Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the request of the Commission on the reevaluation of coccidiostat Elancoban in accordance with article 9G of Council Directive 70/524/EEC

(EFSA-Q-2003-0045)

Adopted on 4 March 2004

SUMMARY

Elancoban is a feed additive intended for the control of coccidiosis, a debilitating protozoal infection in poultry. With a number of other coccidiostats, Elancoban is due for re-evaluation to comply with statutory requirements agreed at an EU-level. The European Commission asked the EFSA to evaluate the product 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 active agent in Elancoban is monensin sodium, a polyether ionophore produced by Streptomyces cinnamonensis that exhibits both antibacterial and anticoccidial activities. Elancoban is the granulated fermentation medium which contains 20 % (w/w) monensin sodium. Evidence generated at the times of its introduction suggested that Elancoban supplementation delivering 100 to 125 mg monensin sodium kg\(^{-1}\) complete feed for chickens for fattening, 100 to 120 mg kg\(^{-1}\) for chickens reared for laying and 90 to 100 mg kg\(^{-1}\) for turkeys, was effective to control coccidiosis. However, a full assessment of the present day efficacy for these species and categories was not possible as only an insufficient number of trials reflecting current production conditions, had been carried out in the last decade.

Tolerance tests showed that Elancoban is safe for the target animals although the margin of safety is very small. There are known interactions of monensin sodium with the drug tiamulin.

Monensin sodium is active mainly against Gram positive bacteria and laboratory studies have shown that they can develop resistance to the additive. However, no evidence was found that exposure to monensin sodium would induce the development of a cross-resistance to other antibiotics used for humans or animals. There was no evidence that colonisation and shedding of enteropathogens were influenced by monensin sodium supplementation.

Monensin sodium is absorbed and metabolized extensively by chicken and turkey and its metabolic fate is similar in both species and also in the laboratory animals (rat) used to assess its toxicity. Unchanged monensin represents a limited fraction of the excreted monensin-related compounds. For practical reasons the skin/fat has been retained as the
target tissue. A great number of metabolites have been isolated from the excreta and tissues of which eight major metabolites have been identified each representing less than 10% of the total residues. Metabolites are produced by either single or combined demethylation, decarboxylation and hydroxylation reactions. It is reasonable to consider that their biological activity is much lower than that of monensin. The kinetic study of the total residues in tissues is available in the chicken but not the turkey (only 0-day withdrawal). The proportion of unextractable but not drug-related residues in tissues increases with the withdrawal time. Although monensin represents a very limited fraction of tissue residues, the FEEDAP Panel proposes it as the marker-residue.

The passage of monensin in the eggs is very limited, non cumulative and declines rapidly when monensin sodium administration to hens ceases. Therefore, the contribution of eventual monensin residues from the first eggs laid by chickens reared for laying to the consumer exposure is considered as negligible.

Monensin sodium is neither genotoxic nor carcinogenic. No indication of embryotoxicity, fetotoxicity or teratogenicity was found. Monensin sodium exhibits inotropic activity, i.e. acute pharmacological effects on the cardiovascular system. It is highly toxic for horses. The lowest NOEL (no observed effect level) identified from the toxicological studies was 1.2 mg per kg body weight per day based on the 2-year chronic toxicity/carcinogenicity assay in mice. Acute pharmacological effects on the cardiovascular system of the dog lead the FEEDAP Panel to identify a lower NOEL for monensin sodium of 0.345 mg per kg body weight per day and an ADI (acceptable daily intake) of 0.003 mg per kg body weight.

The FEEDAP Panel proposes a provisional and uniform MRL for all tissues (0.05 mg kg\(^{-1}\)) and a 3-day withdrawal period for both the chicken and turkey.

Elancoban is very irritant to the eye but not to the skin. It is regarded as a weak sensitiser by skin exposure and a potential respiratory sensitiser. Moreover the dermal and respiratory exposure could lead to systemic pharmacological effect, namely to the heart. Although the granular formulation of Elancoban minimises the release of monensin sodium dust from the product, further protective measures to limit worker exposure at the feed industry level need to be considered.

The use of Elancoban at the recommended dose range is not considered to pose a risk for soil organisms. However, insufficient data was provided to allow the FEEDAP Panel to assess the risk for the aquatic environment (groundwater and surface water) and secondary poisoning.

Key words: Elancoban, coccidiostat, feed additive, ionophore, monensin sodium, anticoccidial efficacy, microbiological risks, target animal safety, consumer safety, ADI, MRL, worker safety, environmental safety

BACKGROUND

According to article 9g of Directive 70/524/EEC as amended by Directive 96/51/EC, additives subject to authorisation linked to a person responsible for putting them into circulation, included in Annex I before 1\(^{st}\) January 1988 should be re-evaluated.

