National Registration Authority
For Agricultural & Veterinary Chemicals

THE SPECIAL REVIEW OF AVOPARCIN

STATUS DOCUMENT

January 2001

NRA Review Series 01.1

National Registration Authority
for Agricultural and Veterinary Chemicals

Canberra
Australia

ISSN No. 1443 2528

This review is published by the National Registration Authority for Agricultural and Veterinary Chemicals (NRA). For further information about the review or the Chemical Review Program, contact:

Manager, Chemical Review
National Registration Authority for Agricultural and Veterinary Chemicals
PO Box E240
Kingston ACT 2604
Australia

Telephone: 61 2 6272 3213
Facsimile 61 2 6272 3551
Email: chemrev@nra.gov.au
NRA web site: http://www.nra.gov

TABLE OF CONTENTS

1. INTRODUCTION

The purpose of this document is to provide information regarding the status of the Australian review of the active ingredient avoparcin.

In 1998, the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) initiated a review of avoparcin, all products containing avoparcin and associated labels as part of its Special Review Program. In September 1999, the primary registrant informed the NRA that it was withdrawing avoparcin from the market place for commercial reasons. The NRA did not proceed with the review, as it was unlikely to have been completed before the anticipated withdrawal. At this time, the residue assessment was the only completed component of the review.

This document summarises what happened during this review, the residue assessment and outlines what data would be required for the future registration in Australia of products containing avoparcin. There are currently no registered products containing avoparcin in Australia.

2. SPECIAL REVIEW OF AVOPARCIN

2.1 Reasons for review

Avoparcin is a glycopeptide antibiotic with a gram positive spectrum of activity produced by fermentation of a strain of *Streptomyces candidus*. It has been in continual use in Australian livestock feeds since 1978 for growth promotion and improved animal feed conversion efficiency in broiler chickens, growing pigs, calves and beef cattle. Avoparcin is also used as an aid in the prevention of necrotic enteritits (*Clostridium perfringens*) in broiler chickens.

Concerns were raised regarding the continual usage of in-feed antibacterials in animals leading to the likelihood of acquired bacterial resistance development in the gut of the animals. More specifically, there had been concerns regarding possible links between the emergence in Australia of Vancomycin Resistant Enterococci (VRE) in humans and the use of avoparcin (a related antibiotic) as a growth promotant in animal feeds.

In 1998, the Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR) was established by the then Minister for Health and Family Services and the Minister for Primary Industries and Energy. JETACAR reviewed broadly the use of antibiotics in food-producing animals, and the occurrence of antibiotic resistance and its importance in human and veterinary medicine.

Also in 1998, the NRA placed avoparcin under reconsideration as part of its Special Review Program in accordance with statutory powers under Part 2, Division 4 of the *Agricultural and Veterinary Chemicals Code Act 1994* (the Agvet Codes). The reconsideration included a review of all aspects of the approval of the active constituent avoparcin, registrations of all products containing avoparcin and the labels of all such products. This action was taken because of concerns that the use of

avoparcin in animals may pose a threat to human health through being a contributing factor in the emergence of VRE.

The review focussed on assessing both published and unpublished data relating to the potential for the development of VRE in animals and humans as a result of the use of avoparcin in animals. In particular, data was requested in the following areas:

- resistance development in animals;
- mechanism and potential for transfer of resistance across species;
- residues - to the extent that residues of avoparcin in meat might contribute to resistance development; and
- efficacy - as a possible guide to resistance development in animals.

The review was to also incorporate JETACAR findings relating to avoparcin. Their report was published September 1999.

2.2 Consultation

Companies with registered products were informed about the Special Review of avoparcin in accordance with section 32(2) of the Agvet Codes. The review was advertised in the June 1998 edition of the NRA's *Agricultural and Veterinary Gazette* in accordance with section 32(1) of the Agvet Codes. Various stakeholders such as State and Territory regulators, government agencies, user groups and medical associations were also invited to make submissions to the review. Six submissions were received from medical organisations and five from State and Territory government agencies.

2.3 Registration status in Australia

Products containing avoparcin have been registered nationally in Australia since 1978. The products registered at the time of commencement of the Special Review are listed in the table below.

