Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission on the evaluation of coccidiostat Kokcisan® 120G

(Question No EFSA-Q-2003-050)

Adopted on 7 May 2004

SUMMARY

Kokcisan® 120G is a feed additive intended for the control of coccidiosis, a debilitating protozoal infection in poultry. In common with a number of other coccidiostats, the additive is due for evaluation to comply with statutory requirements agreed at EU-level. The European Commission asked the EFSA to evaluate the product Kokcisan® 120G and advise the Commission on its efficacy and safety. Within EFSA this task was allocated to the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel). The data provided in the dossier proved insufficient to give conclusive answers to the several of the questions raised by the European Commission.

Kokcisan® 120G containing 12 % salinomycin sodium (SAL-Na) as active substance, added as coccidiostat at a level of 60 mg SAL-Na kg⁻¹ feed for chickens for fattening, is as effective as another SAL-Na as seen in field trials. However, a full assessment of the efficacy was not possible, as no up-to-date floor pen studies have been carried out. Studies on the occurrence of coccidial resistance against SAL-Na from Kokcisan® 120 G were not submitted, however the development of resistance against coccidiostats including SAL-Na is a well known phenomenon effectively counteracted in practice by rotation or shuttle programmes.

Tolerance tests showed that Kokcisan® 120G is safe for the target animal with a small margin of safety (about 1.7). Incompatibilities or interactions with feedingstuffs, carriers and other approved additives are not to be expected based on the known history of the additive. But there exists a well known interaction with some medicinal substances (i.e. tiamulin) justifying a warning label on the product that the simultaneous use of these substances may be contraindicated. SAL-Na at the use level for chickens is poisonous to horses, other equines and turkeys.

SAL is active against certain Gram-positive bacteria, while Enterobacteriaceae are resistant. Increased shedding of Salmonella Enteritidis and Campylobacter spp. is unlikely to occur at the dose used in practical conditions. Induction of resistance and cross resistance was not observed in experimental conditions. The selection of salinomycin resistant enterococci is possible but resistance to salinomycin is not associated with cross resistance to antibiotics used for therapy in human or veterinary medicine.

SAL is absorbed in the chicken intestine and rapidly excreted as a number of polar metabolites via the faeces, but the metabolites in chicken excreta were not identified. SAL itself represents obviously a minor part of the salinomycin related excretion.

Unchanged salinomycin represents also a very minor part of tissue residues. Already after a short withdrawal period unextractable residues become the most important part of total tissue residues. No data are available concerning the separation and identification of salinomycin metabolites in chicken tissues. Therefore no marker residue could be established.
Kinetics of tissue residues in the chicken indicate that skin plus fat is the target tissue. During a 60 hours withdrawal period significant residue depletion in edible tissues could not be observed suggesting the existence of bound residues or the incorporation of SAL-fragments into endogenous compounds. But no attempt has been made to test this hypothesis.

The SAL-Na fermentation product gave a positive result for mutagenicity in one of the in vitro mutagenicity tests, but negative results from in vivo studies of mutagenicity in two different somatic tissues. FEEDAP panel concludes that the SAL-Na fermentation product and salinomycin in Kokcisan® 120G is not genotoxic in vivo.

The lowest NOEL for SAL-Na from Kokcisan® 120G was 0.117 mg kg⁻¹ body weight d⁻¹, based on the results of a 90-day dog study. There was no investigation of chronic toxicity and developmental toxicity was studied in only one species, whereas guidelines require at least one chronic toxicity study and developmental studies in at least two species. Also a NOEL based on pharmacological studies on the heart of the dog was not identified. It could not be excluded by FEEDAP Panel that a lower NOEL might have been identified from one of the outstanding studies. It was therefore not possible to set an ADI.

MRL’s can not be set because of the lack of an ADI. No withdrawal period can be recommended because of the lack of an ADI and MRL’s, and of a satisfactory relation between residue level and withdrawal time.

Sensitive and validated methods for the determination of SAL in feed and tissues are available, The acute inhalation study indicated a toxic hazard associated with inhalation of Kokcisan® 120G. However, the formulation of Kokcisan® 120G minimises dust formation from handling of the product. Consequently, there would be minimal inhalation risk to workers. Kokcisan® 120G was moderately irritating to the eyes and skin and was also a skin sensitizer. It will need appropriate labelling to warn operators of the risks from skin and eye exposure. The risk from systemic exposure can only be fully evaluated from the available data if data for chronic toxicity and developmental studies are supplied.

Based on the information provided on the fate and toxicity of salinomycin, a risk for the terrestrial and aquatic environment by the use of Kokcisan® 120G at the recommended dose range can not be excluded.

Key words:
Kokcisan, coccidiostat, feed additive, ionophore, salinomycin-sodium, anticoccidial efficacy, microbiological risks, target animal safety, consumer safety, ADI, MRL, worker safety, environmental safety
Table of contents

BACKGROUND 5
TERMS OF REFERENCE 5
ANNEX INSCRIPTIOPNNS 6
ASSESSMENT 7

1 Introduction 7
1.1 Salinomycin sodium 7
1.2 Kokcisan® 120G 7
1.3 Mode of action 8
1.4 Stability 8
1.5 Control methods 8

2 Efficacy 9
2.1 Dose titration and confirmation studies 9
2.2 Controlled floor pen studies 9
2.3 Controlled field trials 9
2.4 Studies on the development/incidence of resistance in Eimeria 13
2.5 Study on the quality of animal produce 14
2.6 Conclusions on efficacy 14

3 Safety - Studies on target species 15
3.1 Tolerance 15
3.2 Interactions 15
3.3 Microbiological safety of the additive 16
3.4 Conclusion on the safety for the target animal 17
3.5 Metabolism of SAL 18
3.6 Residues 19
3.7 Conclusion on metabolism and residues 20

4 Safety – studies on laboratory animals 21
4.1 Acute toxicity studies 21
4.2 Genotoxicity 21
4.3 Subchronic oral toxicity studies 23
4.4 Chronic oral toxicity and carcinogenicity studies 24
4.6 Special studies of cardiac effects in dogs 25
4.7 Special studies on farm animals that are not the target species 25
4.8 Determination of the overall no observed adverse effect level (NOEL) 26

5 Safety evaluation for the human consumer 26
5.1 Studies on human gut flora, antimicrobial spectrum and MIC 26
5.2 Proposal for an acceptable daily intake 27
5.3 Proposal for the maximum residue limit 27
5.4 Proposal of the withdrawal period 27

6 Worker safety 27
6.1 Dust formation 27
6.2 Acute inhalation toxicity 27
6.3 Irritation – Skin 28
6.4 Irritation – Eye 28
6.5 Skin sensitisation 28
6.6 Systemic toxicity 28
6.7 Conclusions on worker safety 28
7 Environment 29
7.1 Fate and behaviour 29
7.2 Effect assessment 30
7.3 Risk Characterisation 32
CONCLUSIONS AND RECOMMENDATIONS
DOCUMENTATION PROVIDED TO EFSA 35
PANEL MEMBERS 36
ACKNOWLEDGEMENT 36
REFERENCES 36
ANNEX I 39
Background

The product ‘Kokcisan 120G’ (salinomycin sodium), is intended for the use as feed additive. The Commission received a request for 10 years Community authorisation of this product under the conditions set out in the Annex inscriptions (below):

The company producing ‘Kokcisan 120G’ prepared a dossier that has been submitted through the national rapporteur (United Kingdom) to the Commission. The dossier was checked by the Member States for its compliance with the requirements of Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition. The Member States concluded in the Standing Committee of Animal Nutrition on 18th February 2002 that the dossier fulfilled these requirements.


TERMS OF REFERENCE

EFSA is requested to consider the above mentioned product and to advise the Commission on its efficacy and its safety. In assessing each product on the basis of the dossier presented, EFSA and its Panel on Additives and Products or Substances used in Animal Feed is requested to answer the following questions. Under the conditions proposed for its use as additive in feed,

- Is the efficacy of the product as described in the Annex Inscriptions demonstrated?
- Can the use of the product result in the development of resistance in bacteria to prophylactic or therapeutic preparations?
- Is the product and its metabolites safe for
  - the target animals,
  - the user,
  - the consumers,
  - the environment?
- Can the product be monitored?
## ANNEX INSCRIPTIONS

<table>
<thead>
<tr>
<th>Name and registration number of person responsible for putting additive into circulation</th>
<th>Additive (trade name)</th>
<th>Composition, chemical formula, description.</th>
<th>Species or category of animal</th>
<th>Maximum content kg of active substance of complete feedingstuff</th>
<th>Minimum content</th>
<th>Other provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRKA Aussenhandels GmbH</td>
<td>Salinomycin sodium 120 g kg⁻¹ (Kokcisan 120G)</td>
<td>Additive composition: Salinomycin sodium : 120 g kg⁻¹ Calcium carbonate : 250-400 g kg⁻¹ Sucrose : 80-100 g kg⁻¹ Corn starch : 20 g kg⁻¹</td>
<td>Chickens for fattening</td>
<td>-</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Use prohibited at least 1 day before slaughter Indicate in the instructions for use: ‘Dangerous for equines’. ‘This feedingstuff contains an ionophore: simultaneous use with certain medicinal substances (e.g. tiamulin) can be contraindicated’.</td>
</tr>
</tbody>
</table>
ASSESSMENT

1 INTRODUCTION

Kokcisan® 120G is a feed additive used for prevention of coccidiosis in chickens for fattening containing 120 g salinomycin sodium kg⁻¹ product as active ingredient. It visually consists of light brown granules.

1.1 Salinomycin sodium

Salinomycin sodium (SAL-Na) is a polyether ionophore coccidiostat presently approved as generic substance and listed under Council Directive 70/524/EEC (amended by Council Directive 96/51/EC). The dose approved for chickens for fattening is 50-70 mg kg⁻¹ of complete feed with a withdrawal time of 5 days.

Structural formula of salinomycin sodium (C₄₂H₆₉O₁₁Na, molecular weight: 772.99)

Ethyl-6-[5-{2-(5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyrano-2-yl)-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4,1,5,3]pentadec-13-en-9-yl}2-hydroxy-1,3-dimethyl-4-oxoheptyl]tetrahydroxy-5-methyl-2H-pyran-2-acetic acid, sodium salt. CAS-No: 55721-31-8 (SAL-Na), 53003-10-4 (SAL free acid)

SAL-Na is presently also approved at levels of 30-60 mg kg⁻¹ feed for piglets, and 15-30 mg kg⁻¹ feed for pigs as Sacox 120 microGranulate (Council Directive 91/408/EEC, see also 2004/C 50/01), 20-25 mg kg⁻¹ feed for rabbits for fattening as Sacox 120 (Commission Directive 96/7/EC, see also 2004/C 50/01) and 50 mg kg⁻¹ feed for chickens reared for laying as Sacox 120 microGranulate (Commission Regulation (EC) 1852/2003).

1.2 Kokcisan® 120G

The company submitted a dossier, in order to get a brand specific approval of Kokcisan® 120G under Council Directive 96/51/EC, the Fifth Amendment to Council Directive 70/524/EEC.

Salinomycin from Kokcisan® 120G is produced by a fermentation process of a selected strain of *Streptomyces albus*. The strain is deposited at Centraalbureau voor Schimmelcultures (CBS), Baarn, the Netherlands, under accession number CBS 101071. The sodium salt is generated by the addition of sodium hydroxide at the end of fermentation. Calcium carbonate and talc are added to the fermentation broth, the pH-value is adjusted to 10.5 by addition of sulphuric acid and calcium hydroxide, the mixture is then spray dried, containing between 18 and 28 % SAL-Na (on average 22 %), about 37 % biomass, 19 % calcium carbonate, 19 % talc and 3 % moisture and serves as SAL-Na-concentrate for the formulation of the final preparation. Kokcisan® 120G is composed on an average (range) of approximately 54 (48-65) % SAL-Na-concentrate, 35 (25-40) % calcium carbonate, 2 % cornstarch, and 9 (8-10) % sucrose, the latter being added during a wet granulating process for dust binding.

The granulation process results in a more or less uniform product, 80 % of all particles being between 0.1 and 0.4 mm, particles > 0.8 mm being absent. Particles below 0.045 mm amount only to 0.3 %.

Among salinomycin and related products in Kokcisan® 120G, salinomycin amounts to 88 ± 5 %, 17-epi-20-deoxy salinomycin to 2 ± 1 %, 20-deoxy salinomycin to 5 ± 2 %, 6-ethyl
salinomycin to 6 ± 3 %, and others to < 2 % (elaiophylin in an average of three batches <10 mg kg⁻¹ SAL-Na).

Further information on impurities is given for lead (7 mg kg⁻¹), iron (0.2 g kg⁻²), aflatoxin B₁ (<0.01 mg kg⁻¹), *E. coli* and *Salmonella* (absent), fungi and yeast (less than 100 cfu g⁻¹ product), and the production organism (absent).

The product contains about 2.8 % crude protein, 7.3 % glucose, 5.0 % ether extract and 41.3 % ash (residue on ignition, sulphated ash is 74.9 %). However, detailed information on the composition of the biomass from fermentation, amounting to approximately 20 % in KOKCISAN® 120G, is not given.