In accordance with article 9g of Directive 70/524/EEC the person responsible for each product had to provide a new application for the authorisation for its product, including a monograph and an identification note before the 1\(^{st}\) October 1998. Furthermore, a dossier, as referred to in article 4 of Directive 70/524/EEC, had to be submitted not later than 1\(^{st}\) October 2000.
The Directive requires that the re-evaluation of the dossiers be completed 3 years after the submission of the dossier, this means before 1st October 2003. Fifteen dossiers have been submitted before the deadline of 1st October 2000. Each Member State rapporteur, and the other Member States, checked the dossiers for their compliance with the Guidelines for the assessment of additives in animal nutrition laid down in Council Directive 87/153/EEC as amended by Commission Directive 94/40/EC. The outcome of Member States' check was endorsed at the meeting of the Standing Committee for Animal Nutrition on 29 January 2001.

Seven dossiers for products of the category "Coccidiostats and other medicinal substances" fulfilled the requirements of the guidelines and have therefore been retained for re-evaluation.

**Coccidiostats**

- Decoquinate (DECCOX®)
- Halofuginone (STENOROL®)
- Lasalocid sodium (AVATEC 15%®)
- Monensin sodium (ELANCOBAN®)
- Narasin (Monteban®)
- Salinomycin sodium (SACOX 120 micro-granulate®)
- Robenidine hydrochloride (CYCOSTAT 66G®)

The Standing Committee for Animal Nutrition started the re-evaluation of the safety and the efficacy of these products on 29 January 2001.

This opinion deals with monensin sodium (Elancoban®).

**TERMS OF REFERENCE**

The Commission requests EFSA to consider each of the above mentioned products and to advise it on their efficacy and their safety. In assessing the product on the basis of the dossiers presented, the EFSA 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 Table 1 demonstrated?

* For the product considered, can its use 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?

Table 1. Annex inscriptions
<table>
<thead>
<tr>
<th>Additive (trade name)</th>
<th>Composition, chemical formula, description.</th>
<th>Species or category of animal</th>
<th>Maximum age</th>
<th>Minimum content mg of active substance kg⁻¹ of complete feedingstuff</th>
<th>Maximum content mg of active substance kg⁻¹ of complete feedingstuff</th>
<th>Other provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monensin sodium (Elancoban)</td>
<td>C₃₆H₆₂O₁₁Na (sodium salt of a polyether monocarboxylic acid produced by <em>Streptomyces cinnamonensis</em>)</td>
<td>Chickens for fattening</td>
<td>-</td>
<td>100</td>
<td>125</td>
<td>Use prohibited at least 3 days before slaughter Indicate in the instructions for use: &quot;Dangerous for equines&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Turkeys</td>
<td>16 weeks</td>
<td>90</td>
<td>100</td>
<td>&quot;This feedingstuff contains an ionophore: simultaneous use with certain medicinal substances (e.g. tiamulin) can be contra-indicated&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chickens reared for laying</td>
<td>16 weeks</td>
<td>100</td>
<td>120</td>
<td>Indicate in the instructions for use: &quot;Dangerous for equines&quot; &quot;This feedingstuff contains an ionophore: simultaneous use with certain medicinal substances (e.g. tiamulin) can be contra-indicated&quot;</td>
</tr>
</tbody>
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1. Introduction

ELANCOBAN® is a feed additive used to control coccidiosis in chickens for fattening (broilers), fattening turkeys and replacement layers. It is a brown product speckled with pale straw-coloured particles made of the whole dried and granulated monensin fermentation medium, an antidusting oil (light petroleum oil called also paraffin oil) and a diluent (rice hulls, granular limestone). Monensin sodium is the active ingredient that represents 20 % (w/w) of the granulated monensin medium. The product is distributed in two different formulations: Elancoban 200 (or Elancoban G200) and Elancoban 100 (or Elancoban G100) that contain 200 and 100 g monensin kg⁻¹ respectively (Table 2).

Table 2. Typical composition of Elancoban 200 and Elancoban 100

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>ELANCOBAN 200 g kg⁻¹</th>
<th>ELANCOBAN 100 g kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monensin granulated (equiv. monensin sodium)</td>
<td>930 (200)</td>
<td>500 (100)</td>
</tr>
<tr>
<td>Antidusting oil</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Rice hulls or limestone granular</td>
<td>50</td>
<td>300</td>
</tr>
<tr>
<td>Limestone granular</td>
<td>-</td>
<td>180</td>
</tr>
</tbody>
</table>

The petitioner applies for dose levels of 100-125 mg monensin sodium kg⁻¹ complete feed for chickens for fattening, 100 to 120 mg kg⁻¹ for chickens reared for laying and 90 to 100 mg kg⁻¹ for turkeys. The product should be administered during the fattening period until three days before slaughter of the chicken for fattening but for only until 16 weeks of age only in the chicken reared for laying and the turkey.

