Report of the Scientific Committee for Animal Nutrition on the use of Narasin + Nicarbazin in feedingstuffs for chickens. (Provisional opinion : 10 July 1991).

Terms of Reference (June 1990)

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

1. Has the use as a coccidiostat of Narasin (polyether of monocarboxylic acid produced by *Streptomyces aureofaciens*) plus Nicarbazin (equimolecular complex of 1,3-bis (4-nitrophenyl)urea and 4,6-dimethylpyrimidine-2-ol) under the conditions proposed for its use as an additive for fattening chickens (see background) significant effects on the prevention of coccidiosis in this animal species?

2. Is this use safe for the chickens?

3. Can it be monitored in animal feedingstuffs?

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

5. What is the metabolic fate of the whole product Narasin+Nicarbazin in the chicken? Are the two component products compatible? 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 toxicological 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 Narasin+Nicarbazin? 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

Narasin[1] and Nicarbazin[2] are coccidiostats already included in section D (Coccidiostats and other medicinal substances) of the Annex I list to Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs[3].

The preparation Narasin+Nicarbazin was the subject of an application for admission in the same section D (Coccidiostats and other medicinal substances) of this Council Directive 70/524/EEC (3) under the following conditions of use:

[1] J.O. No. L183, p.15 (11.07.1984)

[2] J.O. No. L310, p.19 (5.11.1986)

[3] J.O. No. L270, p. 1 (14.12.1970)

- Species of animal: chickens for fattening.
- Use level: 40+40 to 50+50 mg/kg of complete feedingstuffs.
- Other provisions: Use prohibited at least five days before slaughter.

Opinion of the Committee

1. Narasin is a polyether antibiotic produced by deep culture fermentation of a strain of *Streptomyces aureofaciens*. Its chemical structure shows it to be a monobasic carboxylic acid containing 5 cyclic ether rings.

   Nicarbazin is an equimolecular crystalline complex composed of 67.4-73.O% 4,4'-dinitrocarbanilide (DNC) and 27.7-30.O% 2-hydroxy-4,6-dimethyl-pyrimidine (HDP).

   In a large number of tests, using various combinations ranging from 10+10 to 60+60 mg/kg of feedingstuff of each substance against 5 *Eimeria* strains producing intestinal coccidiosis and 1 strain producing caecal coccidiosis as well as mixed *Eimeria* infections, a combination of 50+50 mg/kg of complete feedingstuff has been the most efficient with regard to growth, improved feed efficiency, lesion control and oocyst elimination. A clear dose-response relationship has been demonstrated. Efficiency of the combination has been compared with feedingstuff containing 70 mg/kg Narasin or 125 mg/kg Nicarbazin but not with other coccidiostats. Because the 50+50 combination does not eliminate oocysts completely, sufficient remain to induce immunity. The 50+50 combination is effective against resistant, strongly pathogenic single strain infections and mixed infections. However, with this dosage there are the occasional cases where weight gain is reduced and feed efficiency impaired.

2. The use of the 50+50 mg/kg of complete feedingstuff combination is safe for chicken. The occasionally observed smaller weight gain or impaired feed efficiency is acceptable in the absence of any other adverse effects in chicken, when compared to the risk of 30 - 35% mortality from untreated infection.

3. There are adequate analytical methods for assaying the pure drugs and the tissue residues. Narasin is assayed by TLC with bioautographic overlay using *Streptococcus faecium* (limit of detection 0.005 mg/kg). Nicarbazin is assayed mostly by determining the DNC component with HPLC/UV (limit of detection 0.10 mg/kg), less frequently by HDP determination using FID GLC (limit of detection 2 mg/kg).

4. Of the two components only Narasin has antibiotic activity. The evaluation by SCAN is reported in the Fourth Series of Reports. SCAN concluded that none of the gram-positive bacteria, the only kind against which Narasin is active, showed cross-resistance to a variety of clinical antibiotics tested, even when transient resistance to Narasin developed. There appears therefore to be no need for concern over the possible development of bacterial resistance. A separate experiment on the possible effect of Nicarbazin on the antibacterial activity of Narasin against *E. coli, Bacteroides* and *Staphylococci* was negative, showing no change in MIC for the susceptible and non susceptible strains examined, however only little material was used. The normally susceptible *Staphylococci* were found to be unexpectedly resistant to the action of narasin.

