EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

Report of the Scientific Committee on Animal Nutrition

on the use of Semduramicin sodium in feedingstuffs for Chickens for fattening

Adopted on 17 April 2002

1. TERMS OF REFERENCE (JULY 1995)

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

1.1. Does the use of semduramicin sodium ($C_{45}H_{76}O_{16}$ Na, a monocarboxylic acid polyether ionophore produced by fermentation *of Actinomadura roseorufa* Huanh the active substance being "23,27-didemethoxy-2,6,22-tridemethyl-5,11-di-O-demethyl-6-methoxy-22-[(tetrahydro-5-methoxy-6-methyl-2H-pyran-2-yl)oxy]ionomycin A sodium salt", under the conditions proposed for its use as an additive for the feedingstuffs for chickens for fattening significantly prevent coccidiosis in this animal species?

1.2. Is this use safe to the chickens for fattening?

1.3. Can it be monitored in animal feedingstuffs?

1.4. Can it result in development of resistance in bacteria to prophylactic or therapeutic preparations, or exert an effect on the persistence of Gram negative bacteria in the intestinal tract of chickens for fattening?

1.5. What is the metabolic fate of Semduramicin sodium in chickens for fattening? Does this use result in the presence of residues in meat? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

1.6. Do the toxicology studies allow the conclusion that the proposed use does not present risks for the consumer or for the user?

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

2. BACKGROUND

In accordance with the provisions of the Article 3A of Council Directive 70/524/EEC[1], a coccidiostat additive shall be given permanent authorisation only if:

(a) It improves animal production;

(b) At the level permitted in feedingstuffs, it does not adversely affect human or animal health or the environment, nor harm the consumer by altering the characteristics of livestock products;

(c) Its presence in feedingstuffs can be controlled;

(d) (Note: Does not apply to coccidiostat)

(e) For serious reasons concerning human or animal health its use must not be restricted to medical or veterinary purposes.

A new additive may be authorised provisionally only if the conditions stipulated under (b), (c) and (e) are satisfied and if it is reasonable to assume that (a) is also satisfied. The provisional authorisation must not exceed four years.

It has been requested to authorise semduramicin sodium belonging to the group of D (Coccidiostats and other medicinal substances) according to Directive 70/524/EEC according to the following conditions

Species or category of animal	Minimum content	Maximum content	Other provisions
	mg/kg of complete feedingstuff		
Semduramicin Sodium Chickens for fattening	20	25	Use prohibited at least 5 days before slaughter.

Dossiers supporting this request have been provided by the company Pfizer France S.A.

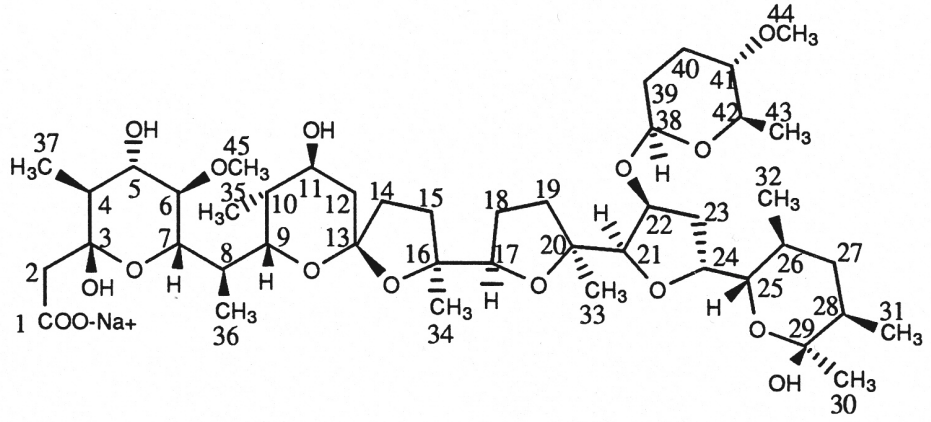
[1] Concerning additives in animal feedingstuffs (OJ No L270, 14/12/70 p.1.) as amended by Council Directive 84/587/EEC (OJ No L319, 08/12/84, p.13.) and last amended by Directive 93/114/EC (OJ No L334 31/12/93, p. 24).

3. INTRODUCTION

3.1. Description of semduramicin sodium

Semduramicin sodium is a monocarboxylic acid polyether ionophore produced by fermentation with a selected strain of *Actinomadura roseorufa* Huang sp. rev. The fermentation process utilises commonly employed fermentation and recovery procedures. The molecular formula of semduramicin sodium is $C_{45}H_{75}O_{16}Na$ and the molecular weight 894.5.