Table 1. Summary of physical properties of SAL-Na

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Condition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>772.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water solubility</td>
<td>57, 294 and 308 mg L⁻¹</td>
<td>pH 5, pH 7 and pH 9 respectively reverse osmosis water</td>
<td>¹</td>
</tr>
<tr>
<td>Log Kₐw</td>
<td>2.4 *</td>
<td>reverse osmosis water /octanol</td>
<td>²</td>
</tr>
</tbody>
</table>

1.3 **Mode of action**

Salinomycin is effective against sporozoites, and early and late asexual stages of coccidia in the intestine of the chicken. The biological activity of SAL is based on the ability of the ionophores to form lipid soluble, dynamically reversible complexes with mono- and divalent cations (preferably the alkali ions K⁺, Na⁺ and Rb⁺). SAL encloses the cation in a hollow ball, in the centre of which the cation is fixed and immobilised. It functions as a carrier by mediating an electrically neutral exchange-diffusion type of cation transport across the membranes. The resultant changes in transmembrane ion gradients and electrical potentials often produce profound effects on cellular function and metabolism.

1.4 **Stability**

Three batches of Kokcisan® 120G stored under room temperature (25°C/60 % relative humidity) for 24 months and under tropical conditions (30°C/60 % relative humidity) for 12 months showed good stability. These data as well as other results from 4 batches of Kokcisan® 120G stored under ambient temperature support a shelf life of 2 years.

SAL-Na from the salinomycin concentrate as well as in pelleted or mash broiler feed is obviously stable under alkali, thermal, UV and oxidative conditions, but very unstable under acid conditions.

Studies on pelleted premixtures and final mixtures demonstrated satisfactory stability of SAL-Na, even after a 20 week storage time (23°C/50 % relative humidity).

1.5 **Control methods**

Specificity and sensitivity of the control methods are satisfactory.

1.5.1 Determination of salinomycin in premixtures and feeds for chickens³

An HPLC method using post-column derivatization with vanillin has been developed to determine salinomycin in premixes and supplemented feeds for chickens. The method has

---

³ Section II. Vol. 7. chapter 5.2.
been validated for linearity of response, recovery, selectivity, repeatability and precision. The limit of quantification (LOQ) has been established to 0.2 mg kg⁻¹ premixes or feed.

1.5.2 Determination of salinomycin residues in chicken tissues⁴

Anticipating that salinomycin might be the marker-residue in tissues, the petitioner proposes a validated liquid chromatography-mass spectrometry (LC-MS) method for the determination of salinomycin in the muscle, liver, kidney and skin/fat of chickens. That method allows to identify and determine salinomycin and to separate it from four other ionophores. The LOQ has been set to 0.001 mg kg⁻¹ for all tissues. Moreover, the stability of salinomycin residues in tissues during the preservation of samples in deep freeze conditions has been established to 6 and 12 weeks for the muscle and kidney, and 29 weeks for the liver and skin/fat.

2 Efficacy

Commission Directive 2001/79/EC requires efficacy data on three stages of target animal experimentation: (a) controlled battery-cage experiments (single and mixed infections), (b) controlled floor pen studies (simulated use conditions), and (c) controlled field trials (actual use conditions).

The continuous use of coccidiostats over a long time as well as the breeding progress may result in the selection of resistant variants of *Eimeria spp*. The above mentioned Directive also outlines: “The dossiers must enable an assessment to be made of the additives based on the present state of knowledge”. In the evaluation of Kokcisan® 120G, FEEDAP Panel took therefore only efficacy studies into consideration, which were conducted not earlier than about 1990.

Only one exception is made by FEEDAP Panel for battery-cage experiments, which serve for the principal discovery or confirmation of the anticoccidial efficacy of an additive against single or mixed infections or for dose titration studies.

2.1 Dose titration and confirmation studies

Kokcisan® 120G is claimed to be effective against 6 species of *Eimeria* (*E. acervulina, E. necatrix, E. brunetti, E. maxima, E. mitis, and E. tenella*).

The anticoccidial activity of SAL-Na against *Eimeria* species infecting the intestine of chickens is well established and has been subject of numerous publications since the substance was first synthesised in the mid-1970s.

A recent experimental infection study⁵ has been conducted to evaluate the efficacy of 60 mg SAL-Na from Kokcisan® 120G kg⁻¹ feed against *Eimeria* field isolates in an experimental challenge study in broiler chickens. The study showed that 60 mg SAL-Na from Kokcisan® 120G kg⁻¹ diet was effective in supporting growth of treated birds and in reducing lesions due to a mixed infection with *E. acervulina, E. necatrix* and *E. brunetti*, but did not reduce lesions due to *E. mitis, E. maxima* and *E. tenella* and did not fully restore growth.

2.2 Controlled floor pen studies

No controlled floor pen studies with Kokcisan® 120G on the efficacy in target species were made post 1990.

2.3 Controlled field trials

Six studies were completed after 1990 and were considered in this evaluation.

---

⁴ Section II. Vol.7. chap. 5.3.
⁵ Vol. 8, VLAS/00/37, 2001, vol. 8
2.3.1 Broiler experiment 1

The field study\(^6\) compared the efficacy of 60 mg of SAL-Na from Kokcisan® 120G kg\(^{-1}\) feed with the same dosage of another SAL-Na (positive control group) on groups of approximately 21 000 male and female broiler chicken each. The trial (without replicates) lasted 42 (female) and 49 (male) days for the Kokcisan® 120G group and 43 (female) and 50 (male) days for the control group, respectively. Broilers were offered feed supplemented with SAL-Na from the beginning of the fattening period until at least 5 days before slaughter (withdrawal period). Feed analysis (feed ingredients, salinomycin content) confirmed that both herds were offered feed of adequate and comparable quality. Mortality, oocyst excretion, intestinal lesion scores and productivity were recorded.

The zootechnical parameters did not show any relevant differences between the groups (Table 2).

### Table 2. Broiler performance data – Controlled field trial 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Positive control</th>
<th>Kokcisan® 120G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality [%]</td>
<td>5.2</td>
<td>3.3</td>
</tr>
<tr>
<td>Body weight gain [g day(^{-1})]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Female</td>
<td>46.7</td>
<td>48.1</td>
</tr>
<tr>
<td>- Male</td>
<td>54.4</td>
<td>54.3</td>
</tr>
<tr>
<td>Feed conversion [g feed g(^{-1}) gain]</td>
<td>2.03</td>
<td>1.90</td>
</tr>
</tbody>
</table>

Oocyst excretion in the Kokcisan® 120G herd peaked in week 3 and 4, and decreased thereafter. Onset of oocyst excretion in the positive control herd (Table 3) was observed later (4\(^{th}\) and 5\(^{th}\) week).

### Table 3. Oocyst counts per g pooled faecal samples – Controlled field trial 1

<table>
<thead>
<tr>
<th>Week of fattening</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>0</td>
<td>0</td>
<td>1950</td>
<td>3100</td>
<td>600</td>
</tr>
<tr>
<td>Kokcisan® 120G</td>
<td>&lt;50</td>
<td>2350</td>
<td>1750</td>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>

Most chicken of both treatments did not show any intestinal lesions attributable to coccidiosis by necropsy at day 28 and 35. Faecal consistency was within the physiological range.

2.3.2 Broiler experiment 2

A field study in Slovenia\(^7\) compared the efficacy of 60 mg SAL-Na from Kokcisan® 120G kg\(^{-1}\) feed (40 000 chickens) with the same dosage of another SAL-Na (positive control group; 40 000 chickens) during a 42 day growing period of broilers, each group with two replicates. Broilers were offered feed supplemented with SAL-Na from the beginning of the fattening period for 37 days followed by a withdrawal period of 5 days before slaughter. Feed analysis (SAL-Na) confirmed that both herds received feed with the intended coccidiostat concentration. Mortality, oocyst excretion and productivity were measured. Statistical analyses were not performed.

The zootechnical parameters did not show any relevant differences between the groups (Table 4).

---

\(^6\) Vol. 9, Daugschies, 2001, Germany, Vol. 9
\(^7\) Vol. 9, Kac and Režek, 1991, vol. 9, p. 103
Throughout the production period no clinical signs of coccidiosis were seen in both groups. No oocysts were found in faecal and litter samples at days 17, 26 and 39.

### 2.3.3 Broiler experiment 3

A field study in Slovenia\(^8\) compared the efficacy of 60 mg of SAL-Na from Kokcisan® 120 G kg\(^{-1}\) complete feed (42,344 chicken) with the same dosage of another SAL-Na (positive control group; 41,561 chicken) during a 42 day growing period of broilers (including 5 days withdrawal), each group with two replicates. Feed analysis (crude nutrients, salinomycin content) confirmed that both herds were offered feed of adequate and comparable quality. Mortality, oocyst excretion and productivity were recorded. Statistical analyses were not performed.

The zootechnical parameters did not show any relevant differences between the groups (Table 5). Clinical coccidiosis was not detected in houses A, B, C and D.

#### Table 5. Broiler performance data – Controlled field trial 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Positive control</th>
<th>Kokcisan® 120G</th>
</tr>
</thead>
<tbody>
<tr>
<td>House B</td>
<td>5.2</td>
<td>4.7</td>
</tr>
<tr>
<td>Mortality [%]</td>
<td>1.90 (House B)</td>
<td>1.91 (House D)</td>
</tr>
<tr>
<td>Body weight at the end [kg]</td>
<td>1.90</td>
<td>1.91</td>
</tr>
<tr>
<td>Feed conversion [g feed g(^{-1}) gain]</td>
<td>1.90</td>
<td>1.91</td>
</tr>
</tbody>
</table>

### 2.3.4 Broiler experiment 4

A field study in UK\(^9\) compared the efficacy of 60 mg of SAL-Na kg\(^{-1}\) feed from Kokcisan® 120G with the same dosage of another SAL-Na (positive control group 1) and with another ionophore anticoccidial additive (positive control group 2) during a 42 day growing period of broilers on a total of 3,360 broilers, each group with 8 replicates (4 pens male chickens, 4 pens female chickens). Relevant statistical analyses were performed. The feeds were not analyzed for anticoccidial content. Mortality, oocyst excretion, visible lesions and productivity were measured.

The broilers of control group 2 had a 3 % lower growth rate (p<0.01) than the broilers of the Kokcisan® 120G group and of control group 1 (Table 6).

#### Table 6. Broiler performance data – Controlled field trial 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Positive control 1</th>
<th>Positive control 2</th>
<th>Kokcisan® 120G</th>
<th>SEM (18 df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality [%]</td>
<td>7.6</td>
<td>8.1</td>
<td>5.8</td>
<td>0.910</td>
</tr>
<tr>
<td>Body weight gain, [kg]</td>
<td>2.28</td>
<td>2.23</td>
<td>2.33</td>
<td>0.014</td>
</tr>
<tr>
<td>Feed conversion [g feed g(^{-1}) gain]</td>
<td>1.98</td>
<td>1.92</td>
<td>1.95</td>
<td>0.021</td>
</tr>
</tbody>
</table>

There was no evidence of coccidiosis in any treatment.

\(^8\) Vol. 9, Viduka, Tuta, Kač, Mrzel, Josipović, 1990, p. 170

\(^9\) Vol. 8, Rose, 1997, p. 55
2.3.5 Broiler experiment 5

A field study in France\(^\text{10}\) compared the efficacy of 60 mg of SAL-Na kg\(^{-1}\) feed from Kokcisan\(^\circ\) 120G per kg feed (15 300 chickens) with the same dosage of another SAL-Na (positive control group; 15 300 chickens) during a 35 day growing period of broilers, each group without replicate. Feeding period with the additives was 4 weeks followed by a withdrawal period of 7 days. Feed analysis (dietary nutrients, SAL-Na content) confirmed that both herds were offered feed of adequate and comparable quality. Mortality, oocyst excretion and productivity were evaluated. Statistical analyses were performed on individual broiler weight.

The zootechnical parameters did not show any relevant differences between the groups (Table 7).

### Table 7. Broiler performance data – Controlled field trial 5

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Positive control</th>
<th>Kokcisan(^\circ) 120G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality [%]</td>
<td>2.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Body weight at the end of the trial [kg]</td>
<td>1.59</td>
<td>1.59</td>
</tr>
<tr>
<td>Feed conversion [g feed g(^{-1}) gain]</td>
<td>1.78</td>
<td>1.83</td>
</tr>
<tr>
<td>Water/feed ratio</td>
<td>2.90</td>
<td>2.96</td>
</tr>
</tbody>
</table>

Pooled faecal samples were collected on day 21 and day 28 (Table 8). The surveys of coccidiosis by oocyst counting and lesion index demonstrate a similar efficacy of both products.

### Table 8. Mean number of oocysts per gram faeces – Controlled field trial 5

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Positive control</th>
<th>Kokcisan(^\circ) 120G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 21</td>
<td>30 600</td>
<td>19 800</td>
</tr>
<tr>
<td>Day 28</td>
<td>948 000</td>
<td>402 000</td>
</tr>
</tbody>
</table>

2.3.6 Broiler experiment 6

A field study\(^\text{11}\) in Spain compared the efficacy of 60 mg of SAL-Na from Kokcisan\(^\circ\) 120G kg\(^{-1}\) complete feed (32 000 chickens) with the same dosage of another SAL-Na (positive control group; 32 000 chickens) during a 45 day growing period of broilers, each group with two replicates. Feed with the additives was given from the beginning of the fattening period until day 39 followed by a withdrawal period of 6 days. Feed analysis (crude nutrients, salinomycin content) confirmed that both herds were offered feed of adequate and similar quality. Mortality, oocyst excretion and productivity were evaluated. Statistical analyses were performed on individual broiler weight.

