

Report of the Scientific Committee for Animal Nutrition on the use of Salinomycin Sodium in feedingstuffs for pigs. (Opinion expressed: 10 July 1991).

Terms of reference (October 1986)

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

1. Has the use of the antibiotic salinomycin (sodium salt of polyether of monocarboxylic acid) at the dosages proposed for feedingstuffs for pigs (see Background) significant effects on the growth of the animal?
2. Is this use 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 salinomycin in the pig? Does the proposed use result in residues in animal tissues? If so, what are the qualitative and quantitative composition and persistence of these residues?
5. Do the toxicological studies of the product allow to conclude that the proposed use does not present risks
  - for the consumer?
  - for the user?
6. Are the products derived from salinomycin and excreted by the pig of the same nature as those excreted by the chicken? If so, 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

In accordance with the provisions of Council Directive 70/524/EEC of 23 November 1970, concerning additives in feedingstuffs<sup>1</sup>, as last amended by the Commission Directive 84/587/EEC of 29 November 1984<sup>2</sup>, the use of salinomycin is authorized at Community level under the following conditions set out in Annex I, Section D, of the Directive:

- Species of animal: chickens for fattening.
- Dosage: 50-70 mg/kg of complete feedingstuff.
- Other provisions: use prohibited at least five days before slaughter.

The use of salinomycin sodium in other animal species, which was also requested, is not authorized in the Community. In this connection, the Scientific Committee for Animal

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<sup>1</sup> O.J. No L 270, 14 Dec 1970 p.1.

<sup>2</sup> O.J. No L 319, 8 Dec 1984 p.13.

Nutrition expressed a provisional opinion in 1982 and, with regard to the chicken, a final opinion in 1984.

Additional files intended for supporting the application for the use of the product as growth promoter in pigs under the conditions mentioned below are presently available:

Use levels:

- piglets up to 4 months: 30-60 mg/kg of complete feedingstuff.
- pigs up to 6 months: 15-30 mg/kg of complete feedingstuff.

Opinion of the committee

Salinomycin is a polyether antibiotic of known structure. It is a monobasic carboxylic acid containing five cyclic ether rings.

1. Salinomycin has been used in a large number of trials both in the rearing of piglets and in fattening pigs in some 14 countries, including some in the EEC. The results are very variable and not easy to interpret because the pig populations involved were not homogeneous and the rearing conditions frequently not standardised. A consistent dose-effect relationship cannot be derived. However, a definite positive though rather flat response was discernible with increasing amounts of salinomycin in the feeds. This supports the existing practical experience of having to use more than the apparent lowest effective dose in view of the large inter-individual variations in biological response and the known differences in animal husbandry practice. A reasonable estimate of the doses giving a significant effect on growth would be 50 mg/kg feed (range 30-60) for piglets up to 4 months and 20 mg/kg feed (range 15-30) for pigs up to 6 months.
2. A six months study in pigs showed some hepatotoxicity at high doses, the NOEL being 2.5 mg/kg b.w. This is well above the dose proposed for growth promotion. Toxic signs following overdose in pigs, apparent after 5 days, are respiratory problems, anorexia, ataxia, inability to stand, haematuria and a mortality of about 10%. Histopathology indicates acute degenerative myopathy.

In conjunction with administered tiamulin there is a dose-dependent increase in toxicity of salinomycin due to a reduction in excretion. The compatibility of tiamulin and salinomycin is influenced mainly by the total combined administered dose/unit time, estimated to be about 6 mg/kg b.w. There should also be an interval of at least 7 days between administration of tiamulin and salinomycin or vice versa, unless doses are of the order of 60 ppm salinomycin and 30-40 ppm tiamulin per pig. Attention is drawn to the possibility of adding a warning remark to the label of ionophores (salinomycin, monensin, narasin, maduramycin, lasalocid) stating that animals, including birds, should not be treated with products containing tiamulin while receiving, and for at least 7 days before or after receiving, feed containing salinomycin because severe growth depression or death may result.