Structural formula



semduramicin sodium

3.2. Physico-chemical characteristics of semduramicin

Physical characteristics: It is a white/off white powder with a particle size of 5-34 µm. Semduramicin is not hygroscopic, there is no evidence of a hydrated crystalline form of the drug

Identification: by thin layer chromatography and infra red spectroscopy

Assay for quantitative measurement: High Performance Liquid Chromatography, potentiometric titrimetry

Sodium content: 2.3-2.8 %

The table below summarises all analytical data for 37 batches of semduramicin. These data indicate that the amounts of impurities found in batches have been relatively low.

Table 1 Summary of impurities found

Known and unknown fermentation products

Individual:	ND[2] - 2.0 %
Total:	0.2 - 3.4 %

Residual solvents

– Methanol	ND² - <0.1 %
– Ethyl acetate	<0.1 - 0.3 %
– Heptane	<0.1 – 0.2 %
– Hexane	ND² - <0.1 %
– Isopropyl ether	<0.1 %
– Total solvents (loss on drying; includes water)	0.1 – 2.0 %
Heavy metals	all <0.004 %

A number of impurity peaks have been observed in the chromatograms of bulk lots of semduramicin sodium (see table below).

Table 2 Summary of known and unknown fermentation products found

RRT[3]	Assignment	**Range % found (37 lots)**
0.9	Descarboxyl semduramicin and/or unknown	ND² - 0.9
1.2	Epimer and /or desmethoxy semduramicin	ND² - 1.6
1.5	Hydroxy-semduramicin	ND² - 2.0
1.7	Unknown	ND² - 0.2
1.9-2.2	Aglycone	ND² - 0.3
2.3	„g“ and „a“ ring desmethyl-semduramicin	ND² - 0.8
2.5	Unknown	ND² - 0.1
2.8	Unknown	ND² - 0.2

3.3. Stability

The stability of bulk semduramicin sodium has been evaluated under a range of environmental conditions including elevated temperature and relative humidity and in the presence of light. In addition, the bulk drug was evaluated under severe conditions to determine potential pathways of degradation. The stability studies showed that there was no loss of potency under any of the conditions tested – up to 75 % relative humidity for 12 weeks, 50 ˚C for 12 weeks and in the light cabinet for 12 weeks. Studies at 37˚C have been completed for 12 months and 30˚C studies have been continued for 36 months. The degradation studies showed that semduramicin is susceptible to acid, but not to alkaline degradation.

Feed pelleting at 90°C resulted in a loss of 5% of semduramicin sodium.

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[2] Not detected, <0.1 %

[3] Relative Retention Time, relative to semduramicin sodium

4. OPINION OF THE COMMITTEE

4.1. Does the use of semduramicin sodium, as defined in 1.1, significatively prevent coccidiosis in chickens for fattening?

Efficacy studies were directed towards demonstrating control of coccidiosis and tolerance in the target species over the life span. The testing procedure consisted of

(1) Preclinical range finding investigations

(2) Rationale for recommended dose range

(3) Efficacy studies in batteries

(4) Efficacy confirmation in floorpen studies

(5) Commercial field studies

(6) Studies on the development of resistance

(7) A comparative efficacy study of pelleted vs meal rations

4.1.1. Range finding studies

Initial explanatory range findings studies were carried out using "Mean Caecal Lesion Score" for efficacy and body weight gain depression for tolerance.

It was concluded that an inclusion level of Semduramicin that would be both efficacious and tolerated would be greater than 15 ppm and less than 30 ppm.

4.1.2. Rationale for the recommended dose range

Two efficacy dose titration studies were conducted in batteries using mixed species infections.

The dose levels in both studies were 20, 25 and 30 ppm and were designed to test the proposed dose range in terms of the two key parameters of anticoccidial efficacy and tolerance when measured together, and in particular, to determine whether overall benefit could accrue above the proposed maximum dose level of 25 ppm (at 30 ppm).

Assessed on the basis of lesion control and bird performance, feed inclusion levels of 20 and 25 ppm Semduramicin were equally effective in the control of coccidiosis caused by infection with the six common *Eimeria* species. Increasing the inclusion level to 30 ppm, though improving lesion control, compromised the birds' performance. It was concluded from these data that Semduramicin provides optimum anticoccidial control within the dose range 20 to 25 ppm in feed.

4.1.3. Efficacy studies in batteries

The purpose of these studies was to define the activity of semduramicin against the six major *Eimeria* species *(E. acervulina, E. maxima, E. tenella, E. brunetti, E. necatrix and E. mitis)*, both singly and in combination. Up to ten strains of each species were tested, all of recent field origin from the US and Europe. Standard battery tests with 8-10 day old chickens were used, the parameters for efficacy being weight gain, mortality and intestinal lesion scores.

