/tonne, but not in those fed 20 or 40 g/tonne. 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 evaluations of use of virginiamycin and other antibiotics as growth promotants were included in the submission (Hays Report, 2000; Zimmerman, 1986). However, these evaluations did not contain data that could be assessed as evidence of efficacy. As mentioned earlier, the Hays Report and the Zimmerman evaluation 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/tonne 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/tonne 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 to 110 lb with 40 g/tonne, but not 20 g/tonne, 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/tonne of virginiamycin improved average daily weight gain from weaning to 125 lb, although higher and lower levels (10 g/tonne and 40 g/tonne) 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/tonne in minimal disease pigs. In one trial pigs were fed virginiamycin from weaning at 32 days to 60 days of age. There was a significantly greater weight gain in pigs fed virginiamycin, but FCE could not be evaluated statistically. In a second trial, boars were fed virginiamycin at 20 g/tonne from 12 to 20 weeks of age. In this trial there was no demonstrable advantage to inclusion of virginiamycin in feed. A series of United States 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 FCE are inconsistent (Pfizer, 2000).

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 five weeks 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 two weeks of treatment. In a second study examining just the first two weeks of treatment the improvement in FCE was not seen (Pfizer, 2000).

A trial examining the effect of inclusion of 25 g/tonne from age 70 to 80 days to age 97 to 107 days, 10 g/tonne till age 154 to 164 days then 5 g/tonne until slaughter at 192 to 202 days found no overall improvement in weight gain, FCE, disease status, mortality 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 were included in the dossier to support claims of efficacy in sheep fed concentrate-rich rations under a range of different Australian conditions. The following assessment 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 80 mg (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 80 mg nor 160 mg 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 four daily drenches with 30 mg virginiamycin were 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 eight 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 for wool production to inclusion of virginiamycin, even at 10 ppm (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 eight 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 80 mg/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

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

### **Methane production by sheep**

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

### **Efficacy in cattle**

Efficacy data were 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).

### **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 one week then at 20 ppm continuously in cattle for eight weeks had little advantage over use for only four 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 four 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).

## Virginiamycin review findings

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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 FCE 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. While there was a significant effect on peak milk production there was no significant effect on total milk production or on milk composition (Pfizer, 2000).

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