Report of the Scientific Committee for Animal Nutrition
on the use of Avoparcin in feedingstuffs for dairy cattle
(Opinion expressed on 29 September 1994)
(Opinion reconfirmed on 24 March 1995)

TERMS OF REFERENCE (January 1993)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions:

1. Has the use of avoparcin ($C_{55}H_6O_{30}N_6C1_3$-glycopeptide produced by *Streptomyces candidus*, the active substance being the mycelium product under the form of sulphate), under the conditions proposed for its use as an additive for the feedingstuffs for dairy cattle significant effects on milk production by dairy cattle?

2. Is this use safe to dairy cows?

3. Can it be monitored in animal feedingstuffs?

4. Can it result in development of resistance in bacteria to prophylactic or therapeutic preparations?

5. What is the metabolic fate of avoparcin in dairy cattle? Does this use result in the presence of residues in milk and milk products? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

6. Do the toxicology studies allow to conclude that the proposed use does not present risks
   - for the consumer?
   - for the user?

7. What are the nature and the persistence of the excreted products derived from avoparcin? Can these products be prejudicial to the environment?

8. In the light of the answers to the above questions, are the proposed conditions of use acceptable?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs[1] the use of avoparcin (E 715, a mycelium product produced by *Streptomyces candidus,* consisting of a $C_{55}H_6O_{30}N_6C1_3$-glycopeptide in the form of sulphate) is authorized at community level under the conditions set out in Annex I,

1 O.J. No. L270 (14.12.70) p. 1

Section A (Antibiotics) of Commission Directive 91/248/EEC[2] as last amended by Commission Directive 92/99/EEC[3].

An entry to Annex II of Directive 70/524/EEC (national authorisation) for a new use in fattening lambs from the beginning of rumination with the exception of pasture-grazed lambs and for dairy cattle was approved by Directive 90/206/EEC[4]. Avoparcin is also authorised at national level under the conditions set out in Annex II, Section A of Commission Directive 91/248/EEC[2] as last amended by Commission Directive 92/99/EEC[3] (see authorisations in Tables 1 & 2).

The Scientific Committee for Animal Nutrition (SCAN) expressed its favourable opinion regarding chickens for fattening, pigs and piglets, milk replacer feeds for calves, and complementary feedingstuffs for fattening cattle in its reports of 11th July 1979 [5] and 1st June 1983[6].

The official method of analysis, by agar diffusion, is published in the Ninth Commission Directive 81/715/EEC of 31 July 1981[7] establishing Community methods of analysis for the official control of feedingstuffs.

In September 1991 a request has been made to change the condition under «other provisions» to fix the minimum and maximum content in the daily ration at 50 and 150 mg. In October 1992 a request has been made to include the authorisation for lambs in Annex I, and in December 1992 a request has been made to include the authorisation for dairy cattle in Annex I , part A (Antibiotics) as follows:

Species or category of animal	Maximum age	Minimum content mg/kg complete feedingstuff	Maximum content	Other Provisions
Dairy cattle	-	4	10	Indicate in the instructions for use: "The dose of avoparcin in the daily ration: -must not exceed 150 mg -must not be less than 50 mg"

New dossiers supporting these requests have been provided by the company

2 O.J. No. L124 (18.05.91) p. 1
3 O.J. No. L350 (01.10.92) p. 83
4 O.J. No. L106 (26.04.90) p. 30
5 Report of the Scientific Committee for Animal Nutrition on the use of Avoparcin in feedingstuffs for Chickens and Pigs (Second Series) 1980 Report 6918 EN Catalogue Nº CDE-NK-80-002-EN-C, p. 22
6 Report of the Scientific Committee for Animal Nutrition on the use of Avoparcin in feedingstuffs for Calves and Fattening Cattle. (Fourth Series, 1984. Report EUR 8769. Catalogue Nº CD-NK-83-010-EN-C, p. 120
7 O.J. No. L257 (10.09.81) p. 38

OPINION OF THE COMMITTEE

1. Avoparcin is already approved for use in feeds for a variety of farm livestock to improve the rate of liveweight gain and the efficiency of feed conversion. The new use of avoparcin in dairy cattle to improve milk yield was the subject of numerous field trials. These consisted of 17 studies involving a total of 433 dairy cows, performed prior to 1987, and 23 trials involving about 1200 animals of numerous breeds, conducted between 1987 and 1992. The trials covered a wide range of production and management systems, feedingstuffs, and stage of lactation. They were undertaken in 9 EEC countries and in Hungary.