Based on the results of the above mentioned studies, two series of studies were carried out at 20 and 25 ppm to study the efficacy of semduramicin. The studies contained various control groups, including medication with authorised ionophore coccidiostats (monensin (100 ppm), salinomycin (60 ppm) and maduramycin (5 ppm)).

Eleven battery studies were conducted to evaluate the efficacy of 20 ppm semduramicin against infections with 39 isolates of the six major pathogenic species of *Eimeria*. At the 25 ppm inclusion level, 30 studies were also conducted against isolates of mixed *Eimeria* species.

The Company data indicated that the anticoccidial efficacy of semduramicin at 25 ppm was significant and was similar to the efficacy of the other three ionophores coccidiostats at their recommended level of use. However, at 20 ppm inclusion level, the anticoccidial efficacy of Semduramicin was only comparable with those of monensin. The reduction of weight gain, a common side effect of the use of coccidiostats, compared with non-infected non-treated group was significant in groups with *E. acervulina* and *E. tenella* infection. Monensin reduced the weight gain significantly more than Semduramicin, in case of *E. acervulina, E. tenella* and *E. necatrix* $P \leq 0.05$).

4.1.4. Efficacy confirmation in floorpen studies

Studies were conducted to evaluate the efficacy of 20 ppm (five studies) and 25 ppm (ten studies) semduramicin in feed against controlled induced infections with mixed field isolates of *Eimeria* spp. during a 49 day period.

In studies with 25 ppm semduramicin, weight gain and intestinal lesion scores were significantly improved and mortality significantly lowered in treated chickens compared with infected, non-treated chickens. In studies with 20 ppm semduramicin in treated chickens compared with infected, non-treated chickens, these differences were not significant. ($P<0.05$).

In general, there were no significant differences between groups treated with semduramicin (25 ppm) and the positive controls listed under *4.1.3*.

4.1.5. Commercial field studies

A total of 11 studies with more than 151,000 chickens were conducted, four of which evaluated 20 ppm and seven 25 ppm semduramicin in feed, in different EU Member States. Treatment was from one day of age to between 30 and 54 days of age, followed by a withdrawal period of between five and ten days (or the mandatory withdrawal period for the control coccidiostats).

Chickens were monitored for mortality, clinical signs of coccidiosis and for clinical and *post mortem* signs of adverse drug effects. *Post mortem* examinations were made on mortalities including routinely culled chickens. There was no clinico-pathological evidence of coccidiosis nor other adverse clinical signs in the chickens given semduramicin.

In two studies with high stocking densities, the litter showed some areas of moistness for chickens given either an approved anticoccidial drug or semduramicin. In one study, the litter of chickens given semduramicin was slightly stickier and the birds slightly dirtier. However, there were no signs of diarrhoea. Furthermore, in the other eight studies, the litter of chickens given either 20 or 25 ppm semduramicin was normal.

These field studies show that the performance of broiler chickens given diets supplemented with semduramicin at either 20 or 25 ppm was similar to that of chickens given contemporary, approved anticoccidial drugs when assessed in terms of survival, total and mean live weights at slaughter, and feed conversion.

4.1.6. Studies on the development of resistance in Eimeria

A series of studies was conducted to evaluate the potential for resistance development by serially passaging a laboratory strain of *Eimeria tenella* through twelve consecutive passages in broiler chickens housed in batteries, either in the presence or absence of semduramicin.

The pathogenicity of both the semduramicin-exposed line and the unexposed line remained stable trough the entire series as indicated by the absence of any significant differences in weight gain or lesion score between groups of infected non-treated birds. Irrespective of the number of passages semduramicin treated birds had significantly ($P \leq 0.05$) greater weight gains and significantly ($P \leq 0.05$) lower lesion scores than corresponding infected non-treated birds but there were no significant differences between treated groups. Semduramicin prevented mortality caused by either of the two lines.

4.1.7. A comparative efficacy study of pelleted vs meal rations

In a study to compare the efficacy of Semduramicin in meal and pelleted rations, three groups received pelleted feed and three

received meal feed. For each feed type, three different treatments were offered. Infected birds had been inoculated with a pathogenic dose of *E. tenella.* Weight gains, lesion scores and mortality of birds receiving Semduramicin in meal or pelleted feed were not significantly different from each other and not significantly different from their corresponding non-infected controls.

4.1.8. Conclusion

Considering the above, it can be concluded that the efficacy of Semduramicin in terms of prevention of coccidiosis is demonstrated at the levels of 25 ppm, under experimental and field conditions. At the level of 20 ppm, although favourable results have been obtained, the efficacy of Semduramicin is not always significantly demonstrated. Additional studies to support the lower level would be needed.

There is no evidence of development of resistance, using *Eimeria tenella* as test strain.

No loss of activity was noted after feed pelleting.