3. Salinomycin is moderately effective only against Gram-positive bacteria. No evidence for selection pressure or selection of indigenous enterococci with multiple-resistance plasmids was found. No cross resistance to 6 antibiotics used in human medicine was found and there is a lack of structural resemblance to chemotherapeutic agents used in human medicine. No resistance was observed in coccidia nor stable resistance by chromosomal mutations in *Staph. aureus in vitro*. Salinomycin alters to a small extent the relative levels of Gram-positive and Gram-negative bacteria in the

intestine of pigs. There appears to be no need for concern over the possible development of bacterial resistance in the Gram-negative bacteria in the digestive tract of the pig.

4. Metabolism studies in mice, rats and chicken using oral doses of <sup>14</sup>C-labelled salinomycin showed that over 90% is excreted in the faeces within 48-72 hours, less than 5% in the urine and negligible amounts in the expired air. Small amounts of <sup>14</sup>C-salinomycin are found in the liver and bile after 48-72 hours. The amounts of radioactivity in rat and mouse tissues, particularly in the liver, were considerably larger than in chicken tissues.

Salinomycin is rapidly metabolized in the gut and liver to numerous metabolites, mainly mono-, di- and trihydroxylated derivatives. The spectra of metabolites in rat and mouse liver were qualitatively and quantitatively similar, the spectrum in chicken liver being qualitatively similar but quantitatively different. Only 2-8% of liver radioactivity was non-extractable in the three species. Metabolism is slowest in the chicken. A similar study with <sup>14</sup>C-salinomycin in dogs indicated similar metabolites to those found in the chicken at about the same order of magnitude.

Salinomycin is rapidly and extensively absorbed and metabolized by pigs when administered by the oral route. An average 83.5% of the radioactivity is excreted in the faeces with little intact salinomycin being present, in urine around 2.1% is excreted. After 4 days, residues in the liver are 0.1 mg/kg and less than the detection limit in muscle, kidney and fat (limit of detection of radiochemical method 0.01 mg/kg). Pig bile contains mainly the di- and trihydroxylated derivatives, while liver also contains other hydroxylated products, notably the monohydroxy derivative also found in rabbit liver.

Extensive residue studies in broilers, turkeys, rabbits and pigs showed no detectable residues after 24 hours withdrawal using a microbiological method (limit of detection 0.01 mg/kg). After single dose administration to pigs 71-88% were found in the gastro-intestinal tract after 12 hours, radioactivity being only detectable in the liver. Repeated dose administration produced a liver residue level of 1.5 mg/kg after 8 hours withdrawal, falling to 0.4 mg/kg after 12 hours (monohydroxy-salinomycin absent, only di- and trihydroxy-salinomycin present), 0.2 mg/kg after 24 hours and 0.06 mg/kg after 60 hours, when analyzed by a radiochemical method with a limit of detection of 0.01 mg/kg. When analyzed by a microbiological assay with a limit of detection of 0.02 mg/kg no residues could be detected after 24 hours in the liver. The half-life of elimination was 2-3 hours during the first 12 hours after withdrawal and about 17 hours thereafter.

Pigs receiving repeated doses of salinomycin-containing mycelium in concentrations up to 200 mg/kg feed produced residues after 24 hours in the cutaneous fat of only 2 animals given 50 mg/kg feed. In another experiment no tissue residues were detected after 16 hours withdrawal. In the opinion of the Committee residues in the liver of pigs have fallen to an insignificant level after 24 hours withdrawal, the ionophoric activity being lower than residues in chicken liver. No residues are detectable in any other tissue of pigs after 12 hours withdrawal.

5. Most of the toxicity studies were performed with the dried mycelium and a few with 87-95% pure salinomycin. Acute toxicity studies in mice, rats, chicken, rabbits, dogs, pigs, bulls and horses showed oral LD<sub>50</sub> values ranging from 21-60 mg/kg b.w.; 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 tests in the rat showed

salinomycin to be moderately irritant. Salinomycin was non-antigenic and caused no immediate or delayed hypersensitivity in guinea-pigs. Subchronic studies were carried out in mice, rats, dogs and pigs. The target organs of toxicity were liver and spleen in mice ( NOEL 3.75 mg/kg b.w.) and rats ( NOEL 2.5 mg/kg b.w.), the nervous system in dogs ( NOEL 1 mg/kg b.w.) and the liver in pigs ( NOEL 2.5 mg/kg b.w.).

