Report of the Scientific Committee for Animal Nutrition on the use of Efrotomycin in feedingstuffs for pigs. (Opinion expressed: 27 July 1990).

Terms of reference (April 1986)

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

1. Has the use of the fermentation product efrotomycin at the dosages proposed for feedingstuffs for pigs under the different climatic conditions existing in the European Community significant effects on growth? Which component of this mixture is the active ingredient? Are pigs of both sexes and all age groups equally affected positively?

2. Is this safe for the pig?

3. Can it result in the development of resistance in bacteria to prophylactic or therapeutic preparations, or exert an effect on the persistence of Gram-negative bacteria in the digestive tract of the pig?

4. What is the metabolic fate of efrotomycin in the pig? Does the proposed use result in residues in animal tissues? If so, what is the qualitative and quantitative composition of these residues?

5. Do the toxicological studies allow the conclusion that the proposed use does not present risks
   - for the consumer?
   - for the user?

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

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

Background

Efrotomycin was the subject of an application for admission in Annex II, Section A (Antibiotics), of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs [1] under the following conditions of use:

Species of animal: pig.
Minimum and maximum doses: 2-16 mg/kg of complete feedingstuff.

Opinion of the committee

Efrotomycin is an antibiotic belonging to the elfamycin family. It is structurally a N-methylhydroxypyridone glucoside produced by fermentation with *Nocardia lactamdurans*. The granular premix formulation [2], containing 32 g efrotomycin activity/kg, has been

[1] O.J. N° L 270 of 14.12.1970, p. 1.

[2] Registered trade name of Merck, Sharp and Dohme.

proposed for use as a growth promoter in swine rations at a final concentration of 2-16 mg active ingredient/kg. Efrotomycin consists of 3 components: about 65-75% A_1, 1-7% B, and 2.5-10% A_z. The components A_1 and A_z are E and Z geometric isomers. Although the stability of the premix at 45°C has not been examined specifically, it is stable at 37°C for about 9 months. The feedingstuff containing 16 mg/kg is reasonably stable for over 3 months at constant humidity. 10% of the activity may be lost in 6 months to 1 year.

The microbiological method of analysis does not give reliable results for the components A_z and B below levels of 16 mg/kg. It, and the HPLC method of analysis, are however reliable for component A_1 down to the level of 4 mg/kg.

1. Efficacy was studied in 37 trials with pigs (23 in UK, France, Germany, Italy, Denmark and 14 in USA, Canada, Brazil) involving a total of 3600 animals including 954 controls, and doses from 2-16 mg/kg of complete feedingstuff. The 23 European trials involved 2612 animals and 540 controls. All these trials were conducted with concentrations of copper between 35-180 mg/kg feedingstuff. In 8 trials 5 different additives (tylosin, olaquindox, virginiamycin, carbadox, avoparcin) were used as positive controls. Daily weight gain and feed conversion efficiency were the zootechnical parameters.

   In the data obtained before 1986 the positive controls often showed no growth promoting effect due to inadequate experimental design. This was not noted in the European trials after 1986. The energy and amino acid composition of the feeds are not stated in many of the trials and the stated doses of efrotomycin frequently do not correspond with those determined by feed analysis. In several trials the number of animals used was small.

   For all European trials the improvement in daily growth was very variable at all tested doses. The same variability was observed for feed efficiency. The dose-response curves in these trials are not linear.

   In 22 trials using doses higher than 4 mg efrotomycin/kg of complete feedingstuff neither growth nor feed conversion index were significantly. Furthermore, in some trials there was no difference in weight gain between 2 mg and 4 mg efrotomycin/kg of complete feedingstuff. In general, results appeared to be better during the early growing period (up to 60 days). The concomitant use of Cu supplementation and of castration in the European trials makes it difficult to assess the specific growth promoting effect of efrotomycin. In some trials males show a better growth response.

2. Studies in the target species using efrotomycin at 5x (80 mg/kg feed), 20x (320 mg/kg feed) and 25x (400 mg/kg feed) the recommended inclusion level revealed no toxicologically relevant effects.
   Transient diarrhoea and perianal erythema were seen at 400 mg/kg feed. No adverse treatment-related effects on body weight gain or feed consumption were noted at 320 mg/kg feed or less after 28 days. Treatment with efrotomycin did not influence reproduction parameters or offspring.