Product(s) and Company	Host	Use	Dose rate of avoparcin
Avotan 100 Feed Supplement *Avotan 100 Granular Feed Supplement* *(100 g avoparcin/kg)* Roche Vitamins Australia Pty Ltd	Unweaned calves	Growth promotant	40 mg/kg milk replacer
	Early weaned calves	Growth promotant	40 mg/kg feed
	Dairy cattle	Milk production	100-150 mg/head
	Beef cattle	Growth promotion	150-250 mg/head
	Broiler chickens	Growth promotion Necrotic enteritis prevention	10 mg/kg feed 20 mg/kg feed
	Growing pigs	Growth promotion	20 mg/kg feed
Premax Calf Booster *(4 g avoparcin/kg)* Ridley Agriproducts Pty Ltd	Calves	Growth promotant	40 mg/kg feed or 80 mg/head/day in liquid

2.4 Residues Assessment

2.4.1 Background

Avoparcin is a glycopeptide antibiotic with a molecular weight greater than 1900. Its chemical structure is as follows:

The current Australian Maximum Residue Limits (MRLs) for avoparcin in food commodities are presented below (Table 1, *MRL Standard*, January 2001). The residue definition is "avoparcin" (Table 3, *MRL Standard*, January 2001). There are no withholding periods associated with the registered products and an Australian Acceptable Daily Intake for avoparcin has not been established.

Commodity	***MRL (mg/kg)***
MO 0105 Edible offal (mammalian)	*0.1
MM 0095 Meat [mammalian]	*0.1
ML 0106 Milks	*0.01
PO 0111 Poultry, Edible offal of	*0.1
PM 0110 Poultry meat	*0.1

*Denotes that the MRL has been set at or about the limit of determination.

2.4.2 Metabolism

There were three studies submitted for consideration which were conducted in rats, pigs and chickens, respectively. All these studies had been submitted for consideration to the Pesticide and Agricultural Chemical Committee, which provided technical assessments prior to the establishment of the National Registration Scheme in 1993. A search of the archival records revealed a further three studies in cattle and

dairy cows, which had previously been submitted or considered during the registration process. The table below summarises the assessment of these studies. In all cases the route of administration was oral.

Animal Species	Analytical Method	Sensitivity	Specificity for Avoparcin	Comments
Rat	Radiometric	Tissues (0.05 mg/kg)	No	< 0.2 % of dose in urine; Blood < 0.05 mg/kg
Pig	Microbiological	Urine (0.4 mg/L)	No	< 1% of dose in urine; Blood < 0.7 mg/kg
Chicken	Microbiological	Blood (0.6 mg/L)	No	Blood < 0.6 mg/L
Cattle	Radiometric	Tissues (0.05 mg/kg)	No	Muscle, liver and fat < 0.05 mg/kg; Blood < 0.05 mg/kg Kidney approximately at LOD* (2 x rate)
Cattle	Radiometric	Tissues (0.05 mg/kg)	No	0.2% of dose in urine Tissues < 0.05 mg/kg
Dairy cow	Radio Immuno Assay	Milk (0.01 mg/kg)	OK	Milk < 0.01 mg/kg

*LOD = Limit of Determination

The rat and cattle studies indicated that no significant radioactivity occurred in either blood or urine, and no residues occurred in tissues. This demonstrates that little avoparcin or its labelled metabolites were absorbed from the gut. The pig and chicken studies indicated that the recovery of avoparcin was incomplete when measured by microbiological assay. It is not possible to determine the fate of the unaccounted for avoparcin using this assay; it could be that avoparcin was metabolised and absorbed from the gut of pigs and chickens.

One of the cattle metabolism studies showed that no detectable residues occurred in muscle, liver, fat or blood but residues slightly above the Limit of Determination (LOD) occurred in kidney (average of 0.06 mg/kg). In both radiolabelled studies with rats and cattle about 0.2% of the total radioactivity occurred in urine. The separate study in dairy cows indicated that no detectable residues of radioactive avoparcin or its metabolites occurred in cattle milk.

The radioactive studies demonstrated that there is very little absorption of avoparcin (parent or metabolites) from the gut of rats, chickens, pigs and cattle, and that tissue residues are negligible in all of these species.

2.4.3 Analytical methods

Four analytical methods were submitted, either as part of a trial report or as a separate report, with one method being Gas Liquid Chromatography (GLC) and the other three being microbiological assays. A further five methods involving microbiological assays and Radio Immuno Assays (RIA) were obtained from the archives of the NRA

and Therapeutic Goods Administration. The assessment of these studies is summarised in the table below.