   The studies were well designed and conducted. The results were analysed using appropriate statistical techniques. Most of the studies have used dose rates of avoparcin of 100 mg/cow/day. In 4 studies a dose-response was demonstrated but the observed differences in milk production did not reach statistical significance.

   Food intake was measured as total dry matter intake in 9 studies and as concentrate intake in 22 studies. In neither case was a significant difference noted between the controls and the avoparcin-treated groups.

   Milk yield was improved significantly by an average of 4%, if all 40 studies are considered, showing a benefit in 90% of the trials. Field scale studies on commercial farms also indicated a milk yield increase of approximately 4.6%.

   Avoparcin had no effect on milk composition, as reflected in the levels of milk fat, protein and lactose content. Other quality parameters such as pH, non-casein nitrogen, non-protein nitrogen, fatty acid composition, refraction value, bacterial count, somatic cell count, alcohol stability, freezing point, souring activity, heat stability or renneting behaviour and milk processing characteristics for the production of cheese or other dairy products were similarly unaffected.

   In the opinion of the Committee the proposed use of avoparcin in dairy cattle produces a significant increase in milk production and has no effect on milk composition, quality and processing.

   Because of the range of intakes or concentrate allocations between cows in a herd there is a need to set a minimum and a maximum inclusion level in feedingstuffs to ensure, that both high and low feed intake animals receive effective doses of avoparcin. Upper limits to the inclusion levels should be set so that no animal receives more that 150 mg/day.

2. The safety of avoparcin for cattle, if used under the conditions laid down in Annex I for milk replacer feeds for calves and complementary feedingstuffs for fattening cattle, has already been evaluated and established by the Committee in its opinion of 1st June 1983.

   The data included the results of 7 studies in lactating dairy cows that showed no adverse effects on breeding and calving performances following intakes of avoparcin between 50 and 150 mg/cow/day over periods of 70-305 days.

The use of avoparcin was therefore considered by the Committee to be safe also for dairy cows.

3. An official method of analysis, by agar diffusion, has been published in the Ninth Commission Directive 81/715/EEC[8] for monitoring the concentration of avoparcin in feedingstuffs and complementary feedingstuffs (limit of detection 2 mg/kg).

4. The development of resistance in bacteria to prophylactic or therapeutic antibiotic preparations following the use of avoparcin in animal feedingstuffs has already been considered by the Committee in the case of chickens, turkeys and pigs. The microbiological effects of the addition of avoparcin to feed for calves and cattle were also already examined by the Committee, which came to the opinion that this additive does not lead to the development of resistance in intestinal bacteria.

   Although no specific microbiological information on dairy cattle has been submitted, the extension of use to dairy cattle poses no additional microbiological problems. In parallel to the findings, that avoparcin did not affect the balance between Gram positive and Gram negative bacteria in the intestinal tract of fattening cattle nor caused a prolongation of Salmonella shedding in veal calves, no different microbiological effects would be expected in dairy cattle.

   There is no recent evidence for any capability of avoparcin to select resistant strains under field conditions.

   The Committee is therefore of the opinion that the use of avoparcin in the feeds of dairy cattle is not likely to result in the development of resistance in bacteria to prophylactic or therapeutic preparations.

5. In its opinion expressed on 11th July 1979 the Committee stated that avoparcin added to feed was virtually unabsorbed by the digestive system in rats, dogs, chickens and pigs. In its present evaluation it noted however the absence of information on the exact nature of the radio labelled material in the gut. Similarly, its use in feed for chickens and pigs did not produce any detectable residues in the tissues. Studies performed on calves, steers and bulls under the proposed conditions as well as at higher doses and with the use of molecules, labelled with ^{14}C in the tyrosine residues, led the Committee to the same conclusions for these animal species in 1983.