4.2. Is this use safe to the chickens for fattening?

Three series of studies have been carried out to investigate the safety of Semduramicin in broiler chickens: exploratory safety margin study, safety margin floor-pen studies and tolerance studies within the recommended dose range.

In the exploratory safety margin study levels of 20, 25, 30 and 35 ppm semduramicin were fed continuously for 21 days to non-infected birds under battery conditions. Non-treated and 60 ppm salinomycin treated groups were used as negative and positive controls, respectively. No increased mortality was observed in the treated groups. Compared to the untreated group, there were no statistically significant differences in feed consumption amongst treatments except for 35 ppm semduramicin group in which consumption was significantly reduced. At the levels of 30 ppm and 35 ppm, the body weight gain was significantly reduced, being -10.5% and -13% respectively compared to the non-treated group. There were no statistically significant differences in feed efficiency between treatments.

In a safety margin floor pen study birds were fed concentrations of Semduramicin of 25, 50 and 75 ppm for 49 days. A few birds receiving either 50 or 75 ppm Semduramicin exhibited juvenile or thin feathering and decreased haemoglobin values. The 49 day body weight gains of birds receiving 50 or 75 ppm were significantly reduced (19 and 46%).

On the basis of both studies performance data, the maximum recommended dose of semduramicin in the complete feeding stuffs of broiler chickens should be 25 ppm, but it must be recognised that at this level of inclusion there is only a narrow margin of safety for the bird.

Compatibility of Semduramicin with tiamulin

It is recognised that in general ionophore polyethers are non compatible with concurrent tiamulin. Indeed, concurrent medication of birds receiving 25 ppm semduramicin in the feed with tiamulin in water (250 ppm) for three days resulted in a modification of performance data *i.e.* depression on body weight gain and deterioration on feed efficiency. Other relevant data as haematology, clinical chemistry, gross pathology and histopathology from treated animals with this combination were not available.

Conclusion

Semduramicin was well tolerated by broiler chickens at the maximum dose level of 25 ppm.

Considering the significant reduction of body weight gain and feed consumption in birds receiving from 30 ppm semduramicin onwards, the margin of safety is very narrow. Therefore careful attention should be given to respect the level of 25 ppm.

Semduramicin in the feed is not compatible with the concurrent use of tiamulin.

4.3. Can it be monitored in animal feedingstuffs?

A method is described for the assay and identity of semduramicin sodium in feeds. The compound is extracted from the feed using an organic solvent mixture then purified using solid phase extraction. It is analysed by high performance liquid chromatography (HPLC) and post-column chemical derivatization with vanillin to produce a chromophore whose concentration is then measured by absorption. The reaction is highly selective for ionophore monocarboxylic acids like semduramicin, salinomycin, maduramycin, narasin or monensin, with no appreciable interference from the feed matrix. The chromatographic separation resolves these five ionophores plus lasalocid (dicarboxylic acid). The linearity of the response in the concentration range compatible with the 20-30 ppm level in feedstufs and recovery from spiked samples are documented and satisfactory.

4.4. Can it result in development of resistance in bacteria to prophylactic or therapeutic preparations, or exert an effect on the persistence of Gram negative bacteria in the intestinal tract of chickens for fattening?

4.4.1. Resistance to Semduramicin

Minimum Inhibitory Concentrations (MICs) data demonstrated high MICs of semduramicin, generally >100 mg/l, against aerobic and anaerobic representative Gram-negative bacteria, including *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella* spp. The Gram-negative aerobic and anaerobic bacteria were not affected by semduramicin. These results confirm the resistance of a broad range of Gram-negative bacterial species to ionophores.

For some Gram-positive bacterial species, *Lactobacillus* spp. (two strains) and *Staphylococcus aureus* (three strains), high MICs (>76 to 100 mg/l) were observed. *Clostridium perfringens* (10 mg/l), *C. difficile* (28 mg/l), *Eubacterium limosum* (22 mg/l) and Groups C and E *Streptococci* (16 mg/l) were more susceptible.

Additional data regarding MICs of strains of *Campylobacter jejuni*, *Yersinia enterocolitica*, *Bifidobacterium* spp., *Clostridium* spp. (*including C. perfringens*), *Enterococcus faecium* and *Lactobacillus* spp. were provided. Ten isolates of human origin and ten isolates of avian origin were tested for each bacterial species, excepted for *Yersinia enterocolitica* for which only isolates of human origin were tested because this species is not normally found in poultry.