Monensin sodium is a polyether ionophore that exhibits both antimicrobial and anticoccidial activities. It is used to improve performance in cattle and to prevent or treat coccidiosis in poultry. Monensin sodium is not used in humans.

Monensin sodium A [2-(5-ethyltetrahydro-5-(tetrahydro-3-methyl-5-(tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl-2-furyl)-2-furyl)9-hydroxy-jmethoxy-a,g,2,8-tetramethyl-1,6-diaoxaspiro(4,5)decane-7-butyric acid ; C₃₆H₆₁O₁₁Na ; CAS: 17090-79-8] (Figure 1) belongs to the monocarboxylic acid polyether chemical family. Characterisation by mass spectrometry and NMR analysis is described.
Monensin sodium appears as a white crystalline solid which physico-chemical properties are summarized on Table 3.

Table 3. Summary of physical and chemical properties of monensin sodium A

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>670 g mol⁻¹</td>
<td>Acid</td>
</tr>
<tr>
<td></td>
<td>692 g mol⁻¹</td>
<td>Sodium salt</td>
</tr>
<tr>
<td>Water solubility</td>
<td>Degraded</td>
<td>pH 4</td>
</tr>
<tr>
<td></td>
<td>4.8 mg l⁻¹</td>
<td>pH 7 buffer</td>
</tr>
<tr>
<td></td>
<td>8.9 mg l⁻¹</td>
<td>pH 9 buffer</td>
</tr>
<tr>
<td>log Kow</td>
<td>4.2</td>
<td>pH 5 Sodium salt</td>
</tr>
<tr>
<td></td>
<td>2.8</td>
<td>pH 7 Sodium salt</td>
</tr>
<tr>
<td></td>
<td>3.8</td>
<td>pH 9 Sodium salt</td>
</tr>
<tr>
<td>pKa</td>
<td>6.65</td>
<td></td>
</tr>
</tbody>
</table>

Monensin acid is produced by *Streptomyces cinnamonensis* or mutants thereof. The original culture is deposited with the American Type Culture Collection (ATCC No 15413). At harvest amyl alcohol is added to the broth to prevent growth of foreign organisms. Monensin is recovered as the sodium salt of four main factors: monensin A, B, C and D (see chemical structure above) which relative biopotency assayed against *Enterococcus faecium* are 1, 0.28, 1.5 and 1.5 respectively. The notifier guarantees the following composition:

<table>
<thead>
<tr>
<th>Monensin composition</th>
<th>weight (%)⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monensin A</td>
<td>&gt; 95</td>
</tr>
<tr>
<td>Monensin A plus B</td>
<td>&gt; 96</td>
</tr>
<tr>
<td>Monensin C plus D</td>
<td>&gt; 2</td>
</tr>
</tbody>
</table>

⁴ HPLC determination

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⁴ Section II, Vol.2 of the company dossier
Monensin granulated is produced by passing the dried mycelium monensin through a pellet mill. If higher potency monensin granulated is desired, crystalline monensin sodium may be added in order to achieve the desired potency.

The fermentation process is performed under sterile conditions and protective measures are taken during the processing of the broth to avoid biological contaminations. Results of trace contaminant (heavy metals, aflatoxins) determinations in granulated monensin comply with the requirements of Directive 2003/100/EC. Dioxin controls are performed on the incoming material during the process and on the final product. Microbiological controls on Elancoban 100 and 200 indicate the absence of the producing strain, an acceptable level for the total microflora and fungi as well as the compliance to mycotoxin contamination limits.

Particle size determinations indicate that less than 0.1 % of the monensin granular material or Elancoban 200 and less than 0.05 % of Elancoban 100 have an aerodynamic particle diameter below the respirable limit value of 10 µm. Less than 10 % of each of the three products have a particle size below 125 µm.