Mortality was slightly higher than normal, mostly due to the incidence of the flaccid neck syndrome, and in the case of the Kokcisan group, due to a bacterial infection not related to the experimental treatments, which responded to the treatment with antibiotics. Body weight at the end of the trial and feed conversion did not show relevant differences between the groups (Table 9).

### Table 9. Broiler performance data – Controlled field trial 6

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Positive control</th>
<th>Kokcisan(^\circ) 120G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality [%]</td>
<td>6.3</td>
<td>8.6</td>
</tr>
<tr>
<td>Body weight at the end of the trial [kg]</td>
<td>2.74</td>
<td>2.79</td>
</tr>
<tr>
<td>Feed conversion [g feed g(^{-1}) gain]</td>
<td>2.01</td>
<td>2.03</td>
</tr>
</tbody>
</table>

\(^{10}\) Vol. 8, p. 71, Guyonvarch, 2000

\(^{11}\) Vol. 8, p. 196, Estevc-Garcia, 2001
Oocyst counts, litter scoring and intestinal lesions suggested an insignificant incidence of coccidiosis during the trial.

2.4 Studies on the development/Incidence of resistance in *Eimeria*

Studies on the development of resistance of *Eimeria* spp. against SAL-Na from Kokcisan® 120G were not submitted.

2.4.1 Recent publications

Resistance to anticoccidials is a widespread phenomenon, which occurs even with the more recent additives. To describe the resistance situation of *Eimeria* spp. clinically important in the production of chickens for fattening in Europe, FEEDAP Panel reviews the results of 4 studies published in the last decade.

The study of Peeters *et al.* (1994) was performed with coccidia from 122 Belgian broiler farms without clinical coccidiosis, where shuttle programs were most commonly used. 146 *E. acervulina*, 65 *E. maxima* and 88 *E. tenella* isolates were tested in 17 sensitivity profiles. Results were related to the anticoccidial program that had been in use. The data clearly indicate that recorded anticoccidial sensitivity is widespread and that most ionophore did not control isolates significantly. The differences between the ionophores tested confirm – according to authors - earlier data of incomplete cross-resistance to polyether ionophorous drugs.

Two studies published in 1997 (Stephan *et al.*.) and 1998 (Daugschies *et al.*.) describe the situation in Germany. Stephan *et al.* (1997) studied the sensitivity of 10 Eimeria field isolates. 9 of the 10 isolates showed resistance, mostly multiple resistance. Partial or complete resistance was shown for maduramycin in 7 field isolates, for monensin in 6, for salinomycin in 5, for nicarbazin in 8 and for halofuginone in 7 isolates. Cross resistance between maduramycin, monensin, and salinomycin occurred in 5 isolates.

Daugschies *et al.* (1998) compared the efficacy of different additives in a commercial broiler farm with a history of suspected drug resistance on approximately 100 000 chicks in three consecutive runs. Coccidia were isolated from indicator birds. Sensitivity profiles were followed in battery trials. Anticoccidials tested were nicarbazin, narasin, halofuginone, salinomycin, metclopinodol plus methylbenzoquate, and monensin. The first run showed resistance in the battery trial against the anticoccidials used in the field (nicarbazin/monensin) and halofuginone.

The most recent publication (Peek and Landman, 2003) studied the Eimeria resistance situation in Dutch poultry production. In 1996, four isolates were selected from farms with clinical coccidiosis problems, four isolates from 1999 and seven isolates from 2001 originated from farms with subclinical disease. The tests were conducted according to Chapman (1998) as in vivo anticoccidial sensitivity tests. The sensitivity profile is based on the reduction of lesion scores compared to the infected untreated control.

*Eimeria acervulina* was more or less resistant against all coccidiostats tested in 1996 (diclurazil, halofuginone, lasalocid, metclopinodol plus methylbenzoquate, monensin, narasin and nicarbazin), and 3 of 4 strains against maduramycin and salinomycin (one showed reduced sensitivity). In 1999 the same species presented a similar resistance pattern, one strain (of four) showed sensitivity against monensin and narasin. In 2001 increased sensitivity was found. Higher sensitivity was found for metclopinodol/methylbenzoquate (7/7), salinomycin and narasin (4/7), followed by nicarbazin (3/7) and monensin (2/7). Resistance was found for lasalocid (5/7), nicarbazin (4/7), diclurazil, monensin, narasin and salinomycin (all 2/7). An *E. acervulina* reference strain, tested in 1999, showed full sensitivity towards all anticoccidial additives tested. The differences between the results of 1996 and 1999/2001 may also reflect the origin of the isolates (1996 from flocks with clinical disease and therefore more virulent strains), but also higher inoculation doses have been applied.
In the broiler farms participating in the Dutch coccidiosis monitoring programme, the incidence of coccidiosis (E. acervulina) was approximately 70 (68) % in 1996, 91 (84) % in 2000 and 73 (67) % in 2001. Despite the obvious resistance, an increase in clinical problems was not observed. The authors suggest that a form of spontaneous vaccination might occur in field.

The FEEDAP Panel concludes that despite the prevailing (partial) resistance of Eimeria spp. against nearly all coccidiostats the benefits of their use are not essentially jeopardized under field conditions. The recent data also confirm that the consequences of developing resistance can successfully be counteracted in practice by rotation (the alternation of coccidiostats from run to run) or by shuttle programmes.

2.5 Study on the quality of animal produce

In a recent study the quality of animal produce was investigated. Two groups of 100 broilers received 60 mg of SAL-Na from Kokcisan® 120G kg⁻¹ feed and from another coccidiostat, respectively, from day 1 to day 35. A third group of 100 broilers received feed without any anticoccidial additive. All birds were housed under commercial conditions and were sacrificed on day 43. In addition to production parameters, meat quality criteria (percentage skeletal rack, wings, breast, shank, thigh, and abdominal fat) and organoleptic parameters (skin colour, abdominal fat, breast and thigh conformity, texture, taste and smell after cooking) were measured.

The zootechnical parameters (mortality rate, body weight, feed conversion) did not show relevant differences between the groups. Also meat quality and organoleptic parameters examined did not differ significantly between the groups under investigation.

2.6 Conclusions on efficacy

Early studies have shown that SAL-Na is effective in preventing coccidiosis in chickens for fattening in the range of 50 – 70 mg SAL-Na kg⁻¹ diet.

However, no recent floor pen trial with Kokcisan® 120G was presented. This does not fulfil the present requirements of the Commission Directive 2001/79/EC. Three significant results on the target animal should be presented to recognise that efficacy is fully demonstrated. The basis for an assessment of the actual efficacy of Kokcisan® 120G in floor pen studies is therefore not given. This omission is of particular significance considering the unsatisfactory data provided by the only recent battery trial with Kokcisan® 120G.

Convincing field studies are difficult to design because negative control groups can hardly be established under practical conditions. The in field effect of an additive can only be tested in comparison with a positive control group (receiving another anticoccidial additive). Although some field trials could not be considered due to high mortality rate or disease occurrence and despite of the lack of satisfactory statistical measures, the results of the remaining investigations indicate that 60 mg SAL-Na from Kokcisan® 120G kg⁻¹ diet is (at least as) effective (as another SAL-Na or another ionophore coccidiostat) in controlling coccidiosis of chickens for fattening under practical conditions.

No confirmation studies for the approved range of 50 to 70 mg SAL-Na kg⁻¹ diet were submitted. All conclusions are focussed on 60 mg SAL-Na from Kokcisan® 120G.

As far as resistance of Eimeria spp. is concerned, no specific data were submitted but FEEDAP Panel assumes that SAL-Na from Kokcisan® 120G would not behave different from other SAL-Na-products. The development of resistance is a well known fact, which is counteracted in practice by rotation (the alternation of coccidiostats from run to run) or by shuttle programmes, in which one coccidiostat follows or precedes the other after e.g. 2 or 3 weeks.

12 Vol. 8, Mrzel, 2001
3 SAFETY - STUDIES ON TARGET SPECIES

3.1 Tolerance

One recent study\(^{13}\) was designed to investigate the tolerance of the target species (5 males and 5 females per group) to the test material at three dose levels (1x, 2x, 2.5x of the recommended dose of 60 mg SAL-Na from Kokcisan® 120G kg\(^{-1}\) complete feed). 10 chickens/group received Kokcisan® 120G for 56 consecutive days at dose rates of 0, 60, 120 and 150 mg SAL-Na kg\(^{-1}\) feed, respectively.

At all dose levels the health status of the birds receiving Kokcisan® 120G was comparable to the control group without SAL-Na. A reduction in feed consumption was observed at all SAL-Na levels. By study day 42 the birds with 60 and 120 mg SAL-Na from Kokcisan® 120G kg\(^{-1}\) diet had consumed about 10 g food day\(^{-1}\) less than the control birds, but for the group with 150 mg SAL-Na kg\(^{-1}\) diet daily feed intake was reduced by about 25 g. This depression of feed intake had become even more evident by the end of the trial. Consequently there was considerable growth rate depression in the group with 150 mg SAL-Na kg\(^{-1}\) complete feed after 56 days.

Clinical chemistry and haematology did not reveal any differences between the control group and the groups with 60 and 120 mg SAL-Na kg\(^{-1}\) feed. In the 150 mg SAL-Na kg\(^{-1}\) feed group there was a non-significant increase in serum-aspartate-amino-transferase and a non-significant reduction in serum-alkaline-phosphatase. All other parameters measured showed results similar to those observed in the control animals.

3.2 Interactions

Studies on the compatibility/interaction of SAL-Na with feedingstuffs, carriers, approved feed additives or other medicinal drugs were not submitted. In long term experience the applicant had not noticed interactions of Kokcisan with feedingstuffs and carriers.

In the supplementary dossier from October 2002 the Notifier outlines that concurrent medication of broiler chickens receiving 60 mg SAL-Na kg\(^{-1}\) feed with tiamulin in water (250 mg L\(^{-1}\)) resulted in a modification of performance data i.e. depression of body weight gain, water intake, deterioration of feed efficiency. Paralysis and even death could occur. Other relevant data as haematology, clinical chemistry, gross pathology and histopathology from treated animals were not submitted.

Clinically important interactions between the ionophore anticoccidials and the antibiotic tiamulin are well known phenomena in chickens, turkeys and other species. Principally the same toxic symptoms were seen after administration of monensin alone or in combination with tiamulin (Hanrahan et al., 1981; Umemura et al., 1985; Van Vleet et al., 1987; Szuces et al., 2000). The interaction depends on dose (Meingassner et al., 1979; Lehel et al., 1995; Weisman et al., 1983a, 1983b) and the ionophore itself (salinomycin concurrently given with tiamulin in feed caused a 60 % loss in a chicken breeder flock (Lin, 1995), co-administration of tiamulin and lasalocid being without adverse effects (Comben, 1984)). Antioxidants obviously reduce the severity of toxic symptoms (Van Vleet et al., 1987; Laczay et al., 1994; Lehel et al., 1995). It was assumed that tiamulin reduces metabolic degradation and excretion of monensin (Meingassner et al., 1979). Later, further toxic interactions with polyethers (mainly monensin) became known for sulphonamides (Frigg et al., 1983), chloramphenicol (Broz and Frigg, 1987), erythromycin, oleandomycin and furazolidone (see reviews by Anadón and Martínez-Larrañaga, 1990, and Anadón and Reeve-Johnson, 1999).

\(^{13}\) Vol. 10, Cunningham, McLean, Mc Lellan, Scotland, 2001/1999
The basis of these interactions is the inhibition of cytochrome P-450 isoenzymes, which plays an important role in the oxidative and reductive metabolism of numerous endogenous and exogenous compounds (also monensin [Nebbia et al., 1999], by tiamulin [Witkamp et al., 1994, 1995, 1996] and macrolide antibiotics [Larry et al., 1983; Watkins et al., 1986]). Monensin itself does not exert significant effects on microsomal liver enzymes (Szucs et al., 2000). Compounds capable of binding or inhibiting these isoenzymes could therefore be expected to give rise to toxic interactions with the ionophore(s).

3.3 Microbiological safety of the additive

3.3.1 Antimicrobial spectrum and MIC studies

For the Gram-positive bacterial strains isolated from poultry, the range of MIC is determined by agar dilution with low (≤10^5 cfu per spot) and high inoculum (>10^6 cfu per spot). For the low inoculum, the ranges are: *Clostridium* spp. [0.5-2 mg L⁻¹], *Staphylococcus aureus* [4 mg L⁻¹] and *Enterococcus faecalis* [1-2 mg L⁻¹]. *E. faecium* [1-16 mg L⁻¹] The strains tested are classified as susceptible, when MIC is below or equal to 8 mg L⁻¹. The Gram-negative bacteria were not inhibited by salinomycin and the MICs for *Escherichia coli, Salmonella Typhimurium, Klebsiella pneumoniae, Pasteurella multocida* and *Pseudomonas aeruginosa* are more than 128 mg L⁻¹.

3.3.2 Ability to select for resistance and cross resistance

The potential for emergence of resistant bacterial strains in vitro after culture was investigated with four bacterial strains (*Bifidobacterium infantis, Staphylococcus aureus, Enterococcus faecalis* and *Clostridium perfringens*). The strains are cultured 10 times in the absence or presence of subinhibitory concentrations of salinomycin. Before subculture, the MICs of the antibiotic panel (salinomycin, cephalothin, erythromycin, ampicillin, lincomycin, enrofloxacin, gentamicin, metronidazole) are determined. There was no evidence of any changes in MICs of the tested antibiotics for the strains exposed to subinhibitory concentrations of salinomycin.