The strains were obtained from human and veterinary laboratories in Belgium, France and the United Kingdom. Species identification of all bacterial isolates was confirmed before testing for MICs. MIC determinations were conducted according to the NCCLS guidelines. The confirm the resistance pattern for the Gram-negative bacterial species, *Campylobacter jejuni* and *Yersinia enterocolitica*, with high resistance against ionophores with MICs > 128 mg/l for all isolates. The distributions of the MICs of the Gram-positive bacteria tested (4 to 16 mg/l for *Bifidobacterium* spp., 2 to 16 mg/l for *Clostridium* spp. including *C. perfringens*, 4 to 32 mg/l for *Enterococcus faecium* and 2 to 8 mg/l for *Lactobacillus* spp.) were very similar to those of the first group of human and avian isolates studied. These results confirm a weak antibacterial activity of semduramicin against some numerically important gut Gram-positive bacterial species; a property shared with other molecules of the ionophore class of coccidiostats.

4.4.2. Development of cross resistance

In a first experiment to study the incidence of antimicrobial resistance of indigenous faecal coliforms two groups of chicks, one non-treated group and one treated at the recommended dose (25 ppm) were constituted and reared from one day old to 56 days. After an adjustment period of two weeks (day 1 to day 14) without treatment, the groups of 12 chicks were reared individually in cages. Before and during the treatment period faecal samples were collected (weekly during treatment) for coliform isolation and antimicrobial susceptibility testing. The MICs were determined against 12 antibiotics representative of the families effective against Gram-negative bacteria (β-lactams, aminoglycosides, chloramphenicol, tetracyclines, quinolones, sulphonamids, trimethoprim) and classified as sensitive or resistant according to the NCCLS.

Resistance to kanamycin and tetracycline was present in: the non-treated and treated group, (5.7% and 4.8% of isolates respectively) prior to the addition of semduramicin to the treated group. The prevalence of resistance remained similar in both groups throughout the experiment.. No resistance was encountered at any time to the

aminoglycosides (gentamicin, amikacin), chloramphenicol or quinolones (nalidixic acid). For other antibiotics no statistically significant differences of the overall study period data were observed between the non-treated and treated groups.

A second study was conducted for seven weeks with a total of approximately 1000 broiler chickens housed in good sanitary conditions. Approximately 200 birds (10 pens each with 20 chickens) were used in each of five treatments, which included semduramicin at either 20 or at 25 ppm (with a withdrawal period from day 44 to day 48) and a control group. Day-old chickens were placed in floor pens and each bird was inoculated with a well characterised strain of *Enterococcus faecium*. On day 10, each bird was inoculated with well characterised strains of *Salmonella typhimurium* DT104 and *Campylobacter jejuni.* Bulk faecal samples were collected on days 13 and 48 and examined for microorganisms able to grow in the presence of selected antimicrobials included in the culture medium at a concentration equivalent to four times the MIC determined for each inoculated strain before inoculation.

For *S. typhimurium*, the antibiotics included were amoxycillin, amoxycillin/clavulanic acid, cefotaxim, gentamicin, tetracycline, nalidixic acid, ciprofloxacin, sulphonamide/trimethoprim and for Gram-positive bacteria, amoxycillin, amoxycillin/clavulanic acid, gentamicin, erythromycin, vancomycin, pristinamycin, teicoplanin, tetracycline, ciprofloxacin and sulphonamide/trimethoprime.

The only resistant bacteria isolated from faecal samples (one sample per pen) at day 48 were *S. typhimurium* showing growth in the presence of gentamicin (14/29 samples) and tetracycline (1/29 samples). For gentamicin resistant isolates were found in 5/10 samples from non-supplemented birds and 9/19 birds trearted with semduramicin. There were no significant differences in in antimicrobial sensitivity between the groups of birds. In the same way, very little modification in the sensitivity of *E. faecium* to erythromycin, pristinamycin, tetracycline and sulphonamides / trimethoprime occurred and there were no significant differences found between the control and the supplemented groups.

Conclusion

The results of these two studies demonstrate that the feeding of chickens for around six weeks with a feed containing 20 or 25 ppm of semduramicin had no effect on the development of resistance to antibiotics used in human and animal medicine in faecal coliforms or in introduced strains of *S. typhimurium*, *E. faecium* and *Campylobacter jejuni.*

4.4.3. Salmonella shedding

Three studies evaluated the effects of semduramicin on salmonella shedding. In the first study chickens received various treatments,

including one group given 25 ppm semduramicin. Challenged birds received by gavage 10^7 cfu of a chicken isolate of *Salmonella typhimurium* resistant to nalidixic acid, semduramicin and monensin. Salmonella shedding for all groups was monitored weekly during the 63 days treatment period. For eight days after the challenge, all birds of the challenged groups excreted salmonellae. From day 10 to the end (day 63) of the experiment more birds excreted salmonellae in the treated group than the non-treated group. Thus at day 63, 7/11 birds excreted salmonellae in the challenged non-treated group, 10/11 in the semduramicin treated group and 11/12 in the control treated group.