Analytical Method	Limit Of Determinations (LOD)s	Specificity for Avoparcin	Suitability
GLC (old technology)	Tissue (0.05 mg/kg)	No	No
Microbiological (broilers)	Liver and kidney (< 0.5 mg/kg) Muscle (< 0.2 mg/kg) Fat (< 0.4 mg/kg)	No	No
RIA (cattle)	Liver, kidney, muscle, fat, milk (0.05 mg/kg)	Probably	Maybe
Microbiological (vealers)	Tissue (0.2 mg/kg)	No	No
RIA (milk)	0.01 mg/kg	Probably	Maybe
Microbiological (cattle)	Liver, kidney, muscle, fat (0.25 mg/kg)	No	No

The analytical method of GLC is considered old-fashioned and was not specific for avoparcin although the sensitivity was good. Microbiological assays submitted were less sensitive than GLC with the LODs ranging from 0.2 mg/kg to 0.5 mg/kg. This could mean that the results obtained using these methods are not meaningful. The specificity of these methods is also not known and it is suspected that they would also respond to other antibiotics. The response of any of the methods to possible metabolites is unknown and therefore indicates that these methods might not be adequate for quantitative analyses. RIA is the method usually used for milk and is far more sensitive and specific. Hence this method could be considered for general use to determine residues of avoparcin. The greatest deficit in analytical methods was the lack of a current instrumental method to determine avoparcin residues in tissues, and the lack of a properly validated and documented method to screen samples for residues.

2.4.4 Residue definition

The current residue definition in Table 3 of the *MRL Standard* (January 2001) is "avoparcin". Following a detailed examination of the metabolism data assessed in Section 2.4.2 above, it was revealed that metabolism could have possibly occurred in the gut of pigs and chickens. This was considered to be important because of the possibility of absorption of any metabolites formed in the gut. The lack of definitive metabolism data, especially as finite residues occurred in the cattle kidney, does not allow the appropriateness of the residue definition to be assessed.

2.4.5 Residue trials

Five reports of residue trials were submitted for consideration. A registrant submitted some data, while past registration data were obtained from archives. Their assessment is summarised in the table below.

Animal Species	Analytical Method	Reported Residues (mg/kg)
Poultry	GLC	Liver and muscle (< 0.05)
Broilers	Microbiological	All < LOD
Broilers	Microbiological	All < LOD
Pigs	Microbiological	All < LOD
Pigs	Microbiological	All < LOD
Pigs	Microbiological	All < LOQ* (rates = 2 or 20 x)
Calves	Microbiological	All < LOD
Cattle	Radiometric	Kidney (0.06 at rate = 2 x) Liver, muscle, fat (< LOD)
Steers	RIA	All < LOD (rate = 4 x)
Dairy cows	RIA	Milk < LOD (rate = 4 x)
Dairy cows	RIA	Milk < LOD

*LOQ = Limit of Quantitation

A variety of methods were used for the analysis of the tissue and blood samples, including GLC for one trial, RIA and microbiological assays, and a ^{14}C study. In most trials, dosage rates were well above label recommendations.
No residues were detected in cattle milk or in the tissues of the species studied except bovine kidney. In the cattle study the LOD was only 0.05 mg/kg and the mean residues in kidney were 0.06 mg/kg. The issue with this finite "residue" is that it was measured as radioactivity. Accordingly, it could not be determined whether the residue was avoparcin and/or metabolite(s). Pig tissues were analysed by microbiological assay and showed no residues with antimicrobial activity above 0.05 mg/kg, even following 20-times the recommended dose. The metabolism studies discussed in section 2.4.2 indicate that the residue was most likely comprised of a metabolite, without any microbiological activity. It is therefore most likely that no residues of the parent compound are present at levels greater than 0.05 mg/kg. The potential for human dietary exposure should therefore be negligible.

No residues were detected in blood samples in any trial. The LODs in blood are not considered low enough to determine whether there were any significant residues.

2.4.6 Fate of residues in processing and storage

No processing data were available for evaluation. One small storage trial was retrieved from the archives that reported the stability of avoparcin in tissues over 42 days at -20^{o}C.

