REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF SALINOMYCIN-SODIUM IN FEEDINGSTUFFS FOR RABBITS FOR FATTENING

(Opinion expressed: July 1992)

TERMS OF REFERENCE (November 1991):

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

1. Has the use of the antibiotic Salinomycin sodium ($C_{42}H_{69}O_{11}Na$, Sodium salt of the monocarboxylic acid 30 of a polyether produced by Streptomyces albus) a favourable effect on the prevention of coccidiosis in the rabbit?

2. Is this use safe for the rabbits?

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 Salinomycin-sodium in the rabbits? Does the proposed use result in residues in animal tissues? If so, what are the qualitative and quantitative composition and persistence of these residues?

6. Do the toxicology studies allow the conclusion 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 Salinomycin sodium? Can these products be prejudicial to the environment?

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

BACKGROUND:

In Accordance with the provisions of Council Directive 70/524/EEC[1] of 23 November 1970 as amended by Council Directive 84/487/EEC of 29 November 1984[2] concerning additives in feedingstuffs, the use of Salinomycin-sodium (E 716) is authorized at Community level under the conditions set out as follows in Annex 1,

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

2 O.J. No. L319 (08.12.84), p. 13

Section D of Commission Directive 91/248/EEC[3] at last amended by Commission Directive 91/508/EEC[4]

A. Antibiotics				
Species or category of animal	Maximum age	Minimum content mg/kg of complete feedingstuff	Maximum content mg/kg of complete feedingstuff	Other provisions
Piglets Pigs	4 months 6 months	30 16	60 30	Indicate in the instructions for use: - Dangerous for equines - This feedingstuff contains one ionophore: simultaneous use with certain medicinal substances (e.g.tiamulin) can be contraindicated

D. Coccidiostats and other medicinal substances				
Species or category of animal	Maximum age	Minimum content mg/kg of complete feedingstuff	Maximum content mg/kg of complete feedingstuff	Other provisions
Chickens for fattening	-	50	70	Use prohibited at least 5 days before slaughter. Indicate in the instructions for use: Dangerous for equines

In 1980, Salinomycin sodium, was the subject of a submission for inclusion in Annex II of Council Directive 70/524/EEC as a coccidiostat for fattening chickens, turkeys and rabbits, and as a growth promoter (group of antibiotics) for piglets, pigs, lambs and fattening cattle. The SCAN expressed its provisional opinion on the use of Salinomycin-Na in feedingstuffs for chickens, piglets, pigs and cattle, in its report of 14 April 1982[5]. According to this report, the use of salinomycin could be admitted provisionally, pending reassessment of the additive when metabolism data become available. More recently, the SCAN expressed an opinion on the use of salinomycin for pigs for fattening[6]. The SCAN is asked to examine a submission to heading D. Coccidiostats and other medicinal substances, according to the following conditions:

Species or category of animal	Maximum age	Minimum content mg/kg of complete feedingstuff	Maximum content mg/kg of complete feedingstuff	Other provisions
Rabbits for fattening	-	15	25	Use prohibited at least 5 days before slaughter. Indicate in the instructions for use: Dangerous for equines

3 O.J. No. L124 (18.05.91) p. 12

4 O.J. No. L271 (27.09.91) p. 67

5 Reports of the Scientific Committee for Animal Nutrition. Fourth Series. EUR 8769, p. 36, Catalogue No. CD-NK-83-010-EN-C

6 Reports of the Scientific Committee for Animal Nutrition. Eighth Series, Luxembourg: Office for Official Publications of the EC, ISBN 92-826-7977-2

OPINION OF THE COMMITTEE:

1. Salinomycin is a polyether antibiotic of known structure. It is a monobasic carboxylic acid containing five cyclic ether rings.In numerous studies the effects of salinomycin have been investigated with regard to its coccidiostatic activity and the resulting improvement of alimentary efficiency and weight gain in the rearing of fattening rabbits affected by gut or liver coccidiosis. Most of the trials were carried out in Germany but also in other EEC and European non-EEC countries on the basis of experimental Eimeria infections with different highly pathogenic strains.