The severity of infection in this study explains the excretion of *S. typhimurium* by the majority of the birds in all treatment groups. Both semduramicin and control medication seem to increase salmonella shedding throughout the study but the small number of birds in each group do not allow this numerical difference to reach statistical significance.

A second study was conducted in individual floor pens more representative of practical conditions with a similar protocol but without the control treated group. After an adjustment period the birds in the challenge groups were inoculated at 10 days of agewith 10^9 cfu of the same *S. typhimurium* strain used in the first experiment. After a 5-day postchallenge interval, challenged birds were assigned to be fed either the treated or non-treated diet for the following 37 days. Individual faecal specimens were tested weekly for *Salmonella*. During the pretreatment period all of the birds of the two groups excreted salmonellae in similar quantities. During the treatment period the prevalence of *Salmonella* shedding decreased with time in both groups. Between day 10 and day 28 of the treatment period, the prevalence was lower in the non-treated group (33.3% to 8.3% non-treated at day 10, 75% to 33.3% non-treated group at day 28). At the end of the study (day 35), four birds in both groups excreted *Salmonella*. After necropsy of all birds, salmonellae were recovered from 2/12 caecal contents (16.7% positive) in the non-treated group and from 6/12 (50% positive) in the treated group.

Considering a bird as *Salmonella*-positive if it was either shedding *Salmonella* or has a positive caecal culture, 5/12 non-treated birds and 7/12 semduramicin-treated birds were *Salmonella* positive on day 35. These proportions are not significantly different. In this second study the rate of excretion of *Salmonella* after experimental inoculation again seems to be higher in birds receiving semduramicin but the number of birds studied are to small for a statistical validation of the results.

A third study was conducted for seven weeks with a total of approximately 1000 broiler chickens housed in good sanitary conditions. Approximately 200 birds (10 pens each with 20 chickens) were used in each of five treatments, which included semduramicin at

either 20 ppm or at 25 ppm (with a withdrawal period from day 44 to day 48) and a control group. The chicks in the three challenge groups were inoculated with 10^7 bacteria via oral gavage of strain of *S. typhimurium* DT104 on day 10. On days 13, 20, 29, 43 and 48, individual swabs were taken from each bird and the prevalence of excretion was determined. On days 13, 43 and 48, bulk faecal samples were taken from each pen and the number of *Salmonella* per gram of faeces was determined. At the end of the study, caecal samples, liver samples and spleen samples from each bird were also examined for the presence of *Salmonella.*

At day 48, the prevalence of *Salmonella* shedding was very similar in the three groups of birds: 83.9% of excretion for the chickens non-supplemented, 84.4% and 91.6% for the chickens supplemented with 20 and 25 ppm of semduramicin respectively. The number of *Salmonella* excreted was also similar in the three groups: between 10^8 and 6.5 x 10^8/gram of faeces. No differences were observed between the three groups in the frequency of infection of liver (15.2 to 18.8%), spleen (5.1 to 5.6%) and caeca (98.9 to 100%).

Conclusion

The first two studies were considered only as possibly indicative and to carry far less weight than the third study because of the limited number of animals tested.

The third study, conducted on approximately 600 broiler chickens, demonstrated that semduramicin does not significantly increase *Salmonella* shedding or the risk of internal organs being colonised by the bacterium.

4.5. What is the metabolic fate of semduramicin in chickens for fattening? Does this use result in the presence of residues in meat? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

A preliminary study was designed to establish the semduramicin plus metabolite levels in plasma, bile and excreta of chickens following a seven day oral administration of the ^{14}C- uniformally labelled compound at the dose corresponding to the intended use *i.e.* 25 ppm in feedstuffs. Total radioactivity measured in plasma at slaughter following a six hour withdrawal period was very low (0.025 μg/ml equivalent semduramicin) and decreased as the withdrawal period increased. The absence of a separate measurement of urine and faeces radioactivity and of bile excretion does not allow the absorption rate of semduramicin to be established. Total radioactivity measured in bile only at slaughter following a six hour withdrawal showed very significant (23.6 μg/ml) levels which indicate that semduramicin would be absorbed to a significant extent, as are similar ionophore antibiotics (SCAN, 1984). The only data concerning excreta indicate an average concentration of 23.3 mg/kg equivalent semduramicin between days 4 and 7.

A comparative metabolism study of semduramicin in the chicken, rat and dog has been carried out following a five day oral administration of the ^{14}C-labelled compound at the dose intended for use in the chicken, i.e. 25 ppm in feedstuffs or 2.3mg/kg bw, and 1mg/kg bw for the rats and dogs. Excreta, bile and liver were examined for total radioactivity and identification of the metabolites. Semduramicin is extensively metabolised with the production of 19 more polar metabolites. The three main metabolites have been identified as:

Metabolite E - O-desmethyl (G-ring) semduramicin
Metabolite F - O-desmethyl (A-ring) semduramicin
Metabolite C - a compound corresponding to the F-ring opening of metabolite F.