   Treatment of non-target species with doses up to 40 mg/kg feed resulted in lower feed consumption and diarrhoea in the first few days, disappearing within one week in sheep and cattle. In horses decreased feed consumption and lower body weight gains were observed with 16 mg/kg feed. Birds (chicken, turkey, duck, guinea fowl, quail) treated with efrotomycin at 4 mg/kg feed showed generally higher weight gain and feed conversion efficiency. Higher doses were not studied. No data on rabbits were available.

3. Efrotomycin is a narrow-spectrum antibiotic, its activity being mainly due to the component A_1. Although the component A_z has the same spectrum of antibiotic activity, its presence is too low to make any significant contribution. Component B has no antibiotic activity. Relatively high inhibitory levels of efrotomycin are required *in vitro*. It may be more effective *in vivo*. Little activity was shown against a large number of bacterial isolates from farms except against *Streptococcus suis, Pasteurella multocida, Clostridium perfringens, and Treponema hyodysenteriae*

   There was little evidence of the development of resistance to efrotomycin in *enterococci, coliforms, Cl. perfringens* and *bacteroides* species. No mutants resistant to efrotomycin developed in *clostridia*.

4. The metabolic studies used the reverse isotope dilution analysis of the substance labelled with ^{14}C at C 7 (limit of detection 0.005-0.01 mg/kg). Tissue residues were assessed by HPLC/UV (limit of detection 0.005 mg/kg) which correlates well with the microbiological assay method.

   Balance studies were carried out in young pigs using ^{14}C-labelled efrotomycin A_1 at 16 mg/kg feed. Recovery studies were carried out over 48 hours only which is an undesirably brief period. 60-77% of radioactivity was recovered in the faeces and urine (less than 2%) after 48 hours with an approximate half-life of 1 day. Efrotomycin A_1 is therefore absorbed to a small extent from the gut, the target organ being the liver.

   Liver residue amounted to 0.16 mg/kg tissue, kidney residue to 0.04 mg/kg and muscle 0.011 mg/kg. No radioactivity appeared in the fat. Live residue had fallen to 0.018 mg/kg and kidney residues to O.O13 mg/kg within 48 hours, the half-life being 0.5-1 day. Muscle tissue was free from residue after 24 hours. 65-70% of the liver residue was efrotomycin A_1, 12-16% was non-extractable. No efrotomycin B was detected in the liver. Solvent extraction caused analytical interference because of partial decomposition of efrotomycin A_1.

   About 66% of the kidney residue was efrotomycin A_1. Urine contained no efrotomycin B, about 30% of the activity being due to efrotomycin A_1. The remainder were polar metabolites. 60% of the faecal radioactivity was efrotomycin A_1 and 20% efrotomycin B, the remainder being breakdown products. The polar urinary and faecal breakdown products were not identified further. Efrotomycin B was therefore not absorbed from the gut.

   The rat metabolizes efrotomycin A_1 approximately similarly to the pig but appears to form fewer polar metabolites. Rat liver contains both efrotomycin A_1 and a smaller percentage of polar metabolites than pig liver.

   Pig stomach contents partially convert efrotomycin A_1 into efrotomycin B *in vitro* and this conversion may also occur *in vivo*.

   Bioavailability was not studied but in view of the low level residues and very short half-life this is not relevant to safety. Similarly, the low level of the residues for polar breakdown makes further identification unnecessary.

5. The toxicological studies involved acute oral and i.p. toxicity in rats and mice. Efrotomycin-Mg-alginate was non-irritant to rabbit skin and eyes. Subchronic toxicity was studied in dogs and rats. Two-year chronic studies in mice and rats produced no evidence of carcinogenic potential. A two-generation reproduction study in rats showed no treatment-related effects on reproductive parameters. Teratogenicity studies in mice and rats produced some evidence of phytotoxicity at high doses tested (2000 mg/kg b.w.) which however caused no maternal toxicity. Mutagenicity was studied in several test systems, both *in vivo* and *in vitro*. The positive result for efrotomycin in one *in vitro* test could not be confirmed in subsequent tests with the same and a new sample of efrotomycin. An additional *in vitro* test for induction of SCEs in cultured CHO cells was negative. An ADI of 0.1 mg/kg b.w. can be established based on the lowest NOEL of 10 mg/kg b.w. found in the subchronic dog study. However the dog is not as sensitive as some livestock animals. Adverse effects were found in pigs at 16 mg/kg b.w.