After administration of Kokcisan® 120G in the diet for 56 days at dose rates of 60, 120 and 150 mg SAL-Na kg⁻¹ feed in three groups of 10 chickens, salinomycin reduced the number of enterococci in the intestinal flora of medicated chickens by comparison with a control group but it had no effect on the number of salinomycin resistant enterococci or on the selection of enterococci resistant to other antibiotics (penicillin G, lincomycin, gentamicin, cephalothin, chloramphenicol, streptomycin, oxytetracycline, minocycline, vancomycin, enrofloxacin)⁰⁶. No significant effects were observed on the *Eubacterium* spp., *Bifido-bacterium* spp., *Bacteroides* spp., *Clostridium* spp., *Enterobacteriaceae* spp. and *Pseudomonas* spp.⁰⁷

3.3.3 Effect on a number of opportunistic pathogens present in the digestive tract and/or on the shedding or excretion of relevant zoonotic micro-organisms

Three groups (control, Kokcisan 120G (60 mg SAL-Na kg⁻¹ feed), positive control group (60 mg of another SAL-Na kg⁻¹ feed) of 96 male broiler chickens each were inoculated, half of each group with *Salmonella Enteritidis* PT4 at day 17 of age, half with *Campylobacter jejuni*. The course of *Salmonella* experimental infections, in birds orally infected with 10⁹ cfu/animal, was similar in treated groups but the percentage of *Salmonella* negative faecal samples at day 25 was statistically significantly lower in the supplemented feed groups compared with the untreated group. At slaughter, the percentage of *Salmonella*

---

14 Wheadon A. Vol 10, p 55-77
15 McConville, ML. Vol 10 p 209-226
16 Mc Conville & Wheadon, 1999a. Vol 10 p 78-116
17 Mc Conville & Wheadon, 1999b, Vol 10 p 117-168
isolation from colon was similar in the three groups. No differences between the groups were observed for Campylobacter.

### 3.3.4 Field studies to monitor bacterial resistance to the additive

In the European Union the development of harmonized antimicrobial resistance and surveillance programmes in animals and animal derived food increased in the last ten years. These programmes, recommended by international bodies (WHO, OIE, EU Commission), were based on Danish experience and followed international guidelines (Franklin et al., 2001). Monitoring the antimicrobial resistance of zoonotic bacteria is requested by the zoonosis directive (Common Position (EC) n°13/2003) and is a part of surveillance networks implemented in the European Union for communicable diseases (Common Decision of 17 July 2003). In the meantime, several European countries have improved the existing system. Several countries such as Denmark (DANMAP 1995-2002, www.vetinst.dk), Norway (Norm-VET 2000-2002, www.zoonose.no), Sweden (SVARM 2000-2002, www.sva.se), France (Sanders et al., 2001), Germany (Guerra, 2003), Spain (Moreno et al., 2000) and the UK (Goodyear, 2002) have implemented programmes for monitoring antimicrobial resistance in commensal bacteria such as *E. coli* and *E. faecium* collected from faeces or caecal content of slaughtered animals. The sampling is, in principle, based on the same epidemiological approach to permit international comparisons (Bywater et al., 2003).

In the monitoring programmes resistance to salinomycin (DANMAP 2000) and narasin (Norm-VET 2002; SVARM 2002) in broiler isolates of *Enterococcus faecalis* and *Enterococcus faecium* was monitored and reported. In Denmark, since 1997, salinomycin susceptibility was monitored in *Enterococcus faecium* and *faecalis* strains collected in poultry, pigs and calves. The salinomycin MIC<sub>90</sub> of *Enterococcus faecalis* increased between 1998 and 2002 in poultry from 0.5 to 8 µg mL<sup>-1</sup>. This reflects a shift in the MIC distribution of *Enterococcus* isolates of poultry origin. In Belgium resistance against salinomycin was frequent among poultry strains of *Enterococcus faecalis* and *Enterococcus faecium* (Butaye et al., 2000). In the Netherlands (MARAN 2002), no salinomycin resistance was reported in *Enterococcus faecium* poultry isolates.

In the studies provided by the Notifier salinomycin MICs for four different bacterial subpopulations (staphylococci, clostridia, lactobacilli, enterococci) were compared in two groups of 10 chicken samples each from farms using Kokcisan® 120G or not. No differences were observed.

The strains tested included strains of enterococci (232) and clostridia (169) collected in UK in the end of 2000 from healthy animals at slaughterhouses (McConville, 2001). The MICs of the enterococcal strains ranged between 0.125 and 8 mg L<sup>-1</sup> (MIC<sub>50</sub>=4 mg L<sup>-1</sup>). The MICs for clostridia ranged between 0.125 and 64 mg L<sup>-1</sup> (MIC<sub>50</sub>=2 mg L<sup>-1</sup>) with a percentage of 3% of clostridia classified as resistant<sup>19</sup>.

### 3.4 Conclusion on the safety for the target animal

120 mg SAL-Na kg<sup>-1</sup> feed is obviously well tolerated by chickens for fattening for 56 consecutive days. However, at 150 mg SAL-Na kg<sup>-2</sup> diet there was a marked reduction in growth rate and feed consumption. Related to the highest level approved, the margin of safety is therefore about 1.7 (120/70).

The known history of use of salinomycin has shown that incompatibilities or interactions with feedstuffs, carriers, other approved additives are not to be expected. On the other hand it is well known from the literature that severe interactions between the polyether ionophor coccidiostats and the diterpene-antibiotic tiamulin as well as other antimicrobi-

---

<sup>19</sup> McConville. M. Vol 10 p 189-208
als (mainly macrolides) may occur. Therefore the simultaneous use of Kokcisan® 120G and certain antibiotic drugs (i.e. tiamulin) is contra-indicated.

In summary, SAL-Na shows a selective antimicrobial action in a concentration range of 0.5 to 16 mg L⁻¹ against some Gram-positive bacterial genera while Enterobacteriaceae are resistant. Induction of resistance and cross resistance was not observed in experimental conditions. Increased shedding of Salmonella Enteritidis and Campylobacter spp. is unlikely to occur at the dose used in practical conditions. The selection of salinomycin resistant Enterococcus faecium strains is possible but resistance to salinomycin is not associated with cross resistance to antibiotics used for therapy in human or veterinary medicine.

3.5 Metabolism of SAL

3.5.1 In the target animal

A GLP-compliant metabolic study has been conducted in chickens using [¹⁴C]-salinomycin²⁰. No information on the labelled position(s) on the molecule was supplied. Male and female animals were orally dosed by gavage, twice daily and for 7 consecutive days, a quantity equivalent to that brought by feeding 70 mg SAL-Na kg⁻¹ feed. Animals (3 males and 3 females) were slaughtered 12, 24, 48 and 60 hours after the last administration. Plasma radioactivity peaked after 2 hours (0.047 μg equiv. mL⁻¹) and 4 hours (0.036 μg equiv. mL⁻¹) for the male and female, respectively, following the first administration. The main results are as follows:

(i) The cumulated excretion of radioactivity measured at the end of the last day gavage represented 92 % and 86 % of the whole administered doses for the male and female respectively. An additional 2 % was excreted during the next two days withdrawal which indicates a very fast excretion.

(ii) The HPLC analysis of the 7th day excreta extracts (46 % and 52 % final recovery) showed that unchanged SAL accounted for 3 % and 9 %, most of radioactivity appearing as an unresolved group of polar metabolites.

A modified analytical procedure²¹ applied to the same pooled 6th and 7th day excreta improved the extraction of the radioactivity (95% and 96% for male and female) and allowed a better separation of metabolites. The main results are the following:

i) Unchanged SAL co-chromatographed with standard SAL represented 66% and 8% of the whole metabolites in the male and female, respectively. If confirmed by an unambiguous identification of SAL, these data would indicate an apparent gender difference which has not been reported either formerly in the chicken (SCAN Report, 1986) or recently in the rat (see below). Moreover the high value found in the male is inconsistent with the very low concentrations of SAL found in the excreta of the rat (see below) and the mice, dog and fowl (SCAN Report, 1992). The experimental condition may appear critical for birds that exhibit very fast intestinal transit. It is noteworthy that the oral dosage of the animals by gavage does not comply with the recommendations of the Commission Directive 2001/79/EC.

ii) Eight metabolites (or groups of metabolites) were separated from male excreta, of which two represented less than 10 % and 6 represented less than 5 % of the total radioactivity. In the female 15 metabolites (or groups of metabolites) were separated of which 2 accounted for 21 % and 19 % respectively, 2 were less than 10 % and 11 less than 5 %. Most metabolites had a higher polarity than SAL. No attempt was made to identify the major ones (>10%) in the excreta of the female birds.

²⁰ Vol.11 p. 1
²¹ Vol.11, p. 110
iii) The analysis of the liver of the animals slaughtered after 12 and 24 hours withdrawal has been carried out after 20 months storage\textsuperscript{22}. It was shown that the unextractable radioactivity accounted for 37-44\% (12 h, two males and one female) and 51\% (24 h, one female) of the total. Due to the limited number of animals tested and the lack of information on the stability of residues during storage these results are of limited significance. No attempt was carried out to identify SAL metabolites in the extractable fraction.

3.5.2 In the rat

A GLP-compliant metabolic study has been carried out in the rat using the same \([14C]\)-labelled salinomycin\textsuperscript{23}. Groups of animals (males and females) were orally administered by gavage 5 mg kg\(^{-1}\) bw, daily for 7 days, then sacrificed 6 and 48 hours after the last dose. Plasma sampling and trapping of the expired air was performed daily, and selected tissues and organs were taken. The main conclusions are as follows:

(i) Peak plasma concentration was reached 6 hours after the first dosage (0.154 and 0.120 \(\mu\)g equiv. mL\(^{-1}\) for the male and female respectively).

(ii) 48 h after the last dose 94\% and 95\% (male and female) of the radioactivity was excreted. Urinary excretion accounted for 2\% and 3\% while the major route of elimination was via faeces (84\% for both sexes). About 0.6\% of the administered dose was expired which indicates the \([14C]\)-labelling positions on the molecule was not entirely stable.

(iii) Tissue distribution indicated the liver is by far the target tissue, followed by the kidney and fat by decreasing order. It appears to be independent of the gender.

(iv) The analysis of the urine and faeces in both male and female indicated that unchanged SAL represented a very minor part of the total residues in the 0-24 h following the first administration while it was absent from the excreta collected on the 6\(^{th}\)-7\(^{th}\) day of administration. The bulk of residues was separated into unresolved groups of polar compounds. If a precise comparison with the corresponding polar metabolites isolated from the chicken excreta (see above) is not possible due to the fact that the analyses have not been conducted in the same conditions, it appears that the polarity of SAL metabolites in the rat excreta is higher than in chickens excreta.

(v) The analysis of liver and kidney metabolites was conducted in similar conditions as those retained for the analysis of the excreta. Better resolution was obtained with the tissues and many metabolites (11 in the liver, 9 in the kidney) were separated which exhibited lower polarity than those in the excreta. As no similar work was performed with tissue residues in the chickens, the comparison of the metabolic fate of SAL in both species cannot be assessed.

3.6 Residues

In the metabolic study conducted in the chicken\textsuperscript{20} data have been obtained concerning the kinetics of the total radioactivity in the tissues along the withdrawal period. SAL, considered as the marker residue by the Notifier, was measured in the same samples using a validated LC-MS method. The results are summarized in Table 10. As no sex difference was observed, the average value of all the animals has been retained. The skin plus fat appears to be the suitable target tissue for monitoring. SAL is present at very low level in the liver, kidneys, muscle and skin plus fat at 12-hour withdrawal but becomes undetectable (0.001 mg kg\(^{-1}\)) after 24 hours with the exception of the skin plus fat (0.002 mg kg\(^{-1}\) at 24-hour).

\textsuperscript{22} Vol.17, p. 313
\textsuperscript{23} Vol. 17, p. 204
Despite wide individual variations it appears that the total residual concentration (radioactivity) does not decrease significantly in liver, kidney and muscle during a 60-hour withdrawal period. These findings may correspond to a long lasting compartment suggesting the existence of bound residues or the incorporation of \([^{14}C]\)-fragments into endogenous compounds. However, no attempt has been made to test these hypotheses, especially in the target tissue. The fact that the whole radioactivity increases in the skin plus fat during the same withdrawal period can not receive a scientific explanation and may be due to a flaw in the experimental conditions.

Table 10.  **Tissue residue kinetics of \([^{14}C]\)-salinomycin in chickens following repeated oral administration of the target dose (70 mg kg\(^{-1}\) feed) and application of a withdrawal period (average of 3 males plus 3 females)**

<table>
<thead>
<tr>
<th>Withdrawal [h]</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRR(^1)</td>
<td>0.063</td>
<td>0.001</td>
<td>0.039</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>SAL(^2)</td>
<td>0.042</td>
<td>&lt;LD (^3)</td>
<td>0.029</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>Liver</td>
<td>0.021</td>
<td>0.002</td>
<td>0.015</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.092</td>
<td>0.005</td>
<td>0.094</td>
<td>0.002</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin plus fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)TRR = total residual radioactivity expressed as equivalent salinomycin in fresh tissue  
\(^2\)SAL = salinomycin concentration (mg kg\(^{-1}\) in fresh tissue)  
\(^3\)LD = limit of detection: 0.001 mg kg\(^{-1}\)

The ionophoric activity of SAL metabolites extracted from the liver of chickens administered SAL supplemented feed (75 mg kg\(^{-1}\)) for 32 days then \([^{14}C]\)-salinomycin for 5 days, has been determined using a \(^{86}\)Rb radiolabeled binding assay. The affinity for rubidium binding of SAL metabolites is approximately 20% that of SAL (Dimenna et al., 1989).