In chicken excreta, the distribution of the extractable radioactivity (about 50%) is as follows: 23.8% metabolite F, 16.2% Semduramicin and a great number of metabolites for a total of less than 10%. Metabolite F is the major compound excreted through the bile. Unchanged Semduramicin was by far (45%) the main residue in the liver after 6-hour withdrawal, each of the other numerous (19) metabolites representing less than 10% of the total radioactivity. Therefore Semduramicin is the marker residue. The metabolic fate of Semduramicin was qualitatively very similar in the rat and dog. Quantitatively, the unchanged compound was the main liver residue in the rat while metabolite F was the main one in dog liver. The residual levels in both species were much higher than those measured in chicken (factors of about 5 and 15 respectively for similar levels of administration).

Two studies were undertaken with chickens that received a 25 ppm ^{14}C-semduramicin (in a crystalline form) supplemented diet for 7 and 11 days respectively, and were slaughtered after 6, 12, 24, 48 and 120 hours withdrawal. Table 3 indicates the total residue figures in the different tissues and organs during the withdrawal period. Both studies showed that the highest residue levels were found in the liver, the total residues being about four times higher than in the fat, the next highest concentration. Therefore the liver is the target tissue. A fast and biphasic decline of the radioactivity occurred. Bound residues accounted for 9, 20, 32, 39 and 45% respectively; their bioavailability was not established.

Table 3 Total residues of ^{14}C-semduramicin in chicken tissues fed a 25 ppm supplemented feed for 7 days (expressed as mg equivalent semduramicin / kg tissue, mean value n= 6)

Time (hrs)	Liver	Kidneys	Muscle	Fat	Skin (+ Fat)
6	.273	.051	.015	.074	.057
12	.112	.027	.007	.027	.022
24	.058	.012	.003	.015	.015
48	.031	.006	.002	.011	.011
120	.019	.004	.001	.010	.009

An additional study was carried out under field conditions where 25 mg/kg unlabelled semduramicin (in crystalline form) was fed to chickens for a 44-day period; (another study involved the use of semduramicin in a crude mycelial form which is not relevant and is therefore not evaluated here). Semduramicin was determined only in the liver of the animals slaughtered after 6, 12, 18, 24, 36 and 48 hours withdrawal of the supplemented feeds using a sensitive (10µg/kg limit of detection) and validated HPLC method (Lynch *et al.*, 1992). The residue data are summarised on Table 4 and compared to the results of the total residue studies reported below. The results confirm that unchanged semduramicin is the marker metabolite.

Table 4 Total residues and unchanged semduramicin residues depletion in the liver of chickens fed 25 ppm semduramicin supplemented feeds (expressed as mg semduramicin equivalent /kg tissue)

Withdrawal time (hrs)	Total residues (I)	Unchanged semduramicin residues	
		(I)	(II)
6	.273	.139	.187
12	.112	.049	.051
18	-	-	.049
24	.058	.022	.022
48	.031	.012	< .010

(I) ^{14}C-semduramicin administered for 7 days, mean value (n=6) (same study as in table 3)
(II) crystalline semduramicin administered for 44 days, mean value (n=3)

As the metabolic pathways and resulting metabolites are very similar in the rat and chickens, toxicity studies in rat are considered appropriate for risk assessment for humans consuming chicken meat. Due to the lack of data on semduramicin residues (unchanged molecule and/or metabolites) in the tissues other than liver, a worse-case scenario would consist in taking into account the total residues as in Table 3 for the calculation of the contribution to the ADI of the consumption of these tissues.

4.6. Do the toxicology studies allow to conclude that the proposed use does not present risks for the consumer or for the user?

A number of studies relevant to the safety of the consumer and user have been reported.

4.6.1. Genotoxicity studies

Various *in vitro* genotoxicity studies were performed. Ames/*S. typhimurium* tests with semduramicin and urine of semduramicin-treated mice, with and without metabolic activation, respectively, were both negative, although no toxicity was seen. Also the gene mutation test using mouse lymphoma cells, (with and without metabolic activation), an *in vitro* test for unscheduled DNA synthesis (UDS) using rat hepatocytes and a test for chromosome aberrations using human lymphocytes (with and without metabolic activation) were all negative. *In vivo* genotoxicity was tested using CD-1 mice

exposed orally to 6 mg/kg bw and no chromosome aberrations were found in bone-marrow cells. All of these tests produced consistently negative results and therefore the compound is considered to be non-genotoxic.

