Complementary report of the Scientific Committee for Animal Nutrition on Question 52 by the Commission on the of Narasin + Nicarbazin in feedingstuffs for chickens

(Opinion expressed on 7 July 1995)

TERMS OF REFERENCE (June 1991)

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:

- 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.

On its provisional report[4] of the Scientific Committee for Animal Nutrition on the use of Narasin + Nicarbazin in feedingstuffs for chickens of 10 July 1991, the SCAN concluded that on the basis of the data provided the use of Narasin+Nicarbazin could be admitted without risks in the feedingstuff for chickens at the levels provisionally authorised of 50+50 mg/kg feedingstuff and with a withdrawal period of at least 5 days before slaughter. In that occasion the Committee considered that it would be desirable to obtain information

- on the metabolic fate of HDP;
- the effect of Narasin on HDP residues;
- the unidentified metabolites of Narasin,
- the biodegradation products of Narasin, Nicarbazin and their metabolites in chicken manure and the soil;
- the fate of HDP in the soil; and
- the effect of administration of the combination on Salmonella shedding.

A complementary registration-file was submitted by the firm to the SCAN.

OPINION OF THE COMMITTEE

Narasin is a polyether ionophore antibiotic. Nicarbazin is an equimolecular "complex" of two synthetic chemicals, DNC (4,4-dinitrocarbanilide) and HDP (2-hydroxy-4,6-dimethylpyridine). The application was for 1:1 mixture of narasin + nicarbazin to be incorporated at 100 mg/kg in feed as a coccidiostat with a 5-day withdrawal period.

1 J.O. No. L183 (11.07.1984) p.15
2 J.O. No. L310 (5.11.1986) p.19
3 J.O. No. L270 (14.12.1970) p. 1
4 See report in 8th Series

Both agents are used singly for this purpose and are listed in Annex 1. The combination product was admitted to Annex 2 in July 1990.

The SCAN has issued a provisional opinion (July 1991) on the use of narasin + nicarbazin in feedingstuffs for chickens. It concluded that additional information was required on the metabolic fate of nicarbazin (HDP), the effect of narasin on the residual status of HDP, the further identification of narasin metabolites, the biodegradation of narasin, HDP and metabolites in chicken manure and soil, as well as on the effect of the association of both compounds on Salmonella shedding, was highly desirable before a final approval.

The examination by the Committee of a new set of data supplied by the applicant lead to the following comments and conclusions:

1. Additional information on the metabolic fate and residues of Narasin and the HDP moiety of Nicarbazin has been produced to improve data on HDP and to study the possible fate interaction of both compounds when administered together.

   Where Narasin is concerned, the parent compound was the major one in the excreta (3.0-3.7%) and fat (56-61%). None of the metabolites represented more than 5% of the total radioactivity excreted or present in the tissues. Using an improved methodological approach when compared to the former studies, a metabolic profile was obtained in the excreta and 15 metabolites were separated and identified as methyl-narasin, tetra-, tri- and di-hydroxy-narasins, as well as di- and tri-hydroxy derivatives of narasin B (di-dehydro-narasin). However it was not clearly established if the 50% unaccounted for radioactivity was distributed between a great number of very minor compounds, as claimed by the applicant.

   A relationship was established between the 7 metabolites isolated formerly and those separated and identified in the present study. Technical difficulties made the identification of liver metabolites unsuccessful. However the metabolic profile was roughly similar to that in the excreta. On the whole, the simultaneous administration of Nicarbazin did not change the metabolic pathways and residue status of Narasin.

   The metabolic fate of C^{14}-Narasin in the rat revealed tri- and di-hydroxy metabolites derived from Narasin and Narasin B, i.e. a very similar pattern to that encountered in the chicken.