### 3.7 Conclusion on metabolism and residues

Indirect evidence is given that SAL is absorbed in the chicken but to an unknown extent. Its excretion is rapid. The very limited excretion of unchanged SAL found for the female is consistent with former findings. The proportion of excreted unchanged SAL is considerably higher in the males. However, the gender difference observed seems questionable.

SAL is metabolized into a number of individual or groups of polar metabolites which represent each less than 10% of the whole radioactivity in the excreta, with the exception of two (about 20%) which have not been identified.

The metabolic fate of SAL in the rat indicates a rapid and major excretion in the faeces, the urine being a minor excretion pathway. Unchanged SAL is absent from the excreta. Groups of polar metabolites have been separated from the urine and faeces but none have been identified and no comparison can be performed with the data from the chicken. The identification of individual or groups of tissue residues has no counterpart in the chicken.

Unchanged SAL represents a very minor part of tissue residues that last until 24 hours withdrawal. After that period unextractable residues become the most important part of the whole tissue residues. No data are available concerning the separation and identification of SAL metabolites in chicken tissues. Therefore no marker residue can be established.

Kinetics of tissue residues in the chicken indicate that skin plus fat is the target tissue. The whole residual radioactivity does not decrease significantly in liver, kidney and muscle over a 60-hour withdrawal period. That suggests the existence of bound residues or the in-
corporation of [14C]-fragments into endogenous compounds. No attempt has been made to test this hypothesis, especially in the target tissue.

4 SAFETY – STUDIES ON LABORATORY ANIMALS

The materials tested in various safety studies were the salinomycin-rich fermentation product, formulated Kokcisan (e.g. acute toxicity and worker safety tests) and purified salinomycin sodium. There was no clear documentation for most studies to indicate whether the fermentation product that was tested was identical to the material currently incorporated into Kokcisan. However, it is assumed that the material provided by the manufacturer for testing in recent studies was relevant to Kokcisan as it is currently manufactured.

4.1 Acute toxicity studies

Acute oral toxicity studies were performed in rats and mice using three formulations of SAL-Na: SAL-Na 28.5% concentrate, SAL-Na 12% preparation before granulation and Kokcisan® 120G. All of the acute toxicity studies were of good quality and well reported except for the study carried out by Miyazaki et al. (1974), which was poorly reported. They were in compliance with current OECD Guidelines and all were GLP compliant.

The LD50 values indicate moderate acute oral toxicity of all the defined salinomycin products and the fermentation product in all of the species tested. When expressed in terms of the salinomycin content, the LD50's were within the ranges: 10.9 to 13.6 mg kg⁻¹ bw for male mice, 8.9 to 12.8 mg kg⁻¹ bw for female mice, 17.0 to 50.7 mg kg⁻¹ bw for male rats and 12.8 to 44.8 mg kg⁻¹ bw for female rats. These oral LD50 values indicate that the products should be categorised as “harmful”.

Clinical signs of salinomycin toxicity included sedation, lethargy and dyspnoea in rats and mice. Paresis of the hind limb and oedema of the head, forelimbs and hind paws was a common occurrence in all of the groups. These effects occurred at all doses irrespective of sex and product type. Examination of rats that died within 24 hours of oral administration of Kokcisan® 120G revealed reddish staining around the nares with blood-tinged frothy discharge, and patchy haemorrhagic lungs, pronounced congestion of the liver, kidneys and spleen, other viscera appeared normal. Rats that survived the treatment to the end of the observation period did not exhibit any of these changes. The Notifier stated that the necropsy findings were consistent with the cardiovascular system being the primary target for the acute toxic action of Kokcisan® 120G.

4.2 Genotoxicity

Studies have been performed on commercial SAL-Na products and on the fermentation product. All of the genotoxicity studies were of good quality and well reported. They were in compliance with current OECD or Good Laboratory Practice (GLP) and quality assurance statements have been included within the dossier. These are summarised in Table 11.

### Table 11. Genotoxicity of salinomycin containing compounds

<table>
<thead>
<tr>
<th>Test Performed</th>
<th>System</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salinomycin 12% mixture for granulate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial gene mutation (Reverse mutation assay)</td>
<td><em>Salmonella typhimurium</em> strains TA 1535, TA 98, TA 97&amp; TA 100</td>
<td>Negative in all strains tested both in the presence and absence of S9 at all concentrations tested: 12.3, 37, 111.1, 333.3, and 1000 μg plate⁻¹.</td>
<td>27</td>
</tr>
<tr>
<td><strong>Salinomycin 12% blended granulate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial gene mutation (Reverse mutation assay)</td>
<td><em>Salmonella typhimurium</em> strains TA 1535, TA 98, TA 97&amp; TA 100</td>
<td>Negative in all strains tested both in the presence and absence of S9 at all concentrations tested: 12.3, 37, 111.1, 333.3, and 1000 μg plate⁻¹.</td>
<td>27</td>
</tr>
<tr>
<td><strong>Other SAL-Na – 12% blended granulate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial gene mutation (Reverse mutation assay)</td>
<td><em>Salmonella typhimurium</em> strains TA 1535, TA 98, TA 97&amp; TA 100</td>
<td>Positive in TA98 at the top dose of 1000 μg plate⁻¹ (doubling of the number of spontaneous revertant colonies) in the presence of S9 but not in its absence. When the test was repeated a dose dependant increase in the number of revertant colonies was observed, although the effect was less pronounced than that observed in the first experiment. Negative in the other strains tested both in the presence and absence of S9 at all concentrations tested: 12.3, 37, 111.1, 333.3, and 1000 μg plate⁻¹.</td>
<td>27</td>
</tr>
<tr>
<td><strong>SAL-Na Fermentation Product (containing 23.4% SAL-Na)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro Mammalian gene mutation (mouse lymphoma assay)</td>
<td>L5178Y mouse lymphoma cells</td>
<td>Negative in the presence and absence of S9.</td>
<td>28</td>
</tr>
<tr>
<td>In vitro Chromosomal Aberration Assay (Metaphase analysis)</td>
<td>Chinese hamster ovary (CHO)</td>
<td>Positive in the presence of a metabolic activation system (S9). Negative in the absence of S9.</td>
<td>29</td>
</tr>
<tr>
<td>In vivo Micronucleus test in bone marrow erythrocytes</td>
<td>CD-1 mice (oral route)</td>
<td>Negative</td>
<td>30</td>
</tr>
<tr>
<td>In vivo DNA-repair assay : rat liver unscheduled DNA synthesis (UDS)</td>
<td>Alpk: ApfSD rats (oral route)</td>
<td>Negative</td>
<td>31</td>
</tr>
</tbody>
</table>

---

27 Filipie, 1995, Vol. 12, p. 47  
29 Murie, E. 1998. Vol. 12, p. 147  
30 Holmstrom and Innes, 1999, Vol. 12, p. 179  
4.2.1. In vitro genotoxicity
Most of the 12 % SAL-Na mixtures gave clearly negative results in the bacterial mutagenicity tests, however one positive result (a doubling in the number of revertants) was obtained in the TA 98 assay treated with a competitive SAL-Na (12 % blended granulate) at the top dose, 1000 μg plate⁻¹. When this assay was repeated a dose dependant increase in mutagenic activity was observed, although the extent of this activity was to a lesser degree than that observed in the first experiment. The applicant has suggested that these inconsistent results may be due to the presence of a genotoxic component within the granulate mixture (not salinomycin), however this idea has not been further investigated in assays using extracts of the active compound or pure salinomycin. Conflicting results were obtained in the in vitro mammalian assays. Salinomycin fermentation product (containing 23.4 % SAL-Na) was not mutagenic in the presence and absence of metabolic activation in the mouse lymphoma assay. However it was clastogenic in the presence of metabolic activation in duplicate experiments performed with the chromosomal aberration test (metaphase analysis) in CHO cells.

4.2.2. In vivo genotoxicity
SAL-Na fermentation product (23.4 % SAL-Na) was negative for genotoxicity in the in vivo rat liver UDS assay and in the mouse bone marrow micronucleus assay. These results indicated that the clastogenicity seen in vitro was not expressed in vivo.

4.2.3. Conclusion on genotoxicity
The SAL-Na fermentation product (23.4 % SAL-Na) gave a positive result for mutagenicity in one of the in vitro mutagenicity tests, but negative results from in vivo studies of mutagenicity in two different somatic tissues demonstrated that the in vitro mutagenicity was not expressed in vivo. Further reassurance was given by negative results in bacterial mutagenicity tests for other preparations that contained 12% SAL-Na. It was concluded that the SAL-Na fermentation product in Kokcisan® 120G was not an in vivo mutagen. It was further concluded that salinomycin from Kokcisan® 120G was not genotoxic in vivo.

4.3 Subchronic oral toxicity studies
4.3.1 Rats
In a 90-day study in Wistar rats (10 sex⁻¹ group⁻¹), two formulations of SAL-Na, Kokcisan® 120G and another additive (each containing SAL-Na at a nominal concentration of 120 g kg⁻¹ product), were administered in the diet to achieve the following dosages: 0, 0.459, 1.379 and 4.137 mg product kg⁻¹ feed (which corresponded to 0, 0.055, 0.165 and 0.496 mg SAL-Na kg⁻¹ bw day⁻¹, respectively). This treatment did not induce any clinical signs of toxicity or changes in body weight gain. Several deaths (up to 4 rats per sex per group) occurred as a result of intubation errors. One male was euthanased after a subcutaneous tumour was observed. Some statistically significant changes in haematological parameters and clinical chemistry values were reported. However they were not considered to be treatment-related changes as they were transient, were not time- or dose-related and the values reported were within the normal physiological range. This study was performed to OECD guidelines but was not GLP compliant.

As salinomycin did not cause treatment-related adverse effects at any of the doses tested, the NOEL was the highest dose tested: 0.496 mg SAL-Na kg⁻¹ bw day⁻¹.

4.3.2 Dogs
In a GLP and OECD compliant 90 day oral study in dogs groups of Beagle dogs (4 sex⁻¹ group⁻¹) were administered a daily oral gelatine capsule of SAL-Na fermentation product

---

32 Murn and Zalar, 1999, Vol. 13, p. 1
Opinion on the additive Kokcisan  - 24 of 41 -

(FP) (containing 23.4 % SAL-Na) to achieve the following dosages: 0, 0.2, 0.5 and 1 mg FP kg⁻¹ bw day⁻¹ for a minimum of 13 weeks. Control animals received an empty gelatine capsule. One male from the 1 mg kg⁻¹ bw day⁻¹ salinomycin sodium FP group was killed in extremis (prematurely on day 22 of the study). This animal had difficulty standing and was dragging its hind limbs (effects indicative of muscle weakness), it also had breathing difficulties (heavy breathing), pale mucous membranes and increased heart rate. Histological examination of the sciatic nerve of this animal showed axonal (Wallerian) degeneration. This effect may be related to the treatment and would explain some of the clinical signs reported in the animal. Histological examination also showed a slight dose dependant reduction in uterine physiological hyperplasia, and altered oestrus cycling. However the applicant states that these effects were due to chance because historical controls from 90 day studies performed at the research facility displayed a similar distribution of oestrus cycling. A slight but statistically significant drop in bodyweight gain was observed amongst the females dosed with 0.2 or 1 mg FP kg⁻¹ bw day⁻¹, and there was also a significant increase (above control) in spleen weights in females in these dose groups. However, these effects were not considered to be treatment-related as they were not observed in the males and were not dose-related. Necropsy examination did not reveal signs of toxicity in any of the major organs.

The no observed effect level for SAL-Na fermentation product (FP) established by this study was 0.5 mg FP kg⁻¹ bw day⁻¹. This is equal to an NOEL of 0.117 mg SAL-Na kg⁻¹ bw day⁻¹.

4.4 Chronic oral toxicity and carcinogenicity studies

No long term/carcinogenicity studies have been submitted. The Notifier argues that several coccidiostats of the ionophore class (salinomycin, narasin, monensin and lasalocid, maduramycin) are approved feed additives, that for all these substances no evidence is given for any mutagenicity or carcinogenicity. The Notifier states further, that SAL-Na from Kokcisan® 120G does not show a mutagenic potential and that the clastogenicity seen in vitro was not expressed in vivo. The Notifier concludes: “Based on the above, and taking into account the requirement of Directive 96/15/EC to avoid unnecessary repetition of toxicological testing in vertebrate animal, there would appear to be no justification for conducting carcinogenicity testing on SAL-Na” from Kokcisan® 120G.

But Commission Directive 2001/79/EC outlines that “a chronic toxicity study, which may include examination of carcinogenicity, must be carried out in at least one rodent species”. Exemptions for a carcinogenicity study are given: Consistently negative results in genotoxicity tests, no structural relation to known carcinogens and no effects indicative of potential neoplasia in chronic toxicity assays.

FEEDAP Panel accepts in case of SAL-Na from Kokcisan® 120G the exemptions given by Directive 2001/79/EC for not conducting a carcinogenicity study. But FEEDAP Panel also confirms the necessity of at least one chronic toxicity study particular in case of a fermentation product (with a high amount of undefined biomass). Also the consequences of a potentially lower NOEL from a chronic toxicity study for an ADI and subsequently for the safety of the consumer has to be considered.