6. Decay of the active principle in the excreta is slow in aqueous solution in the dark but rapid in sunlight and varies with pH. It is particularly rapid at acid pH but, even at pH 9, some 94% have decayed in sunlight after 10 hours. Days is also slow in sandy and clay soil. Efrotomycin shows little mobility in the soil and there is a theoretical possibility of accumulation unless it is destroyed by photodegradation. Data on biodegradation in sediment/water systems or activated sludge are not available. The partition coefficient indicates that bioconcentration in aquatic and terrestrial organisms is unlikely. The physical properties make dispersion in the environment by evaporation unlikely. Germination trials showed slight phytotoxicity only against maize and no significant inhibition of root and shoot growth in mono- and dicotyledons. Efrotomycin was not toxic to earthworms at concentrations up to 1000 mg/kg soil. A concentration of 31 mg/l had no effect on *Daphnia magna* and 0.13 mg/l did not affect algal growth. The LD_{50} for *Salmo gairdneri* was greater than 100 mg/l. No negative effects were noted regarding nitrification up to 20 mg/kg in the soil. Soil methanogenesis showed a slight reduction and concentrations above 100 mg/l reduced methane production in waste water treatment plants. No tests with faeces containing efrotomycin and its degradation products were performed. A Stauber Heubach test on the granulate, the premix and a complete pig feed containing 16 mg efrotomycin/kg showed that the premix produced only 1% of the dust generated from the granulate. No efrotomycin was detected in the dust from the pig feed.

7. The zootechnical data relating to the European trials give only weak support to the efficacy claims for efrotomycin when used in conjunction with copper supplementation and castration of males. No data are available to judge whether the different climatic conditions within the European Community affect the growth promoting action of efrotomycin. The US trials are adequate for substantiating the efficacy of efrotomycin in the absence of copper supplementation. Both the A_1 and the B components appear to be more effective in younger animals and best in uncastrated males. Consideration of the various efficacy trials showed that the effective dose was about 4 mg/kg of complete feedingstuff. Studies in the target species have shown that doses up to 16 mg/kg feed are safe for pigs.

   There was no evidence for the development of resistance to prophylactic or therapeutic preparations or of the persistence of Gram-negative bacteria in the digestive tract of the pig nor of any effect on Salmonella shedding. Efrotomycin A_1 is absorbed to a small extent, undergoes biotransformation and is found in the liver at very low levels. The half-life of these residues is about 24 hours. Bioavailability is not relevant for the safety of these residues in view of their low level and short half-life.

The toxicological profile of efrotomycin has been fully studied, the ADI of 0.1 mg/kg b.w. being based on the dog study. There is an adequate safety margin between the residues in the liver and the ADI. On this basis the use at the level of 4 mg/kg of complete feedingstuff does not present any risks to the consumer. The Stauber-Heubach test confirmed the absence of significant risk from dust inhalation from the premix or complete feedingstuff.

Efrotomycin is photodegradable, shows little mobility in soil and has therefore a theoretical potential for accumulation unless it is destroyed by photodegradation. It does not bioconcentrate nor does it evaporate into the environment. It is not phytotoxic nor toxic to earthworms, *Daphnia*, fish and vegetable forms of aquatic life. It does not affect significantly nitrification or methanogenesis. No data on the effect on meat quality are available.

In the light of the above the Committee is of the opinion that the use of efrotomycin is acceptable at a level of 4 mg active ingredient/kg of complete feedingstuff. The amount of efrotomycin added to the premix or the complete feedingstuff should be stated in terms of efrotomycin A_1, the only component analyzed in feed and residues.

REFERENCES

Dossiers supplied by Merck, Sharp and Dohme.

Jacks, T.M., Frazier, E., Judith, F.R., Olson, G. (1988). Effect of efrotomycin in feed on the quantity, duration and prevalence of shedding and antibacterial susceptibility of Salmonella typhimurium in experimentally infected swine. *Amer. J. Vet. Res., 49:* 1832-1835.