   Although direct experimental data on a possible biliary route of excretion are lacking, the absence of measurable blood and tissue residues at any dosage, seen in earlier studies on rats, chickens, pigs and cattle, using radioactivity and microbiological assays, makes it very unlikely that gastrointestinal uptake and extremely rapid biliary excretion could occur.

   The new date from lactating cows, given orally 150 mg/head labelled avoparcin sulphate for 5 consecutive days, showed no transfer of any radioactivity into the milk up to 48 hours after withdrawal of treatment (limit of detection of radioactivity

8 Ninth Commission Directive 81/715/EEC of 31 July 1981 establishing Community methods of analysis for the official control of feedingstuffs (O.J. No. L257 (10.09.81) p. 38)

assay 0.01 mg/kg). It has been questioned, whether 5 days of treatment were sufficient to obtain a steady state situation in this experiment.

However, in other experiments, in which cows were given either 600 mg unlabelled avoparcin/head/day for 3 days, or 50 and 100 mg/head/day for 60 days, or 100 mg/head/day for 9 months, and in which steady state conditions must have been reached, radioimmunoassays (limit of detection 0.01 mg/kg) showed no measurable avoparcin in the milk.

Tissue analyses showed in all oral studies with different species that blood contained no measurable amounts of avoparcin (limit of detection 0.01 mg/kg) nor any residues in carcass and almost complete excretion in the faeces within 24 hours.

A study in man with 200, 400 and 600 mg/kg diet showed traces of avoparcin activity in the blood only at the highest dose level with less than 0.2% of the dose appearing in the urine. Taking these data into account the Committee is of the opinion, that no residues harmful to the consumer are likely to occur either in the tissues or in the milk of dairy cows following oral administration of avoparcin in the feed.

6. The toxicological studies, previously submitted for establishing the safety of avoparcin for calves and fattening cattle, have been evaluated already and considered satisfactory evidence for the absence of any risks for consumer and user by the Committee in 1979 and 1983.

The pharmacological profile showed avoparcin to be essentially devoid of any significant activity when tested in standard pharmacological screens, applied to antibiotics, for cardiovascular, neurological, renal, endocrine, analgesic, antidepressant, immunosuppressant, antineoplasic and general metabolic effects.

The earlier (1977 & 1979 see references) subchronic studies in rats and dogs, though not quite following GLP protocols showed no adverse effects (NEL) at all doses tested. The NEL lay between 70 mg/kg b.w. for dogs and 85 mg/kg b.w. for rats.

A recent 91-days oral subchronic study in cattle showed no adverse effects at all dose levels tested. The NEL was 180 g/ton feed equivalent to 5.14 mg/kg b.w./day.

Long-term oral feeding studies are available in rats and mice.

The rat study was carried out on the F_{1a} generation of a multi-generation reproduction study using dose levels of 0, 5, 20 and 80 mg/kg b.w. avoparcin. It showed no significant dose-related differences nor any indication for the induction of specific tumours. Because of the observed significant reduction in body weight at the top dose the NEL was considered to be 20 mg/kg b.w. and on this basis an ADI of 0.2 mg/kg b.w. could be established.

Two oral feeding studies in mice, both extending over 2 years, were also reported. The apparent excess of liver tumours and associated hepatic lesions in the high-dose males of the earlier study was not confirmed in the second later study. Because of

the observed reduction in weight gain in females of the top dose group the NEL was considered to be 28 mg/kg b.w.

A three-generation reproduction study in rats, using dose levels of 0, 5, 20 and 80 mg/kg b.w. in the feed, showed some reduction in pup viability and, consequently, in the lactation index at different dose levels in the F_1 and F_2 generations. Weaning weights of the F_3 pups were significantly lower at the top dose than in the controls. Because of the observed reduced weight of F_3 pups in the highest dose group the NEL was considered to be 20 mg/kg b.w.

Avoparcin was shown not to be teratogenic in two rat studies.

Several *in-vitro* mutagenicity studies, comprising *Salmonella* reverse mutation assays, a point mutation assay in CHO cells, and assay for UDS in primary rat hepatocytes, and Rec assays in *B. subtilis*, as well as an *in-vivo* cytogenetic assay in rat bone marrow confirmed the absence of any genotoxic potential.