4.5 Reproduction Toxicity

4.5.1 Fertility study in rats
A GLP-compliant two-generation rat reproduction study was performed using ‘SAL-Na fermentation product’. A certificate of analysis stated that this test material had a salinomycin content of 234 g kg\(^{-1}\) and a moisture content of 16.3 g kg\(^{-1}\), but the chemical identity of the bulk of the material was not defined. The test material was fed to the rats (Sprague-Dawley) at levels that achieved dietary concentrations of SAL-Na of 0, 100, 400 and 1200 mg kg\(^{-1}\). These concentrations delivered dosages of SAL-Na of 0, 0.55-1.51, 2.1-6.2 and 6.9-20.5 mg kg\(^{-1}\) bw d\(^{-1}\) in males and 0, 0.77-2.40, 2.8-10.4 and 9.7-29.8 mg kg\(^{-1}\) bw d\(^{-1}\) in females. These diets were fed to groups of 28 males and 28 females of the F0 generation and 24 males and 24 females of the F1B generation. At 1200 mg kg\(^{-1}\) feed there was decreased body weight gain in both sexes and some of the females adopted a hunched posture, there was a decreased number of pups born, pup weights, and litter weights were reduced and there was reduced testis weight in the F1B generation. At 400 mg kg\(^{-1}\) there were slight reductions in maternal body weight gain and pup weight. No treatment related effects on the F2 generation pups were reported. The NOEL for this study was a concentration of SAL-Na of 100 mg kg\(^{-1}\) feed, which is equal to dosages of SAL-Na in the ranges of 0.55-1.51 mg kg\(^{-1}\) bw d\(^{-1}\) in males and 0.77-2.40 mg kg\(^{-1}\) bw d\(^{-1}\) in females.

### 4.6. Special studies on cardiac effects in dogs

A group of 10 anaesthetised dogs were each given a single intravenous injection of 0.15 mg SAL-Na kg\(^{-1}\) bw (Fahim et al., 1986). The effects of salinomycin were compared with those of adrenaline. Arterial and coronary sinus blood samples were taken prior to the salinomycin injection, and at 5, 10 and 20 minutes after injection. Haemodynamic measurements were taken at the same times plus at 90 minutes after injection. The treatment with SAL-Na caused profound cardiovascular effects including increases in blood pressure (arterial pressure and left ventricle systolic pressure), cardiac output and heart beat rate, but these parameters had returned to normal after 90 mins. Calculated values for left ventricular hydraulic work were increased. The oxygen content of blood was increased in the coronary sinus but not in arteries. Plasma catecholamine concentrations were increased. The authors suggested that SAL-Na caused increases in cardiac output and pressor, chronotropic and inotropic actions as a result of the release of endogenous catecholamines into the plasma, whereas the increase of coronary blood flow was due to a non-adrenergic relaxation of the coronary blood vessels. It was not possible to identify a NOEL for this study as only one dose level was used and as oral dosing was not used.
Several reports were found in the literature showing salinomycin poisoning in turkey flocks. Levels between 24 and 37 mg salinomycin kg\(^{-1}\) inadvertently fed to turkeys resulted in mortality rates between 23 and 90 % (Halvorson et al., 1982). Feed concentrations between 15 and 30 mg salinomycin kg\(^{-1}\) caused mortality rates between 1.6 and 15.8 % in breeder herds (Stuart, 1983). Similar findings are described in case reports by Reece et al. (1985). Potter et al. (1985) could demonstrate that salinomycin became more toxic as the age of turkeys increased.

In a more recent report (Franz et al., 1992), feed contaminated with 57.9 mg salinomycin kg\(^{-1}\) killed 2300 turkeys out of a flock of 5400. Characteristically, clinically affected birds lay in sternal recumbency with both legs extended. Pathological examination showed acute toxic myopathy of the skeletal muscles and atrophy of the spleen. Andreasen and Schleifer (1994) reported losses of 21.7 % from (600) 48 week old breeder turkeys fed salinomycin at levels between 13 and 18 mg kg\(^{-1}\).

4.7.2. Horses

Toxicity of the ionophores to horses and other equines is worldwide known since decades. Kamphues et al. (1990) described anamnesis and clinical signs (mostly severe myocardial degeneration) of horses from five stables. The Notifier did not submit special studies. Rollinson et al. (1987) observed six cases of accidental salinomycin poisoning in horses. The range of clinical signs included anorexia, colic, weakness and ataxia. More recently, Nicpon et al. (1997) reported accidental poisoning of 24 horses fed 2 -3 kg feed containing 61 mg SAL-Na kg\(^{-1}\). Only six horses survived. The most characteristic clinical change appeared as paralysis of the hindlimbs.

4.8 Determination of the overall no observed adverse effect level (NOEL)

Table 12 gives a review of the different NOEL’s from the relevant studies together with the highest doses applied.

Table 12. Summary of NOELs from various toxicological studies

<table>
<thead>
<tr>
<th>Study</th>
<th>NOEL for this study (mg kg(^{-1}) d(^{-1}) of SAL-Na)</th>
<th>Lowest dose to show effects (mg kg(^{-1}) d(^{-1}) of SAL-Na)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subchronic (90 day) toxicity study in rats</td>
<td>0.50</td>
<td>No higher dose used</td>
</tr>
<tr>
<td>Subchronic (90 day) toxicity study in dogs</td>
<td>0.12</td>
<td>0.23</td>
</tr>
<tr>
<td>Two-generation reproductive study in rats</td>
<td>0.55</td>
<td>2.1</td>
</tr>
<tr>
<td>Developmental toxicity study in rats</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

The overall NOEL for SAL-Na from Kokcisan\(^{\text{\textregistered}}\) 120G was about 0.12 (0.117) mg kg\(^{-1}\) d\(^{-1}\), based on the results of a 90-day dog study. Serious adverse effects were seen in one of four male dogs in the top dose group. The possibility that the adverse effects were related to the treatment with SAL-Na could not be excluded.

There was no investigation of chronic toxicity and developmental toxicity was studied in only one species, whereas guidelines require at least one chronic toxicity study and developmental studies in at least two species. It cannot be excluded that a lower NOEL than 0.117 mg kg\(^{-1}\) d\(^{-1}\) might have been identified from one of the outstanding studies, if a full data package had been supplied. Also a NOEL based on pharmacological studies on the heart of the dog using an oral route of delivery was not identified.

5 SAFETY EVALUATION FOR THE HUMAN CONSUMER

5.1 Studies on human gut flora, antimicrobial spectrum and MIC

The study with salinomycin sodium used a total of 99 bacterial strains from 10 genera (Bacteroides, Bifidobacterium, Clostridium, Enterococcus, Eubacterium, Lactobacillus,
Peptococcus, Peptostreptococcus, Escherichia coli, Proteus) regarded as dominant in the human faecal microbiota. The study confirms the high MIC value for aerobic Gram-negative species (>256 µg mL⁻¹) and the activity against Gram-positive strains. The most salinomycin sensitive organisms were anaerobic Gram-positive strains particularly Peptostreptococcus spp (MIC₉₀ = 0.5 µg mL⁻¹).

5.2 Proposal for an acceptable daily intake

It is expected that SAL-Na as a polyether ionophore will have pharmacological effects on physiological processes that are sensitive to disrupted ion transport (e.g. the functioning of the heart and nervous system). It is noted that sub-chronic toxicity study in dogs identified a NOEL of 0.117 mg SAL-Na kg⁻¹ body weight day⁻¹ based on possible neurotoxic effects. Cardiac effects were investigated in dogs in a special study that used only intravenous dosing. No electrocardiogram measurements were made in any of the repeat dose toxicity studies. Therefore, it was not possible to identify a NOEL for possible cardiac effects following oral exposure.

It is noted that the toxicological data package is incomplete. There are areas of toxicity that have not been covered by the reports in the dossier on Kokcisan® 120G. There are no reports on chronic toxicity and developmental toxicity has been studied in only one species. It would only be possible to set an ADI if the outstanding data were to be supplied.

5.3 Proposal for the maximum residue limit

MRL’s cannot be set because of the lack of an ADI but also a lack of proper evaluation of salinomycin residues (a marker residue).

5.4 Proposal of the withdrawal period

No withdrawal period can be recommended because of the lack of an ADI and MRLs, and of a convincing relation between residue levels and withdrawal time.

6 WORKER SAFETY

6.1 Dust formation

Kokcisan® 120G is granulated to reduce dust formation. Its dusting potential, as determined using the Stauber-Heubach method, is normally in the range 0.3 to 0.5 g m⁻³. The maximum dusting potential reported outside this range was 0.8 g m⁻³. The particle size distribution of Kokcisan® 120G is such that all of the granules have diameters smaller than 800 µm, with most particles (84.9 %) being between 300 and 400 µm. Only 0.3 % were smaller than 45 µm. Thus there were no or minimal numbers of particles of respirable size in the product. In contrast however, 50.8 % of the dust particles that were suspended in air during the Stauber-Heubach testing were of diameter less than or equal to 10 µm.

It may be concluded that normal handling of Kokcisan® 120G would not produce respirable dust.

6.2 Acute inhalation toxicity

In an acute inhalation study two groups of Sprague-Dawley rats (5/sex) were exposed (by snout only) to Kokcisan® 120G which was tested as a micronised powder aerosol at an atmospheric concentration of 1.19 mg L⁻¹ and 0.19 mg L⁻¹ for 4 hours. The particle size distribution at the highest concentration was 22.5 % particles at <4.2 µm. The group exposed to 1.19 mg L⁻¹ showed shallow and gasping respiration during the exposure period.

37 Anderson BT. 1999. Vol. 18, p. 32
and closed eyes, unsteady gait and cold temperature immediately after exposure. Based on a respiratory volume of 0.2 litres per min in males and 0.15 litres per minute in females this concentration equates to a dose level of approximately 25 mg kg\(^{-1}\) bw\(^{-1}\). The lower atmospheric concentration of 0.19 mg L\(^{-1}\) produced less severe sign of toxicity e.g. subdued behaviour in all of the rats for up to 2 hours after exposure.

6.3 Irritation – Skin

Three rabbits given a 4 hour application of 0.5 g Kokcisan\textregistered 120G under a semi-occlusive dressing developed moderate skin irritation. Slight to moderate erythema was seen for up to 9 days after patch removal. All three rabbits had zero scores after 18 days\(^{38}\).

6.4 Irritation – Eye

7.9 mg, 8.3 mg, 8.1 mg of Kokcisan\textregistered 120G was instilled into the conjunctival sac of three New Zealand white rabbits each and the treated eye was examined for evidence of irritation at periods up to 22 hrs after treatment. The treatment caused severe conjunctival redness, chemosis and discharge in one of the rabbits for the first few days after treatment but the effect was not permanent and the rabbit recovered (zero scores) by day 22. One of the other two rabbits displayed only a slight conjunctival redness at 1 hour whilst the other animal developed a slight to moderate conjunctival redness and chemosis up to 24 hours after treatment. This animal also had a slight discharge at 1 hour\(^{39}\).

6.5 Skin sensitisation

The Magnusson-Kligman maximisation test was performed with female albino guinea pigs (Dunkin-Hartly strain). Following dose-range finding studies, each animal (30 = 10 controls and 20 test group) was given intradermal injections at six different regions of the scalp up to a final concentration of 1 mg kg\(^{-1}\) of Kokcisan\textregistered 120G. This treatment led to skin irritation. Each of the animals was then given a topical challenge application of Kokcisan (at a concentration of 25 mg kg\(^{-1}\)) on the scapular region.

Following the challenge phase, 8 out of the 20 animals (40 \%) in the test group showed a slight reaction (score of 1) at 24 hours and two of these animals still had a reaction score of 1 by 48 hours whilst none of animals in the control group exhibited a reaction. These responses were weak but they did indicate the potential for Kokcisan\textregistered 120G to cause a slight sensitising effect\(^{40}\).

6.6 Systemic toxicity

There is no genotoxic hazard associated with exposure to salinomycin. However, the risk to exposed workers of systemic toxicity cannot be fully evaluated as chronic toxicity and developmental toxicity have not been fully investigated.

6.7 Conclusions on worker safety

Acute oral toxicity studies showed that Kokcisan was of moderate toxicity.

The results of the acute inhalation study indicated a toxic hazard associated with inhalation of Kokcisan\textregistered 120G. However, the formulation of Kokcisan\textregistered 120G minimises dust formation, so that little dust should be generated from normal handling of the product. Consequently, there would be minimal inhalation risk to workers.

In animal experiments, Kokcisan\textregistered 120G was moderately irritating to the eyes and skin and was also a skin sensitiser.

\(^{38}\) Edgar F. and Donald E. 1998. Vol. 18, p. 71

\(^{39}\) McEwan M and Donald E. 1998. Vol. 18, p. 92

\(^{40}\) Edgar F. and Donald E. 1998. Vol. 18, p. 115
The risk from systemic exposure cannot be fully evaluated from the available data.

7 ENVIRONMENT

The active ingredient is not a physiological/natural substance of established safety for the environment. The additive is also not intended for companion animals. Consequently, the Phase I assessment has to be made to determine the predicted environmental concentration.

In Phase I and II a total residues approach will be taken and a maximum initial PEC will be calculated, based on the assumption that the additive is excreted 100% as parent compound (worst case assumption).

Distribution to other compartments is also based on parent substance properties, as long as no data on relevant metabolites are submitted.

7.1 Fate and behaviour

7.1.1 Fate in manure

No data on the fate of salinomycin in manure of the target animals have been submitted.