   The infections were performed with higher numbers of oocysts than occurring under natural conditions. In most experiments the administration of the salinomycin-medicated feed started 3 days before infection. A certain dose-effect relationship could be derived showing that doses below 18 mg/kg of complete feedingstuffs (ppm) were not sufficiently effective particularly in the case of liver coccidiosis. Doses of 20 to 25 ppm proved to be efficient against gut and liver coccidiosis in these experiments. These doses did not totally eradicate coccidiosis in all experiments but markedly reduced oocyst shedding, diminished the extent of liver lesions and the accompanying increase of liver weight, decreased the number of dead animals, prevented body weight losses or even caused weight gain, and improved alimentary efficiency in infected animals. Under these conditions the necessary number of oocysts remained as an antigenic stimulus for the development of immunity. The efficacy of salinomycin was equal or even superior when compared to other coccidiostats like clopidol, lasalocid or monensin whereas robenidine proved to be more favourable for the control of intestinal coccidiosis. Doses over 50 ppm exerted negative effects on the performance of infected animals. Under natural conditions normally less severe infections can be expected which perhaps may be controlled with lower doses.

   It can be concluded that salinomycin concentrations of 20-25 mg/kg of complete feedingstuffs are effective for the prevention of coccidiosis in rabbits and for protection from subsequent losses of growth performance. Thus, the incorporation of salinomycin at these dosages can exert a favourable effect on the production of rabbits for fattening challenged with coccidiosis. Dosages < 18 ppm are not sufficiently effective to control coccidiosis.

   The higher dosage range of 25-35 ppm as previously given in question 57 cannot be recommended since these doses do not provide a remarkable improvement in efficiency but increase the risk of adverse reactions in prophylactically treated non-infected animals due to the low margin of safety of salinomycin in rabbits as outlined below.

2. The acute oral LD_{50} of salinomycin for rabbits could be calculated as 21 mg/kg body weight which is equivalent to approximately 500 mg/kg in complete feedingstuffs.

The target animal safety of salinomycin as a food additive has been checked in various studies by treating infected and non- infected rabbits with salinomycin doses up to 125 ppm added to 8 different mixed feed components. In non-infected animals salinomycin induced a dose-dependent growth depression, decrease of feed intake and reduction of alimentary efficiency which was clearly apparent at a dose of 50 ppm and was not dependent on the mixed feed components used.

At lower doses depressant effects could also be observed in several experiments depending in some cases on the mixed feed components used. Salinomycin induced a linear reduction of food intake, weight gain and alimentary efficiency even at doses of 12.5 - 20 ppm whereas in other experiments a marginal improvement of growth performance could be demonstrated. In infected animals, however, the depressant effects of salinomycin were outweighed at doses up to 50 ppm by its coccidiostatic action resulting in reduced weight losses or even weight gains in the diseased animals thus improving the performance of the livestock production under these conditions.

The results suggest that the proposed dosage range of 15-25 ppm of salinomycin has a narrow margin of safety.

For a risk-benefit assessment these negative effects on non-infected animals have to be compared to the beneficial effects of salinomycin on rabbits challenged with coccidiosis which were obvious at doses of 20-25 ppm. It should be stated also that in chickens ionophores and other antiprotozoals exert depressant effects. In contrast with chickens, however, where the use of coccidiostats is an obligatory prophylactic measure to avoid disease, the incidence of coccidiosis in rabbits is lower. Therefore the use of coccidiostats is not foreseen in farms which are free of coccidiosis problems.

In order to investigate this problem of poor target animal safety the applicant should be asked to provide new information on the efficiency of lower salinomycin doses under field conditions.

The results in regard to embryotoxic effects are contradictory. In a teratogenicity study in rabbits slight materno/foetotoxic effects (increased resorption sites) could be observed at doses of 0.63 and 1.6 mg/kg which correspond to a dosage of 16 or 40 ppm in medicated feed. The NOEL was 0.25 mg/kg (6.25 ppm). Thus, embryotoxic doses are lower than the anticoccidial doses of 20-25 ppm. In field trials in one study only was a reduced litter size observed at 25 ppm whereas in other experiments doses of 20-75 ppm exerted no apparent negative effects on does or litters.

Since the first mentioned study was carried out under appropriate and defined experimental conditions it has strongly to be supposed that salinomycin may exert materno/foetotxic effects in the proposed dosage range. This effect could be overlooked in the field trials as embryolethality is generally high in rabbits. On the basis of these data salinomycin should not be used in pregnant rabbits. This problem needs no further evaluation for the present application which only claims the use in rabbits for fattening.

3. For monitoring of salinomycin in animal feedingstuffs analytical methods for extraction and purification, microbiological assays, colorimetric reactions and thin layer chromatography with satisfactory sensitivity (20 ppb) are available.

4. Salinomycin is moderately effective only against Gram positive bacteria. As the intestinal flora in rabbits usually contains a number of clostridia it is of special interest that the MIC of Cl. perfringens was 0.1 µg/ml. Consequently, the normal intestinal flora will undergo a change which might influence normal digestion in rabbits. The efficiency trials, however, demonstrate in infected rabbits an improvement of the animals performance and feed conversion efficiency. Therefore it can be supposed that the altered competition pressure will be without adverse influence on digestive processes and growth of the animals.