Tissues were fortified with avoparcin at 0.5 to 4.0 μg/g and were then stored at –20^{o}C for up to 42 days until analysis. The results are shown in the table below.

Tissue	Fortification level (μg/g)	Percent recovery at number of days at -20^{o}C		
		0	14	42
Muscle	4.0	102.5	101.3	101.4
	2.0	105	100	92.5

	1.0	102	99	88
	0.5	102	93	85
	Average	102.9	98.3	91.7
Liver	4.0	101.3	106.3	100
	2.0	100	99	97.5
	1.0	100	97	97
	0.5	96	99	80
	Average	99.3	100.3	93.6
Kidney	4.0	98.8	103.8	102.5
	2.0	104	105	92.5
	1.0	105	110	96
	0.5	100	99	90
	Average	102.0	104.5	95.3
Fat	4.0	100	96.3	106
	2.0	107.5	102.5	97
	1.0	102	110	90
	0.5	100	100	92
	Average	102.4	102.2	96.3

These results show that avoparcin is stable in all tissues for two weeks when stored at -20^{o}C with little change over six weeks. No data were available for storage stability of avoparcin in milk.

2.4.7 Other aspects considered

There was no information submitted during this review concerning the fat solubility of avoparcin.

There are no current registrations of this veterinary drug in the USA or in the EU, and hence there are no MRLs existing in those countries. The absence of detectable residues in meat should mean that there are no risks to trade with the use of avoparcin in chickens, cattle and pigs.

2.4.8 Conclusions from residue data assessment

- Useful metabolism data were not available for evaluation.

- Radiometric studies demonstrated that less than 0.2% of the administered dose was absorbed systemically.

- Radiometric studies, when conducted at twice the label rate, reported bovine kidney residues of 0.06 mg/kg. No other tissue residues were reported even when up to 20-fold the label rate was administered (in this case, tissues were analysed by microbiological assay).

- The analytical methods (with the exception of the RIA method used only for milk) did not demonstrate specificity for avoparcin. Hence the current residue definition cannot be quantitated in tissues.

- The microbiological methods have neither the specificity nor the sensitivity to address the residue definition or the MRLs.

- The available metabolism, analytical and residue data do not satisfy contemporary requirements. Despite this, the radiometric studies provide compelling evidence that avoparcin residues are non-detectable in tissues (LOD = 0.05 mg/kg) and milk (LOD = 0.01 mg/kg). As avoparcin residues are highly unlikely to enter the human food chain, it is concluded that such residues are unlikely to play a role in the emergence of VRE in humans.

2.5 Suspension of review

During September 1999, Roche Vitamins Australia (Roche) informed the NRA that it was withdrawing avoparcin from the market for commercial reasons. Roche indicated that sales would cease on 31 December 1999 and that the bulk of the remaining stock (about 11 tonnes) will be consumed by end users before 1 July 2000. The company decided not to renew the registration of its products after 30 June 2000 and the approval of labels for the containers also ended at this time. The NRA determined that the existing stock of products could continue to be supplied at retail until 31 December 2000. However, it was illegal to import new stock into Australia after registration of the products ended on 30 June 2000.

When informed of the withdrawal, the other registrant, Ridley Agriproducts, also allowed the registration of its product to lapse after 30 June 2000 with the above conditions.

The NRA did not proceed with the Special Review of avoparcin as it was unlikely to have been completed before the anticipated withdrawal of avoparcin from the market place. However, the NRA indicated to the registrants that the review would be reactivated if the registration of products containing avoparcin were renewed after 30 June 2000.

There are currently no registered products containing avoparcin on the market in Australia.

3 REQUIREMENTS FOR THE FUTURE REGISTRATION OF PRODUCTS CONTAINING AVOPARCIN

Any company interested in the registration of products containing avoparcin must provide data in accordance with the NRA's *Guidelines for Registering Ag and Vet Chemicals*. In addition to this, they must also provide a risk analysis of microbial resistance safety as outlined in the *Guidelines for Registering Vet Chemicals, Part 10, Special Data Requirements*. These guidelines are available on the NRA web site at http://www.nra.gov.

As the Special Review of avoparcin was not completed there have been no regulatory outcomes. The conclusions resulting from the residues assessment (section 2.4.8) should be considered as a guide for possible additional data requirements.