7.1.2 Fate in soil

Adsorption

The adsorption of [14C]-salinomycin sodium was determined in clay loam (pH 7.3, 1.6% organic carbon, 30% clay), sandy loam (pH 6.0, 1.6% organic carbon, 17% clay) and loamy sand (pH 4.7, 1.8% organic carbon, 6.6% clay) according OECD 106. In all three soil types the Freundlich constant were similar and in the range of 0.81-1.0. The Koc value for clay loam, sandy loam, loamy sand was 172, 274 and 646, respectively. The adsorption appears to increase with a decrease in pH.

Biodegradation

The degradation of radiolabelled salinomycin was investigated in three fresh field soils at a nominal temperature of 20°C. Samples of sandy clay loam (pH 5.5-6.5), loamy sand (pH 4.5-5.5) and clay loam (pH 7-8) were treated with [14C]-salinomycin at a rate approximately equivalent to 1.6 mg kg⁻¹. The samples were incubated in the dark for up to 120 days under aerobic conditions. At intervals throughout the incubation period samples were removed for analysis of total radioactivity (0, 4, 8, 16, 32, 64, 100 and 120 days). Characterisation of radioactivity in soil extract were carried out by using reverse-phase HPLC and normal-phase TLC. Chromatographic analysis indicated that salinomycin was rapidly degraded into a number of components in each soil. Several metabolites where found but not identified. Only one metabolite, described as unknown A, was observed at concentrations higher than 10% A.R. with a maximum of 14% in loamy sand at 32 days after application decreasing to 5% at 120 days. The recalculated DT₅₀ values for sandy clay loam, clay loam and loamy sand were 7.9, 15.3 and 17.6 days respectively.

Fate and behaviour in water

No data on the fate and behaviour of salinomycin in water have been submitted.

7.1.2.1 Conclusion

The Koc value of SAL varies between 172 to 646. Because of the pH dependency the lowest Koc value is used for the risk assessment. The DT₅₀ of salinomycin in soil is between 8

---

42 Keirs, D.C., Cunningham, D. and MacIsaac, E. (2000). Section 5.6, vol. 19., p. 188
and 18 days. One major unidentified metabolite is formed. Its biological activity is unknown.

### 7.1.3 Predicted environmental concentrations (PEC)

The methodology for the calculation of the maximum PEC soil, groundwater and surface water is shown in Annex 1. The calculated values are given in Table 13.

**Table 13.** Predicted environmental concentration of salinomycin in soil, groundwater and surface water.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Concentration</th>
<th>Trigger value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vulnerable area</td>
<td>Non vulnerable area</td>
</tr>
<tr>
<td>Soil</td>
<td>330 μg kg⁻¹</td>
<td>680 μg kg⁻¹</td>
</tr>
<tr>
<td>Groundwater</td>
<td>100 μg L⁻¹</td>
<td>220 μg L⁻¹</td>
</tr>
<tr>
<td>Surface water</td>
<td>10 μg L⁻¹</td>
<td>22 μg L⁻¹</td>
</tr>
</tbody>
</table>

#### 7.1.4 Conclusion

The Phase I PEC trigger value for soil and groundwater are exceeded. Therefore, a Phase II assessment is considered necessary.

The Phase II Tier A considers the total residue calculated in Phase I (initial maximum concentrations) together with an overall effect assessment based on toxicity data for the parent compound. This is a level of safety regardless the duration of exposure, accepting that the parent compound is the most toxic compound.

### 7.2 Effect assessment

#### 7.2.1 Toxicity to soil organisms

Effects on plants

Effect of salinomycin sodium on the emergence and growth of seedlings of wheat (*Triticum aestivum*), radish (*Raphanus sativus*) and mung bean (*Phaseolus aureus*) was studied in sandy loam soil (pH 6.5) mixed with horticultural grade sand at nominal exposure concentration of 1, 10 and 100 mg salinomycin kg⁻¹ soil⁴³, see Table 14. The test period was 14 days after at least 50% emergence.

Based on both emergence and growth rate, Radish appeared to be the most sensitive species. Radish seeds exposed to 10 and 100 mg kg⁻¹ ha⁻¹ very low emergence levels. Wheat and mung bean seeds exposed to 100 and ≥ 10, respectively, took longer to emergence than the control. Since the growth rate was significantly inhibited at the lowest concentration tested, no NOEC could be determined (see Table 14).

**Table 14.** LC₅₀ (emergence) and EC₅₀ (growth) for different plants

---

Species | Emergence | Growth |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₅₀ (mg kg⁻¹)</td>
<td>NOEC (mg kg⁻¹)</td>
</tr>
<tr>
<td>Wheat</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Radish</td>
<td>5.6</td>
<td>1</td>
</tr>
<tr>
<td>Mung bean</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

Effect on earthworms

The acute toxicity of salinomycin sodium fermentation product (containing 23.4% salinomycin sodium as the active ingredient) to *Eisenia fetida andrei* was determined in exposure rate ranging from 12.5 - 200 mg salinomycin kg⁻¹ dw soil for 14 days at 20 °C, according to OECD 207. An artificial soil was used composed of 70% sand, 20% kaolinite clay and 10% sphagnum moss peat (pH 6.3-6.4). The LC₅₀ was 71 mg kg⁻¹ dw. Body weight was significantly reduced at 100 mg kg⁻¹.

Effects on soil micro-organisms

Effect of salinomycin sodium fermentation product (containing 23.4% salinomycin sodium as the active ingredient) on respiration and nitrification was studied in sandy loam (pH 6.8) according OECD 216 and 217. Soil samples were fortified at a rate of 2.3 mg salinomycin kg⁻¹ and incubated at 20 °C and 40% of the maximum water holding capacity in darkness for 28 days under aerobic conditions. Samples in the nitrogen transformation test were amended with 0.5% lucerne meal. At intervals of 0-3 h, 7, 14 and 28 days the soil respiration (CO₂ formation) and nitrate content was determined. Up to 28 days no treatment effect were measured > 25%. Therefore, the NOEC for effect on soil respiration and soil nitrification is established at > 2.3 mg salinomycin kg⁻¹ soil.

7.2.2. **Toxicity to aquatic organisms**

Effect on algae

The effect of salinomycin sodium fermentation product (containing 23.4% salinomycin sodium as the active ingredient) upon the growth of the freshwater green alga *Selenastrum capricornutum* was assessed over a 72 h period. The study was conducted in accordance with OECD (1984) Guideline 201. The measured salinomycin sodium concentrations tested were 0, 0.28, 0.51, 0.93, 1.88, 3.89, and 7.67 mg kg⁻¹. The 0-72 h EC₅₀ values for salinomycin sodium on area under growth curve (i.e. inhibition of biomass growth) and average specific growth rate were 2.09 and 3.01 mg L⁻¹, respectively. The NOEC was 0.93 mg L⁻¹.

Effect on crustaceans

A 48 hour acute toxicity (EC₅₀) study was conducted with *Daphnia magna* exposed to salinomycin sodium fermentation product which contained 23.4% salinomycin sodium as the active ingredient. The test was carried out in accordance with OECD (1984) Guideline 202. Analyses were conducted to confirm that salinomycin sodium concentrations were maintained over the test period at all concentrations The mean EC₅₀ for immobilisation after 48 hours was 13.3 mg L⁻¹.

Effect on fish

---

44 Knight, B. and Dickson, J. (1999). Section 5.9, vol 20
46 Knight, B. and Dickson, J. (1999). Section 5.14, vol 20
47 Knight, B. and Dickson, J. (1999). Section 5.12, vol 20
The acute toxicity (96 h LC50) of salinomycin sodium fermentation product (containing 23.4% salinomycin sodium as the active ingredient) was tested in rainbow trout (Oncorhynchus mykiss). The test was conducted in accordance with OECD (1992) Guideline 203. Exposure concentrations were 0, 0.739, 1.27, 2.17, 4.54 and 8.77 mg L\(^{-1}\). The 96h-LC50 was 1.66 mg L\(^{-1}\) salinomycin sodium.\(^{48}\)

### 7.2.3 Bioaccumulation

No data on bioaccumulation have been submitted. In reverse osmosis water the LogK\(_{ow}\) was 2.4. No K\(_{ow}\) values could be determined in pH 5, 7 and 9 buffers as salinomycin sodium was not detectable in these solutions.\(^{49}\) However, since salinomycin is a weak acid, knowledge about the pH dependency of the Log K\(_{ow}\) is considered essential for the environmental risk assessment.

### 7.2.4 Conclusion

The available information for the soil compartment covers the three key relevant taxonomic groups: plants, soil dwelling organisms and soil micro-organisms and therefore the environmental risk for the soil compartment can be determined. Based on the lowest L(E)C50 of 1.3 mg salinomycin kg\(^{-1}\) found for plants, the PNEC for soil organisms is 13 \(\mu\)g salinomycin kg\(^{-1}\) using a safety factor of 100.

The lowest toxicity value for the aquatic compartment was found for fish with a LC50 value of 1.7 mg salinomycin L\(^{-1}\). By applying a safety factor of 1000, the PNEC for aquatic organisms is 1.7 \(\mu\)g salinomycin L\(^{-1}\).

### 7.3 Risk Characterisation

#### 7.3.1 Risk for soil organisms

The risk for soil organisms can be estimated comparing the calculated PEC with the PNEC. This comparison is shown in Table 15. The PEC/PNEC ratio for both vulnerable and non-vulnerable areas is above 1, which indicates a risk for soil organisms.

<table>
<thead>
<tr>
<th>Location</th>
<th>PEC(_{soil}) (\mu)g kg(^{-1})</th>
<th>PNEC (\mu)g kg(^{-1})</th>
<th>PEC/PNEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vulnerable areas</td>
<td>330</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Non vulnerable areas</td>
<td>680</td>
<td>13</td>
<td>52</td>
</tr>
</tbody>
</table>

There is evidence that SAL is intensively metabolised (with no metabolites excreted in > 10%), which could refine the PEC for both vulnerable and non-vulnerable areas. However, the metabolism data are equivocal (see section 3.4) and needs to be clarified first. At present, without any other additional information on the fate and behaviour and toxicity of SAL, a risk for terrestrial environment can not be excluded.

#### 7.3.2 Risk for groundwater

Based on the formula in Annex I, the PEC groundwater for both vulnerable and non-vulnerable areas is 104 and 215 \(\mu\)g L\(^{-1}\), respectively. However, based on preliminary calculation with the FOCUS model PEARL, taken the fast degradation of salinomycin into account, it is not expected that the groundwater trigger of 0.1 \(\mu\)g L\(^{-1}\) will be exceeded.

---

\(^{48}\) Knight, B. and Dickson, J. (1999). Section 5.13, vol 20

7.3.3 Risk for aquatic organisms

The risk for aquatic organisms can be estimated comparing the calculated PEC with the PNEC. This comparison is shown in Table 16. The PEC/PNEC ratio for both vulnerable and non-vulnerable areas is above 1, which indicates a risk for aquatic organisms. Due to lack of data, at present also the risk for sediment-dwelling can not be excluded.

As mentioned in section 7.3.1, PEC refinement might be possible providing that the discrepancy in the metabolism data can be clarified.

Table 16. Comparison of the PEC and PNEC for the aquatic compartment

<table>
<thead>
<tr>
<th></th>
<th>PEC surface water μg L⁻¹</th>
<th>PNEC μg L⁻¹</th>
<th>PEC/PNEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vulnerable areas</td>
<td>10</td>
<td>1.7</td>
<td>6</td>
</tr>
<tr>
<td>Non vulnerable areas</td>
<td>22</td>
<td>1.7</td>
<td>13</td>
</tr>
</tbody>
</table>

CONCLUSIONS AND RECOMMENDATIONS

From the assessment of the data submitted for Kokcisan® 120G, FEEDAP Panel draws the following main conclusions:

PRESENT EFFICACY

SAL-Na from Kokcisan® 120G is active against chicken coccidia. However, the data package for an actual assessment of the efficacy considering only trials, which were completed after 1990, does not fulfil the present requirements of Commission Directive 2001/79/EC. For chickens for fattening not any floor pen trial (instead of three) was available for consideration. Although the results of the field trials for chickens for fattening indicate, that SAL-Na from Kokcisan® 120G/kg⁻¹ diet is an effective coccidiostat, a full assessment of the up-to-date efficacy of Kokcisan® 120G was not possible.

All present efficacy trials submitted refer to 60 mg SAL-Na kg⁻¹ complete feed. The dose range 50 – 70 mg SAL-Na kg⁻¹ feed (presently approved) is not confirmed by actual data.

No data specific for SAL-Na from Kokcisan® 120G on resistance of Eimeria in chickens for fattening were submitted. From recent literature data FEEDAP Panel concludes that the efficacy of SAL-Na in field is not essentially reduced despite of a high prevalence of resistance due to effective management practices (rotation, shuttle programmes).

Experimental evidence is given that SAL-Na from Kokcisan® 120G does not influence the quality of chicken produce.

RESISTANCE OF BACTERIA

SAL is active against certain Gram-positive bacteria, while Enterobacteriaceae are resistant. Increased shedding of Salmonella Enteritidis and Campylobacter spp. is unlikely to occur at the dose used under practical conditions. Induction of resistance and cross resistance was not observed in experimental conditions. The selection of salinomycin resistant enterococci is possible but resistance to salinomycin is not associated with cross resistance to antibiotics used for therapy in human or veterinary medicine.

SAFETY FOR THE TARGET SPECIES

The margin of safety deduced from tolerance studies is about 1.7 (120/70 mg SAL-Na kg⁻¹ feed) for chickens for fattening.