   In vitro only a slight increase in MIC-values of Staph aureus (SG 511) was observed which was of short duration and can be regarded as insignificant. Testing of cross-resistance to 7 adequate antibiotics revealed only a slight increase in the MIC of monensin. In vivo in pigs feeding of salinomycin slightly enhanced the number of resistant E. coli. The test was performed with 8 different antibiotics and chemotherapeutics.

   In conclusion the results show that the use of salinomycin does not result in the development of resistant bacteria and does not cause any cross-resistance to other antibiotics and chemotherapeutics commonly used in medical treatment.

5. Pharmacokinetic patterns and residue formation after oral administration of salinomycin to rabbits were investigated with ^{14}C-labeled salinomycin. This was rapidly and extensively absorbed from the rabbit's gut. Elimination occurred mainly via the faeces (56-80% within 3 to 8 days). Eight to 15 % was recovered in urine. These values were slightly influenced by coprophagy. No label was present in the expired air. Salinomycin equivalents rapidly appeared in the bile and entero-hepatic recirculation is suspected. Maximum tissue concentrations of labelled salinomycin in a 15 day oral dosing study were achieved by 24 hours and remained constant thereafter. Hence cumulation is not to be expected under the proposed conditions of use.

   Salinomycin undergoes metabolism in the liver yielding a spectrum of numerous metabolites, mainly mono-, di- and trihydroxylated derivatives similar to those of the rat and mouse. In the bile the metabolite profile was comparable to that of rat and pig. Parent salinomycin was never detected in bile nor was there any significant sex difference in metabolite profiles.

   The liver was the target organ for residues with a maximum value of 4 µg/g salinomycin equivalents. Residues were also detectable in kidney, fat, muscle and gut wall. At the recommended dose in all tissues but the liver the concentrations have fallen to or below the limits of detection (0.01 µg/g) by 48 hours after withdrawal. As in the pig, the residue depletes from liver rapidly initially ($t_{½}$: 24 h) and then more slowly ($t_{½}$: 8 days). There was no influence of coprophagy. In the microbiological assay parent salinomycin reached the limits of detection (0.01 µg/g) at 24 to 48 hours after withdrawal.

In the case of the 15-day feeding study in the rabbit, of the four major hepatic metabolites of salinomycin three were undetectable after 24 hours of withdrawal. The remaining metabolite, monohydroxy-salinomycin (the major metabolite in rat also), was still present at 0.29 ppm salinomycin equivalents after 12 days of withdrawal (detection limit 0.01 ppm). This was the major metabolite from 72 hours of the accumulation period. From 72 hours onwards the relative proportions of the four major metabolites remained constant up to the 15 day limit of the feeding study. The decline of parent substance was, however, more rapid in the case of the study which employed 42 days of medication, suggesting enhancement of elimination. The zero time level was however 15 to 40 times greater than in the 7 days studies suggesting that the presence of parent salinomycin is highly dose-dependent (1.22 rather than 0.8 mg/kg body weight/day). Even at this the concentration of label in the short term study (carried out in France) was 5 to 15 times greater and should therefore be preferred for the ADI/withdrawal period calculation.

6. Most of the toxicity studies were performed with the dried mycelium and a few with 87-95% pure salinomycin. Acute toxicity studies in different animal species (mouse, rat, rabbit, chicken, dog, pig, bull, horse) revealed oral LD_{50} values ranging from 21-60 mg/kg body weight. For mice, rats, chickens and rabbits the signs of toxicity were mostly neurological. Pigs, bulls and horses were increasingly sensitive in that order, toxic effects occurring mostly in the liver and myocardium. Acute dermal toxicity in the rat showed salinomycin to be moderately irritant. Salinomycin was non-antigenic and caused no immediate or delayed hypersensitivity in guinea pigs. Subchronic toxicity studies were carried out in mice, rats, dogs and pigs. The target organs for toxicity were liver and spleen in mice (NOEL 3.75 mg/kg b.w.) and rats (NOEL 2.5 mg/kg b.w.).

   Chronic toxicity was studied in mice over 24 months and in rats over 30 months. Most of the observed adverse effects concerned organ weights and changes in clinical biochemistry findings. No evidence of carcinogenicity could be observed. The NOEL was <1.4 mg/kg b.w. in the mouse study and 2.5 mg/kg b.w. in the rat study.