Two-year chronic toxicity studies were carried out in mice and rats and also a study over 2 1/2 years in rats using mycelium. Most of the observed adverse changes concerned organ weights and clinical biochemistry values but there was no evidence of carcinogenicity. The NOEL was <1.4 mg/kg 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 foetal toxicity in mice with a NOEL 4 mg/kg b.w. Rabbits showed increased resorption most probably due to maternal/foetal toxicity with a NOEL of 0.25 mg/kg b.w. but no teratogenicity was observed.

The bioavailability of <sup>14</sup>C-salinomycin in pig liver was found in rats to be 10-31% of the administered dose. The ionophoric activity in pig liver was markedly lower than in chicken liver due to lower amounts of salinomycin. Genotoxicity was absent when salinomycin was tested in a bacterial system, a host-mediated assay in mice, a recessive lethal assay in *Drosophila* and a gene mutation test in mouse lymphoma cells. From the available toxicological studies an ADI of 0.003 mg/kg b.w. may be determined on the basis of the NOEL in the rabbit embryotoxicity study. It may be concluded from a consideration of the toxicity studies, the ADI of 0.003 mg/kg b.w. and the very low residues in pig liver that the proposed use of salinomycin does not present a risk to the consumer. As regards the user it should be noted that the substance is a dermal irritant.

6. 1.4-2.8% of the radioactivity of <sup>14</sup>C-labelled salinomycin fed to pigs are excreted in the urine and 81-86% appear in the faeces within 4 days, with small amounts of intact salinomycin present. Only about 1-5% of the microbiological activity in the feed is found in the excreta from broilers. Similar excretion patterns were found in other species (fowls, rats, mice, dogs). Because most of the metabolites have no antibiotic activity it appears likely that pig excreta, like chicken excreta, carry only very low antibiotic activity. There are no data on the toxicity of the faecal metabolites in pig manure.

There are no data on the stability of salinomycin in pig excreta during storage. Since salinomycin rapidly disappears from chicken manure, the concentration dropping from 0.04 ppm to 0.01 ppm within 6 days at room temperature, one can assume similar rates of disappearance from pig excreta.

The half-life of salinomycin in soil was 40-50 hours when measured microbiologically (limit of detection 0.01 mg/kg), only 1% remaining after 21 days, thus constituting practically no risk of accumulation. No data are available on leaching from soil or on the breakdown products in soil. Only very low concentrations of salinomycin are likely to be found in soil when using pig manure as fertilizer.

Salinomycin and its metabolites have a low toxicity for *Daphnia*

(EC<sub>50</sub> 24 h: 43 mg/l), no effect being observed at 21.5 mg/l. Fish toxicity (Golden orf-*Leuciscus idus*) was also low (LD<sub>50</sub> 96 h: 30 mg/l). No effect was observed at 22.4 mg/l.

Total methane production from fresh pig manure from treated pigs was increased by 5% over that from untreated pigs. Salinomycin in the faeces of cattle reduced methane production by about 15%. At a salinomycin level of 8 mg/kg in soil (300 times the maximum concentration possible in soil under practical conditions) nitrification was slightly delayed. However no similar data are available for the effect of actual pig manure, i.e. of the metabolites, although most are devoid of antibiotic activity.

Plant growth was slightly inhibited at doses of 12.5-200 mg salinomycin/m<sup>2</sup> for carrots, kohlrabi, chinese cabbage, potatoes and sugar beet. No uptake into plants was noted at 14 mg/kg soil. These tests were done with pure salinomycin and not with manure containing metabolites. These observations indicate that possible harm to the environment is unlikely at the concentrations reached by normal use of pig manure. However, data on the effect of the metabolites are lacking.

7. In the light of the available evidence the Committee is of the opinion that the use of salinomycin in the feedingstuffs of pigs at the proposed levels could be admitted. A similar warning remark regarding interaction with tiamulin should be included on the label of ionophore treated feed and tiamulin preparations.

## References

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