Incompatibilities or interactions with feedingstuffs, carriers or other approved additives are not to be expected based on the known history of salinomycin. On the other hand, severe interac-
tions between the ionophores (including SAL-Na) and certain antimicrobials (e.g., tiamulin) were reported. Therefore the simultaneous use of Kokcisan® 120G and certain antibiotic drugs is contra-indicated.

SAFETY FOR NON-TARGET ANIMAL SPECIES

Salinomycin in doses routinely applied to chickens for fattening is toxic to horses, other equines and turkeys.

SAFETY FOR THE CONSUMER

Salinomycin is absorbed but to an unknown extent in the chicken and rapidly excreted. Unchanged salinomycin represents probably only a small fraction of the metabolites excreted, despite considerable gender differences observed which seem questionable to FEEDAP Panel due to methodological insufficiencies.

Salinomycin is metabolized to a number of individual or groups of polar metabolites, however none of them has been identified in chicken excreta.

Unchanged salinomycin represents a very minor part of the total tissue residues. After 24 hours unextractable residues predominate. No data are available concerning the separation and identification of salinomycin metabolites in chicken tissues. Therefore the marker residue can not be established.

Kinetics of tissue residues in the chicken indicate that skin plus fat could be the suitable target tissue. No significant whole residue depletion is observed after 60 hours withdrawal in any of the edible tissues. No attempt has been made to assess the nature of the residual radioactivity.

SAL-Na nor the fermentation product used for the production of Kokcisan® 120G were not genotoxic in vivo.

The lowest NOEL for SAL-Na from Kokcisan® 120G was 0.117 mg kg⁻¹ bw d⁻¹, identified from the results of a 90-day dog study.

Chronic toxicity has not been investigated and developmental toxicity was studied in only one species, whereas guidelines require at least one chronic toxicity study and developmental studies in at least two species. Furthermore, a NOEL has not been identified for pharmacological effects. It cannot be excluded that a lower NOEL than 0.117 mg kg⁻¹ d⁻¹ had been identified from one of the outstanding studies. Toxicity of SAL-Na from Kokcisan® 120G can therefore not be assessed. No ADI could be calculated because an overall NOEL could not be established.

SAFETY FOR THE USER

Acute oral toxicity studies showed that Kokcisan® 120G was of moderate toxicity. It will need to be labelled ‘R22 – harmful if swallowed’.

The results of the acute inhalation study indicated a toxic hazard associated with inhalation of Kokcisan® 120G. However, the formulation of Kokcisan® 120G minimises dust formation, so that little dust should be generated from normal handling of the product. Consequently, there would be minimal inhalation risk to workers. Nevertheless it would be prudent to take normal precautionary measures (e.g., use a face mask).

In animal experiments, Kokcisan® 120G was moderately irritating to the eyes and skin and was also a skin sensitizer. FEEDAP Panel supports the data sheet recommendations for precautionary measures.

The risk from systemic exposure can be fully evaluated from the available data if data for chronic toxicity and developmental studies are supplied.
SAFETY FOR THE ENVIRONMENT

In absence of relevant data on the relevant metabolites, all calculations are based on the assumption that the additive is excreted 100 % as parent compound.

Based on the information provided on the fate and toxicity of salinomycin, it can not be excluded that the use of Kokcisan at the recommended dose range poses a risk for the terrestrial and aquatic compartments.

MONITORING

FEEDAP Panel concludes that an ADI for SAL can not be identified at present with the data provided by the Notifier for Kokcisan® 120G.

As comparison of the metabolic profiles of the chicken with those of the rat cannot be established, it can not be assumed that the risk for the consumer from the exposure to SAL residues in chicken tissues has been duly evaluated through the toxicological studies performed on laboratory animals.

FEEDAP Panel is also unable to identify a marker residue due to the lack of data on the nature of tissue residues. A relation between withdrawal time and residue concentrations in edible tissues could not be shown either. Therefore the FEEDAP Panel cannot propose either MRL's or withdrawal time (in case an ADI could be fixed). In case that salinomycin could be established as marker residue, a validated specific and sensitive method for analysing SAL in chicken tissues is available.

RECOMMENDATIONS

Feed containing Kokcisan® 120G should be labelled with a warning statement: Avoid simultaneous administration with tiamulin, and monitor for possible adverse reactions when used concurrently with other medicinal substances.

A reference to the toxicity of SAL-Na to horses, other equines and turkeys should be given in the instructions for use.

Precautionary measures should be taken to protect workers as foreseen in the safety data sheet.

DOCUMENTATION PROVIDED TO EFSA

EFSA recognises that three applications for Brand Specific Approval of salinomycin sodium as coccidiostat have been submitted during a limited time period, one as a re-evaluation according to Article 9G of Directive 70/524/EC and two as requests for authorisation according to Article 4 of the same Directive. The data sets of each company were submitted to EFSA for scientific evaluation approximately at the same time. Since these applications are each specific for the applicant company it is not possible due to confidentiality reasons to combine data without consent from the applicants. Therefore EFSA has made an effort to encourage the three companies to share data for the parts dealing with the substance itself (salinomycin sodium). During this effort confidentiality of data was strictly applied. As an outcome of these negotiations two companies agreed to share some data. This is reflected in each of the three opinions on salinomycin coccidiostats and in drawing up each opinion the FEEDAP Panel was only allowed to access the brand specific data unless stated otherwise.

1. Original Kokcisan® 120 G (salinomycin sodium) dossier presented by KRKA, d.d., Novo mesto, Slovenia
2. Supplementary dossier from October 2002
3. Further response to questions on application for brand specific approval of Kokcisan 120G from January 2004


PANEL MEMBERS

Arturo Anadón, Margarita Arboix Arzo, Georges Bories, Paul Brantom, Joaquim Brufau de Barbera, Andrew Chesson, Pier Sandro Cocconcelli, Joop de Knecht, Noël Dierick, Gerhard Flachowsky, Anders Franklin, Jürgen Gropp, Anne-Katrine Haldorsen, Ingrid Halle, Alberto Mantovani, Kimmo Peltonen, Guido Rychen, Pascal Sanders and Pieter Wester

ACKNOWLEDGEMENT

The Scientific Panel on Additives and Products or Substances used in Animal Feed wishes to thank Dr. Derek Renshaw for the contributions to the draft opinion.

REFERENCES


Annex I

Method to determine the predicted environmental concentrations (PEC)

PEC soil

The amount of chicken manure spread on land depends on the nitrogen emission standard and the nitrogen content of the manure. According to the dataset for animal nutrition used by the SCAN, the annual feed consumption for broilers is 29 kg DM and the corresponding annual nitrogen excretion is 0.394 kg. From this it follows that for 1 kg excreted nitrogen, 83 kg feed (88% DM) is consumed. 1 mg kg\(^{-1}\) in feed and 100% excretion of coccidiostats thus yields 83 mg residue in 1 kg nitrogen (see risk assessment in the opinions of the Scientific Committee on Animal Nutrition (2003) on the use of Zn and Cu in feedingstuffs).

Different regulations exist in various member states for soil fertilisation by manure, and consequently environmental exposure to excreted residues will differ. The European Directive established a maximum level of nitrogen of 170 kg N ha\(^{-1}\) per year in vulnerable zones. For non vulnerable soils, a maximum nitrogen amount of 350 kg N ha\(^{-1}\) per year has been used. In Spaepen et al. (1997) a value of 600 kg N ha\(^{-1}\) per year is reported for Italy. This value, quoted as “Personal communication”, is not considered reliable. A maximum value of 350 kg N ha\(^{-1}\) per year is recommended for the Po valley by the Po Basin National Authority and lower values are recommended for other Italian agricultural areas. Both possibilities will be considered in this assessment.

It is assumed that in normal agricultural practice manure from broilers is only spread on arable land and not on grassland. Since most of the manure in stable is produced towards the end of the animal cycle and the additive is administrated for the production lifetime of the birds, biodegradation in manure is initially not taken into account. The concentration in soil (arable land) is calculated assuming soil density of 1500 kg m\(^{-3}\), mixing depth of 0.2 m and a the worst case of one single annual emission using the following equations:

\[
PEC_{soil} = \frac{PEC_{manure} \cdot Q}{\rho_{soil} \cdot CONV_{area\_field} \cdot DEPTH_{field}}
\]

<table>
<thead>
<tr>
<th>Input</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\rho_{soil})</td>
<td>bulk density of soil</td>
<td>1500 kg m(^{-3})</td>
</tr>
<tr>
<td>(DEPTH_{field})</td>
<td>Mixing depth with soil</td>
<td>0.2 m (arable land)</td>
</tr>
<tr>
<td>(CONV_{area_field})</td>
<td>conversion factor for the area of the agricultural field</td>
<td>10000 m(^2) ha(^{-1})</td>
</tr>
<tr>
<td>(Q)</td>
<td>nitrogen immission standard</td>
<td>[kg ha(^{-1}) yr(^{-1})]</td>
</tr>
<tr>
<td>(PEC_{manure})</td>
<td>Concentration in manure expressed per amount nitrogen</td>
<td>[mg kg(^{-1})]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Output</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PEC_{soil})</td>
<td>highest concentration in the soil</td>
<td>[mg kg(_{soil})(^{-1}) ]</td>
</tr>
</tbody>
</table>

PEC groundwater

The \(PEC_{groundwater}\) (PEC\(_{gw}\)) is calculated using the procedures recommended in the EU technical guidance document (ECB, 2003) and by the RIVM (Montforts, 1999). The PEC\(_{gw}\) was calculated using the following equations:

\[
PEC_{gw} = PEC_{pore\_water}
\]
\[ \text{PEC_{porewater}} = \frac{\text{PEC}_{\text{soil}} \cdot RHO_{\text{soil}}}{K_{\text{soil-water}} \cdot 1000} \]

\[ K_{\text{soil-water}} = F_{\text{air}_{\text{soil}}} \cdot K_{\text{air-water}} + F_{\text{water}_{\text{soil}}} + F_{\text{solid}_{\text{soil}}} \cdot \frac{K_{p_{\text{soil}}}}{1000} \cdot RHO_{\text{solid}} \]

\[ K_{p_{\text{soil}}} = F_{\text{o}_{\text{soil}}} \cdot K_{\text{o}} \]

\[ K_{\text{air-water}} = \frac{V_{\text{P}} \cdot MOL_{\text{W}}}{SOL \cdot R \cdot TEMP} \]

### Settings of the module for ground water.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density of fresh soil</td>
<td>RHO_{\text{soil}}</td>
<td>1700 kg m(^{-3})</td>
</tr>
<tr>
<td>Density of soil solids</td>
<td>RHO_{\text{solid-soil}}</td>
<td>2500 kg m(^{-3})</td>
</tr>
<tr>
<td>Fraction air in soil</td>
<td>FAIR_{\text{soil}}</td>
<td>0.2 m(^3) m(^{-3})</td>
</tr>
<tr>
<td>Fraction water in soil</td>
<td>FWATER_{\text{soil}}</td>
<td>0.2 m(^3) m(^{-3})</td>
</tr>
<tr>
<td>Fraction solids in soil</td>
<td>FSOLID_{\text{soil}}</td>
<td>0.6 m(^3) m(^{-3})</td>
</tr>
<tr>
<td>Weight fraction organic carbon in soil</td>
<td>FO\text{C}_{\text{soil}}</td>
<td>0.02 kg kg(^{-1})</td>
</tr>
<tr>
<td>Temperature at air-water interface</td>
<td>TEMP</td>
<td>285 K</td>
</tr>
<tr>
<td>Gas constant</td>
<td>R</td>
<td>8.314 Pa m(^{3}) mol(^{-1}) K(^{-1})</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>VP</td>
<td>[Pa]</td>
</tr>
<tr>
<td>Molar mass</td>
<td>MOL_{\text{W}}</td>
<td>[g mol(^{-1})]</td>
</tr>
<tr>
<td>Water solubility</td>
<td>SOL</td>
<td>[mg l(^{-1})]</td>
</tr>
<tr>
<td>Partition coefficient solids and water in soil (v/v)</td>
<td>K_{\text{soil-water}}</td>
<td>[m(^3) m(^{-3})]</td>
</tr>
<tr>
<td>Partition coefficient solids and water in soil (v/w)</td>
<td>K_{p_{\text{soil}}}</td>
<td>[dm(^3) kg(^{-1})]</td>
</tr>
<tr>
<td>Partition coefficient air and water in soil</td>
<td>K_{\text{air-water}}</td>
<td>[m(^3) m(^{-3})]</td>
</tr>
</tbody>
</table>

### PEC surface water

The PEC surface water is calculated for the additives according to the method described by the RIVM (Montforts, 1999). Here it is assumed that substances not adsorbed to soil particles may be present in the soil water and thus be prone to enter surface water during rainfall. The concentration in the surface water will be influenced by the amount of rainfall relative to the interstitial pore water and subsequent dilution by the receiving water. It is assumed that catchment areas tend to be proportional in size to the receiving stream therefore no account is taken of the size of the catchment or receiving water. Further dilution occurs on entry of the interstitial pore water into receiving water. This dilution factor can be established on the basis of the persistence of the chemical. As a worst case default a factor of 1 (no dilution) is considered for persistent compounds and 10 for non persistent compounds.

The possibility also exists that manure containing the additives remaining on the top soil surface after application can enter surface waters due to run-off associated with rainfall. At present this superficial loading of the aquatic compartment is not considered because appropriate models for feed additives are not available.
REFERENCES