   A two generation reproduction study in rats showed a NOEL of 5 mg/kg b.w. Embryotoxicity and teratogenicity studies in mice and rabbits revealed maternal and fetal toxicity in mice with a NOEL of 4 mg/kg b.w. Rabbits showed increased resorptions most probably due to maternal and fetal toxicity with a NOEL of 0.25 mg/kg b.w. but no teratogenicity was observed.

   Mutagenicity and genotoxicity were absent when salinomycin was tested in bacterial systems, host-mediated assay in mice, recessive lethal mutation tests in Drosophila melanogaster, hepatocyte unscheduled DNA-synthesis and a mouse lymphoma forward mutation assay.

   Due to the similar profile of metabolites in rodents and other animal species these toxicity studies have a predictive relevance for target animals and for the assessment of the risk to consumers.

From the available toxicity study an ADI of 0.0025 mg/kg b.w. or 0.15 mg for a 60 kg weighing human being may be determined on the basis of the NOEL in the rabbit embryotoxicity study.

Residue studies revealed in all tissues a rapid depletion of the more toxic parent salinomycin below the detection limit (0.01 µg/g) within 24 hours using a bioassay method. In different studies with radioactively labeled material the following residue concentrations could be measured: pilot study (2.5 times the recommended dose): 0.724 µg/g liver and 0.05 µg/g kidney which equals a total amount of 74.9 µg salinomycin equivalents in 100 g of liver plus 50 g of kidney.

In the major residue study (at the recommended dose of 20 ppm) relevant residue concentrations could also be detected only in liver which declined from about 4 µg/g (day 0 of withdrawal), 2.2 µg/g (day 2) to 1.3 µg/g (day 8) and 0.29 µg/g at day 12. From this study it appeared that considerable levels of residues are still present in the liver of rabbits after 12 days of withdrawal which are markedly higher than the maximum residue levels of 0.06 µg/g in pig liver after 60 h of withdrawal. After 2 days of withdrawal in the residue fraction only the monohydroxylated metabolite was detectable and this can be regarded as less harmful. The very slow depletion of this metabolite is rather uncommon for a hydrophilic compound and casts doubt on the real nature or origin of this residue.

The reasons for such a long persistence of residues need further clarification. Moreover, it should be mentioned that only one animal was sacrificed at each sampling point. Therefore the data basis is very limited, does not cover interindividual variation and is not adequate for the determination of the proposed withdrawal period. The situation is further complicated by the divergent results at day 5 (the proposed withdrawal period) and day 8 with liver residues of 0.66 µg/g and 1.31 µg/g respectively. Both values are not fitted by the kinetics of residue decay in the liver calculated from the experimental data with significant statistical adjustment. The residues at day 5 and 8 are lower than the ADI-value of 150 µg/person. However, the residues at day 8 (131 µg/100 g liver) are rather close to the ADI-value.

It has further to be considered that liver coccidiosis may decrease the rate of residue depletion.

In regard to the safety for the user it should be noted that salinomycin is a dermal irritant.

7. Because of the lack of data specific for rabbits, evaluation of the environmental impact of salinomycin used in rabbits has to rely on two previous reports on studies in chickens and pigs.

   As described under No.5 salinomycin yielded similar patterns of metabolites in different animal species including rabbit, rat, mouse and pig. Within 3 to 8 days 56-80% of the radioactive dose appeared in the faeces and 8-14.6% in urine with a slight increase of faecal excretion by coprophagy. From the metabolism studies it can be assumed that only a very small fraction of ingested parent salinomycin (1-5%) appears in rabbit excreta, as was proved in broilers and other animal

species. Applying data from other species it can be presumed that most ingested salinomycin appears in the faeces as di- and trihydroxylated metabolites and as a large number of minor metabolites which have no antimicrobial activity.

The dossiers do not provide data on the stability of salinomycin in rabbit manure but only refer to the data obtained in chicken. Salinomycin disappeared rapidly from chicken manure (from 0.04 ppm to 0.01 ppm within 6 days) at room temperature and one can assume similar rates of disappearance from rabbit excreta. Together with a half-life of salinomycin in soil of only 40-50 hours (only 1% remaining after 21 days) there seems to be no risk of accumulation in fertilized surfaces. Salinomycin and metabolites have a low toxicity for Daphnia, no effect being observed at 21.5 mg/l. Fish toxicity was also low (no effect observed at 22.4 mg/l in Golden orf-*Leuciscus idus*). No data on methane production or nitrification by rabbit excreta were available.

Due to the low antimicrobial activity remaining and according to the results obtained with cattle and pig manure, harmful effects can be excluded. Plant growth was slightly inhibited only at relatively high concentrations of pure salinomycin and no relevant uptake by plants could be observed. Appropriate tests, however, with metabolite-containing manure were absent.