   The metabolic fate of C^{14}-HDP was studied separately in chickens dosed for 6 consecutive days with a double dose (125 ppm). Most of the radioactivity excreted (85%) was identified unambiguously as HDP. The residual concentrations found at zero withdrawal time were in decreasing order: in the kidneys, liver, muscle, skin and fat. Most of the radioactivity in the kidneys and liver (89 and 84% respectively) was unchanged HDP based on comparative chromatographic behaviour. In a second study C^{14}-HDP (50 ppm) was administered with or without Narasin (50 ppm) in the diet of chickens for 5 consecutive days. It was confirmed that HDP was

the main compound in the excreta. It represented 71, 67 and 84% of the total extractable (90%) residues in the tissues with the highest concentrations, i.e. the kidneys (0.73-0.83 ppm), liver (0.44-0.47 ppm) and muscle (0.36-0.38 ppm), at zero withdrawal time.

The rest of the radioactivity extracted was distributed among different compounds each one representing less than 5% this precluded any further identification. No difference was observed in the distribution of the radioactivity when narasin was administered or not. No additional data are produced, but the fact that the requested dosage is decreased from 125 to 50 ppm is expected to reduce these residue figures which were already within the acceptable limits for human consumption.

2. The question of the biodegradation of narasin, nicarbazin and metabolites in manure and soil remains unanswered. The applicant claims the unpracticability of this study due to technical difficulties in identifying minor components derived from the original compounds or metabolites, in such complex media. In fact, the new data on Narasin identify the parent compound (3-5%) plus 15 metabolites, none of which representing more than 5% of the excreted material, and it is claimed that the 50% unaccounted for radioactivity is distributed between many very minor compounds. Moreover the co-administration of nicarbazin did not change the metabolic pathways. Where HDP is concerned the metabolic study indicates that metabolites represent only 15% of the excreted compounds. The relevance of additional efforts in identifying these minor compounds and their biodegradation products is very questionable when considering the impractability of establishing their ecotoxicological significance. The only point to be considered and for which no data was supplied is the biodegradation of HDP, this compound being the major one excreted in the droppings.

3. A study on salmonella shedding was performed on three groups of 8 chickens. Groups 1 and 2 were challenged with nalidixic acid resistant *S. Tiphymurium* and fed with and without a narasin containing diet (80 ppm) respectively. Group 3 was not challenged and not fed narasin. This group served as an environmental control of cross-infection during the study.

   None of the chickens shed salmonella before the challenge. None of the birds showed any signs of clinical salmonellosis. Up to day 12 post challenge the challenged control birds excreted salmonella. After day 14 the excretion became more scarce, and on day 56 only one from the control group was still excreting. The last excreting from the group that received narasin was on day 49 when one bird excreted salmonella.

   At the end of the experiment (day 56) no salmonella bacteria were isolated from liver and spleen, while the caecal contents were positive from 37.5% of control group (3 birds) and 25% of the Narasin group (2 birds). The colon contents were positive in 12.5% of both groups (1 bird of each group). No statistically significant differences were observed. Therefore narasin does not prolong the salmonella shedding in chickens.

4. Additional studies not requested by SCAN have been produced that concern skin sensitisation and mutagenicity. The study concerning MIC values of narasin for Staphylococcus and Streptococcus species isolated from poultry have not addressed the issue of the former SCAN opinion expressed in 1990 which stated that the normally susceptible Staphylococci were found to be unexpectedly resistant to the action of narasin in the presence of nicarbazin.

   In the modified Buehler sensitisation study narasin + nicarbazin caused slight dermal irritation (erythema and edema) but no sensitisation. In an in vitro chromosome aberration test in Chinese hamster ovary cells Narasin + Nicarbazin was negative at concentrations of 10, 25 or 50 ìg/ml with and without metabolic activation.

5. With respect to the new questions and answers it can be concluded that no appreciable interaction occurs between the compounds when given as a mixture, whether it is metabolism, residue status or salmonella shedding which are considered. Moreover the complementary data on the metabolic fate of HDP and the improved identification of narasin metabolites illuminate a weak part of the original application without arising new points of concern.

   Therefore the Committee is able to confirm its former conclusions that narasin + nicarbazin may be used in feedingstuffs for chickens under the proposed conditions without causing any harm to the human consumer.

   However, with reference to the previous opinion of the SCAN[5] certain insufficiencies remain, namely the lack of data on the fate of HDP in the manure and soil.

5 See 8th Series of reports (1992) page 48