4.6.2. Toxicity studies

Laboratory animal studies for toxicity were carried out in various species for various periods and with various relevant endpoints. In acute toxicity studies, semduramicin was classified toxic to rats and mice with (oral) LD_{50} values between 5 and 100 mg/kg and very toxic by inhalation with an (inhalatory) LC_{50} of 67 mg/m^3 (rats). No evidence for irritating properties was found for rabbit eye or skin. In beagle dogs, five weeks, six months and one year toxicity studies were reported which revealed changes indicative of muscle damage, consistent with the well known ionophore-type toxicity, and retinal changes, but no clear effects on the heart. The lowest NOAEL was 0.3 mg/kg/day. Studies in rats revealed marginal and non-specific effects (decreased serum protein and sodium levels) at 0.25 mg/kg/day in a two year toxicity/carcinogenicity study and there was no evidence for carcinogenicity. Thus the overall lowest NOAEL was established at 0.125 mg/kg, the next lowest dose in this two year study. In studies with rats and rabbits, no indication of significant reproductive toxicity or teratogenicity was apparent, the NOAEL being 0.5 mg/kg/day based on maternal/fetotoxicity.

4.6.3. Risk for the consumer

In conclusion from these studies the lowest NOAEL of 0.125 mg/kg body weight was established in the two year rat study. Applying a safety factor of 100 this results in an ADI of 1.25 µg/kg body weight (*i.e.* 0.075 mg/day for a 60 kg person). Based on a "reference" diet (*i.e.* 300 g meat, 100 g liver, 10 g kidney and 90 g fat/skin) and taking into account the five day withdrawal period proposed by the Company this would result in a daily exposure to Semduramicin residues of 0.003 mg, which represents 4% of the ADI. It must be emphasised that this calculation is based on the radioactive residue data (Table 3), and assuming all radioactivity is unchanged Semduramicin. This estimation represents a maximum worst case scenario, and thus the risk for the consumer can be considered negligible.

4.6.4. Risk for the user

The risk for the user is mainly from inhalatory exposure since there is significant inhalatory toxicity (LC_{50} in rats is 67 mg/m^3). However, the additive comes in an antidust formulation that provides a significant reduction in dusting properties to acceptable limits under workplace conditions. Additional precautionary measures (dust mask) are, however, still recommended.

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

4.7.1. Nature and persistence of the excreted products

Excreta from chickens fed a feed supplemented with labelled semduramicin (25ppm) contained a mean level of 23 mg/kg radioactive material. Unchanged semduramicin was 1.59 mg/kg. Several other peaks were seen in HPLC traces, but these were not further identified. The major unidentified metabolite accounted for approximately 2.1 mg/kg, which is below the 20% of the ingested dose and therefore not considered a "major metabolite" for further assessment.

The DT_{50} of Semduramicin in soil is more than 30 days. No data on degradation in slurry have been submitted.

4.7.2. Effect on environment

Since the K_{ow} of semduramicin is low, the risk of bioconcentration and secondary poisoning is expected to be negligible.

Using the submitted data, the following NOECs can be established.

Table 5 No Observed Effect Concentrations in environmental organisms

NOECs (or alternative parameter)	Species / Parameter / Test
10 mg / l	Algae, growth
0.33 mg / l	Daphnids, reproduction
100 mg / kg	Earthworms, biomass
32 mg / l	Rainbow trout, 96h LC50
0.32 mg/kg	Plants, soy bean germination
100 mg / kg (agar)	Soil microorganisms

4.7.3. Conclusion

The Committee considers that, on the basis of these data, harmful effects on the environment are not expected.

4.8. General conclusion

On the basis of the data provided on semduramicin sodium for use as feed additive for chickens for fattening against coccidiosis, the Scientific Committee on Animal Nutrition concludes that under the proposed conditions of use, the safety of the product is demonstrated for the target animal, the user, the consumer and the environment.

The efficacy of the product could only be demonstrated consistently at a level of inclusion of 25 ppm. At the level of 20 ppm, although favourable results

have been obtained, the efficacy of Semduramicin is not always significantly demonstrated. Additional studies to support the lower level would be needed.

5. REFERENCES

Dossier (responses to questions raised by the committee of experts, 1996)

Dossier(responses to questions raised by the Committee of Experts and SCAN, November 2001)

SCAN Reports, 4th series, 1984. Report on the use of narasin as feed additive in chickens, p. 41, 1984.

Lynch M.J., Frame G.M., Ericson J.F., Illyes E.F. and Nowakowski M.A. 1992. Semduramicin in the chicken. Tissue residue depletion studies. ACS Symposium Series 503. Xenobiotics and food producing animals, p.49.