   No explanation was offered for this observation. It would therefore be desirable to repeat the experiment or to provide an explanation for the change in the MIC values. Because preparations effective against gram-positive bacteria frequently extend Salmonella shedding through an imbalance in the intestinal flora, it could be desirable

to determine the effect of the combination on Salmonella shedding. In a separate experiment Nicarbazin was shown to have no antibacterial effect on a selection of bacterial strains.

5. Nicarbazin is largely absorbed by the chicken, HDP is excreted mainly in the urine and DNC mainly in the bile and faeces. HDP is rapidly metabolized and disappears from all tissues after a 5-day withdrawal period (limit of detection 0.3-0.4 mg/kg tissue). No identification of metabolites is available. DNC is metabolized slowly with residues persisting mainly in the liver, amounting to 0.06-0.08 mg/kg tissue DNC equivalents after 11 days withdrawal. HDP therefore contributes only 4.5% of the total Nicarbazin residues. Use of ^{14}C-labelled DNC in the chicken indicates the existence of 2 main metabolites which are acetylated amines arising from the reduction of one or both nitro groups. A third minor metabolite results from cleavage of the carbanilide group followed by reduction and acetylation of the nitro group. Excreta consist mainly of DNC plus small amounts of the 3 metabolites. Liver residues consist mainly of about 80 % DNC, less than 12% metabolites (M1 + M3) and some 3.3% unidentified matter. Metabolism in the rat is similar to that in the chicken.

   Narasin is well absorbed and largely excreted in the bile. Excreta contain 6 major metabolites which incorporate the dihydroxy and trihydroxynarasin structure. In total they account for only 20% of the parent antibiotic activity and for a very weak ionophoric activity. The metabolism in the rat is similar. At zero withdrawal time there is no measurable radioactivity in muscle and a rapid decline in other tissues. After three days the liver and skin contain 0.04 and 0.025 mg/kg Narasin equivalents respectively. 5% of the total liver radioactivity and 50% of the total radioactivity in fat at zero withdrawal time was Narasin. None could be detected after 3 days withdrawal using a microbiological assay (sensitivity 0.005 mg/kg).

   Narasin does not change the metabolic pathway of DNC although the metabolic rate seems to be changed. Residues are higher by 25, 50, 38, 41 and 35% in liver, muscle, kidney, fat and skin respectively which implies an effect on metabolic flux rates. 90 % of DNC disappears after 3 days and 98% after 5 days in all tissues. Residues after 5 days are less than 0.025 mg/kg DNC equivalents in muscle, fat and skin, 0,22 mg/kg in liver and 0.14 mg/kg in kidney. After 7 days liver residues amount to 0.06 mg/kg, kidney residues to 0.03 mg/kg. Unchanged DNC is the major residue (over 80%) in fat and skin but less in muscle as determined by HPLC (limit of detection 0.05 mg/kg). 60% of liver radioactivity at zero withdrawal time and 45% after 5 days are DNC. The corresponding kidney residues are 25% and 12% respectively.

   No data are available on the effect of Narasin on HDP residues. Although no direct data on the effect of Nicarbazin on Narasin metabolism are available, there is evidence that the residue pattern of Narasin is not modified.

   In chickens, raised with 125 mg/kg feed of Nicarbazin, a steady state residue level of 240-390 µg/kg liver and 8-10 µg/kg muscle is still found after 3-4 weeks withdrawal due to cross contamination from stable Nicarbazin residues in the litter. Despite the low tissue residues and the absence of significant differences there is a trend to increasing residue levels with time. Moreover the use of an average of 4 chickens as the experimental unit per time period makes statistical interpretation difficult. Comments are therefore required on this situation.

6. Narasin and nicarbazin have been individually assessed for their toxicity in the chicken, the rat and the mouse. These data are summarized in the Fourth Series of Reports of SCAN. The ADI then established for Narasin was 0.0038 mg/kg b.w. and

for Nicarbazin the ADI was 0.2O-O.24 mg/kg b.w. The ADI applies to the mixture of DNC + HDP. Separate ADIs were not established for DNC or HDP. Studies in chickens with various combinations showed that the NOEL was 62.5 + 62.5 mg/kg feed. Studies in laboratory animals involved acute toxicity, dermal and ocular toxicity but hypersensitivity and inhalation toxicity with the granular material were not tested. A 90-day study in rats, using various combinations, showed no treatment-related effects except for a slight reduction in body weight for the 60+60 combination.

Neither Narasin nor Nicarbazin have shown any carcinogenic potential in long-term studies. Extensive mutagenicity studies have shown no evidence of genotoxicity. Since the tissue residues were not qualitatively different from those arising from use of the single compounds, long-term studies o n the combination are not considered necessary.