Although lacking detailed data on rabbit excreta, these observations indicate that possible harm to the environment is unlikely at the concentrations reached by the normal use of manure from rabbits treated with salinomycin.

8. In the light of the available evidence the Committee is of the opinion that

   - a salinomycin dosage of 20-25 mg/kg complete feedingstuffs is effective in protecting rabbits from coccidiosis and in improving alimentary efficiency of infected animals even under conditions of more severe experimental infections than occurring under natural conditions. Dosages < 18 mg/kg of feedingstuffs are not proved as sufficiently effective to prevent liver coccidiosis.

   - in non-infected animals this dosage range and even lower doses of at least 12.5 ppm of complete feedingstuffs may exert in some cases depressant effects. In infected animals these negative effects are outweighed by the anticoccidial effect up to doses of 50 ppm.

   - to address the problem of the poor safety in healthy target animals at a dosage of 20-25 mg/kg of complete feedingsstuffs new information should be provided on the efficiency of lower salinomycin doses under field conditions.

   - the results of studies on materno- and embryotoxicity are contradictory. There are strong indications of embryotoxic effects at doses below 20 mg/kg of complete feedingstuffs which might have been overlooked in the field trials. This problem is not of relevance to the present application, viz the use of salinomycin in rabbits for fattening. It will need clarification if approval is sought for the use of salinomycin in rabbits for breeding.

- The information content of the residue studies is very limited and not adequate to establish the proposed withdrawal period of 5 days as only one animal was sacrificed at each sampling point. A more detailed residue study with a higher number of animals should clarify the residue situation especially in the critical period of 5-8 days.

- the long persistence of residues in the liver is rather uncommon for the putative monohydroxylated metabolite. The slow depletion, long persistence and the true nature or origin of this liver residue needs a thorough explanation.

- an ADI-value of 0.0025 mg/kg can be derived from the embryotoxicity study in rabbits.

- the potential for dermal irritancy should be noted on the label.

- a similar warning remark regarding interaction with tiamulin should be included on the label of ionophore-containing feed and tiamulin preparations.

- the use of salinomycin under the proposed conditions can be regarded as harmless for the environment.

In order to allow the Scientific Committee for Animal Nutrition (SCAN) to answer question 57, the applicant firm is requested to provide the following additional information:

1. Efficiency of salinomycin in fattening rabbits under field conditions at doses below 20 mg/kg[7] (see background § 1).

2. A more detailed residue study with a higher number of animals (see background § 2).

3. Further explanation of the slow depletion, long persistence and true nature or origin of a monohydroxylated metabolite as the main persistent residue in the liver (see background § 3).

BACKGROUND:

1. Salinomycin doses of 20-25 mg/kg [1] proved to be sufficient to protect rabbits from coccidiosis and subsequent growth depression. A sufficient efficacy, in particular against liver coccidiosis, could not be demonstrated at doses < 18 mg/kg.

   In non-infected animals, salinomycin induced a dose-dependent growth depression, reduced feed intake and diminished feed efficiency which was clearly apparent at a dose of 50 mg/kg and was not dependent on the mixed feed components used. Depressant effects could also be observed even at doses of 12.5 - 20 mg/kg.

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[7] Milligrams or 10^{-3} g/kg of complete feedingstuffs

In infected animals, however, the depressant effects of salinomycin were outweighed by its coccidiostatic action at doses up to 50 mg/kg. The results suggest that the safety margin of salinomycin in non-infected animals is rather narrow at the proposed doses of 20-25 mg/kg so that marginal negative effects on the growth performance of healthy animals cannot be excluded.

2. In the major residue study only one animal was sacrificed at each sampling point. Therefore the data basis does not cover between-animal variation and is not adequate for the determination of the proposed withdrawal period. The liver residue values determined in the critical period of 5 to 8 days lie far outside the curve of residue decay.

   An ADI-value of 0.0025 mg/kg b.w. could be derived from the embryotoxic NOEL in the embryotoxicity study in rabbits.

3. From the kinetic studies it appeared that considerable levels of residues of 0.29 µg/g are still present in the liver of rabbits after 12 days of withdrawal which are markedly higher than the maximum residue levels in pig liver of 0.06 µg/g after 2.5 days of withdrawal. After 2 days of withdrawal in the residue fraction only the monohydroxylated metabolite was detectable. The very slow depletion of this metabolite is rather uncommon for a hydrophilic compound and causes some doubts on the true nature or origin of this residue.