The spray-dried and fermentation intermediate products were mildly irritant. No allergenicity was reported from tests on human volunteers.

The results of the toxicological, pharmacological and residues studies allow the Committee to conclude that the proposed use of avoparcin in dairy cattle does not present any risk for the consumer and user.

7. A recent study demonstrated that faeces from dairy cows fed radio labelled avoparcin contain 45-70 mg/kg radio labelled material of which 89% is unchanged avoparcin. Information on the identity of the breakdown products derived from avoparcin in the soil or on the half-live of avoparcin in slurry is not available. Earlier studies on the degradation of avoparcin in beef-cattle manure as distinct from dairy cow manure indicated a half-life for avoparcin of approximately 15 days and residues in manure after ageing of less than 5 ppm (the limit of detection).

   Other earlier studies in a model ecosystem showed that after 37 days about 92% total radioactivity remain after the application of soil mixed with radio labelled avoparcin. Some 57% of this total radioactivity remained unextracted in the soil and only 11% of undegraded avoparcin can be extracted from it, suggesting a possible half-life of 12 days. These studies also showed that avoparcin was not a potential bioaccumulator.

   The newer degradation studies in manure confirm the previously observed absence of phytotoxicity for 6 different vegetables, the absence of any herbicidal effects and of bioaccumulation in the plants.

   The assessment of data on the environmental effects of avoparcin excreted in the faeces of cattle and pigs led the Committee to conclude, that under the proposed conditions of use avoparcin could not have harmful effects on the environment.

8. In the light of the answers to the questions by the Commission and provided, that the dosing regime for avoparcin is designed so as to provide an average of up to 100

mg/head/day in a herd by adjusting the dose range in the daily ration the use of avoparcin in the feedingstuffs for dairy cattle is acceptable.

REFERENCES

Dossiers supplied by Cyanamid:

Report of the Scientific Committee for Animal Nutrition on the use of avoparcin in chickens and pigs. In: Report of the Scientific Committee for Animal Nutrition, 2nd Series. Luxembourg: Office for EC Publications. Report EUR 6918, Catalogue N° CD-KN-80-002-EN-C. pp: 22-24.

Report of the Scientific Committee for Animal Nutrition on the use of avoparcin in the feedingstuff for calves and fattening cattle. In: Report of the Scientific Committee for Animal Nutrition, 4th Series. N° CD-NK-83-010-EN-C. pp: 120-129.

TABLE 1:
Present Authorisations of Avoparcin in Annex I. Part A "Antibiotics"

Species or category of animal	Maximum age	Minimum content	Maximum content	Other Provisions
		Mg/kg of complete Feedingstuff		
Chickens for	-	7.5	15	-
fattening	16 weeks	10	20	-
Turkeys for fattening	4 months	10	40	-
Piglets	6 months	5	20	-
Pigs	6 months	15	40	-
Calves Cattle for fattening	-	15	30	Indicate in the instructions for use: "The quantity of avoparcin in the daily ration must not exceed 103 mg for 100 kg of body weight and 4.3 mg for each additional 10 kg of body weight"

TABLE 2
Present Authorisations of Avoparcin in Annex I. Part A "Antibiotics"

Species or category of animal	Maximum age	Minimum Content Mg/kg complete feedingstuff	Maximum content	Other Provisions
Lambs from the beginning of rumination with the exception of pasture-grazed lambs	16 weeks	10	20	-
Dairy cattle	-	4	10	Indicate in the instructions for use: "The quantity of avoparcin in the daily ration: -must not exceed 100 mg -must not be less than 50 mg"

TABLE 3.
Request (December 1992) for the authorisation of avoparcin for dairy cattle in Annex I, part A (Antibiotics)

Species or category of animal	Maximum age	Minimum Maximum content content Mg/kg complete feedingstuff		Other Provisions
Dairy cattle	-	4	10	Indicate in the instructions for use: "The quantity of avoparcin in the daily ration: - must not exceed 150 mg -must not be less than 50 mg"