A teratogenicity study in rats showed no teratogenic potential, the no-effect-level with respect to maternal and foetal toxicity being 0.75+0.75 mg/kg b.w.

Bioavailability was not studied because of the very low residues.

The Committee considered that the use of the combination did not present any toxicological risk for the consumer or the user.

7. The only data available on the substance and metabolite levels in chicken excreta concern the two constituents separately. Excreta contain primarily parent DNC and small amounts of 3 metabolites. At the recommended dosage of 50 mg/kg feed Nicarbazin about 40 mg/kg of drug related material in the excreta could be estimated, of which about 28 mg/kg excreta is DNC equivalents and 12 mg/kg HDP equivalents. Almost 99% of Narasin appears in the excreta, hence a 50 mg/kg feed dosage would result in 46 mg/kg excreta of which 30% or 14 mg/kg excreta is parent narasin, while 20% or 9 mg/kg excreta are hydroxylated metabolites. The fate of the remaining 50% is not known. The antibiotic activity of the excreted products is however low. There are no data on the biodegradation of Nicarbazin or its metabolites in chicken manure. Narasin does not degrade in chicken manure to any significant extent even after 18 months storage.

The DNC component of Nicarbazin is very stable in soil with a half-life of about 49 weeks. The degradation products have not been determined. There is no leaching from treated soil as demonstrated by radioactivity measurements and absence of formation of $^{14}CO_2$. The behaviour of the water soluble HDP is not documented nor is there any information available on the degradation products of HDP.

Narasin degrades rapidly in soil, some 92% within 4 weeks, particularly in the presence of Nicarbazin. A similar kinetic profile is obtained with chicken litter containing Narasin. The degradation products of Narasin are unknown. Basic soils leach more Narasin but, as it is practically insoluble in water, there is unlikely to be any significant passage into surface waters.

The acute NOEL for the combination is 0.5 mg/l for *Daphnia magna*, the 48 h EC_{50} is 20.65 mg/l. The acute NOEL is 1.80 mg/l for the Bluegill and 0.16 mg/l for the Rainbow trout.

No drug-related phytotoxicity is noted when seeds of corn, soybean, wheat and tomato are fertilized with chicken excreta from birds fed the 50+50 combination. Levels over 16 mg/kg soil reduce growth and cause sublethal toxicity after 7 days in

Lumbricus terrestris. Toxicity is almost entirely attributable to Narasin. Metabolites have not been specifically investigated.

Nitrification is not affected and no effects on methanogenesis are observed.

The Committee concluded that in view of the relatively low concentration of parent drugs and metabolites in chicken excreta, the low solubility of Narasin and the DNC component of Nicarbazin in water, the indirect evidence of lack of bioaccumulation of Narasin, and the absence of data on significant environmental toxicity, no adverse effects on the environment would be expected under ordinary conditions of use. Data on the fate of HDP and the unidentified metabolites of Narasin are desirable.

8. On the basis of the above data the Committee is of the opinion that the use of Narasin+Nicarbazin can be admitted without risks in the feedingstuff for chickens at the levels provisionally authorized of 50+50 mg/kg feedingstuff and with a withdrawal period of at least 5 days before slaughter. It would be desirable to obtain information on: the metabolic fate of HDP; on the effect of Narasin on HDP residues; on the unidentified metabolites of Narasin, on the biodegradation products of Narasin, Nicarbazin and their metabolites in chicken manure and the soil; on the fate of HDP in the soil; and on the effect of administration of the combination on Salmonella shedding.

REFERENCES:

- Dossier supplied by Eli Lilly
- Report of the Scientific Committee for Animal Nutrition on the use of Nicarbazin in feedingstuffs for poultry and narazin in feedingstuffs for chickens. Fourth Series (1984). Report EUR 8769. Catalogue N° CD-NK-83-010-EN-C. Opinion expressed : 14 April 1982; pp.41 and 51.
- Second report of the Scientific Committee for Animal Nutrition on the use of narazin in feedingstuffs for chickens. Fifth Series (1986). Report EUR 1041EN. Catalogue N° CD-NK-86-003-EN-C. Opinion expressed : 8th February 1984; p.4.
- Second report of the Scientific Committee for Animal Nutrition on the use of Nicarbazin in feedingstuffs for fattening chickens. Fifth Series (1986). Report EUR 1041EN. Catalogue N° CD-NK-86-003-EN-C. Opinion expressed : 5 July 1985; p.33.