1.1. Mode of action

Monensin sodium acts as an ionophore, i.e. a chemical substance that complexes monovalent cations with the lipophilic structure that facilitates the transport of the bound ion through biological membranes. Coccidial sporozoites exposed to monensin sodium in the intestinal lumen exhibit considerable swelling, large vacuoles, pitting and holes in the surface suggesting extreme osmotic damage which is potentially lethal. Development of a sporozoite that successfully invades a host cell is inhibited as the ionophore continues its destructive process. Monensin sodium selectively destroys intracellular sporozoites while remaining relatively non-injurious to the host cell (Chapman, 1993). It has been shown that monensin sodium has an effect on second generation merozoites but not upon developing gametocytes. Monensin sodium is primarily coccidiocidal in action and active against *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. mivati*, *E. necatrix* and *E. tenella* of the chicken and *E. melagrimitis*, *E. pallida*, *E. dispersa* and *E. adenoeides* of the turkey.

1.2. Stability

A number of shelf life studies has been carried out to evaluate, in different conditions, the stability of Elancoban 100 (2 years at 25°C; 3 months at 50°C and 6 months at 40°C; 36 months at 25°C and 37°C) and Elancoban 200 (2 years at 25°C; 1 year at 37°C; 1 year at 25°C). An HPLC but also a microbiological method has been used to measure an eventual decrease in monensin sodium concentration or bioactivity. Monensin sodium appears very stable, no significant losses being measured after 2 years storage at 25°C. Similar results have been obtained for premixes with minerals.

No monensin activity is lost during feed processing including pelleting conditions. Four chicken feed batches supplemented with 100 or 121 mg kg⁻¹ monensin sodium have been stored at 25°C or 37°C and 75 % relative humidity for 3 months. No significant loss of monensin sodium was measured which validates the notifier's proposal of 3 months and 25°C for the shelf life of monensin sodium supplemented feed.

The homogeneity of Elancoban 100 tested on eight batches and five samples per batch has shown a coefficient of variation of ± 10 % which is acceptable. The
homogenity of a chicken feed supplemented with Elancoban 100 (six samples and six determinations per sample) gave a coefficient of variation of 6 %.

1.3. Control methods

1.3.1. Determination of monensin sodium in poultry feed

A method has been published describing the detection and quantification of monensin sodium in raw material (Elancoban 100 or 200), premix and animal feed based on liquid chromatography with post column derivatization with vanillin and visible wavelength detection (Rodewald et al., 1992). The method proved to be specific for monensin A and B when tested for potential interferences of other ionophores (narasin, salinomycin, lasalocid) and 18 different current feed additives and medicinal drugs. The limit of detection (LOD), established as the absolute amount required to generate a signal-to-noise ratio of 3, was estimated to 0.3 mg monensin A kg\(^{-1}\). The limit of quantification (LOQ) was 5 mg kg\(^{-1}\). An interlaboratory study conducted in the United States, Canada, France and Germany confirmed the efficacy and reliability of that method for the determination of monensin sodium in premixes and feeds and established lower LOQ (0.08 mg kg\(^{-1}\)) and LOD (0.04 mg kg\(^{-1}\)) (Coleman et al., 1997).

1.3.2. Determination of monensin in poultry tissues

The method described above (1.3.1) has been adapted and extended to the determination of monensin in poultry tissues, then validated\(^2\). Its specificity was that already mentioned. The LOQ was established to 0.025 mg kg\(^{-1}\) for the muscle, liver and skin/fat while the LOD was 0.005 mg kg\(^{-1}\).

A more sensitive immunoaffinity chromatography/chemiluminescent ELISA method has been developed (Godfrey et al., 1997) that allows to determine monensin in chicken tissues with LODs around 0.001 mg kg\(^{-1}\) in the fat, liver, kidney and muscle, but 0.002 mg kg\(^{-1}\) in the skin. That method has not been validated yet.

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 and the genetic progress in the daily body weight gain has changed the chickens’ performance and consequently the efficacy of coccidiostats should be assessed using recent data.

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 this evaluation of Elancoban, FEEDAP Panel took therefore only efficacy studies into consideration conducted after about 1990.

An exception is made 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.

\(^2\) Vol.2, ref.24
2.1. Dose titration and confirmation studies

Judged by the prevention of mortality and body weight gain, a concentration of 121 mg monensin sodium from Elancoban kg\(^{-1}\) complete feed was shown to be effective against *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. mivati*, *E. necatrix* and *E. tenella*, although some lesions and oocysts were produced in treated birds (Shumard and Callender, 1968). Chapman (1976) found that monensin sodium from Elancoban was effective on the basis of body weight gain at 125 mg kg\(^{-1}\) feed but that complete suppression of oocyst production and lesions was only achieved with 250 mg kg\(^{-1}\). Although increased incorporation rates of monensin resulted in better coccicidal conditions, as indicated by reduced mortality, oocyst output and faecal score, body weight gains were poor. The dose of 121 mg kg\(^{-1}\) seems to be a compromise between the amount of monensin allowing a normal development of the birds and an adequate anticoccidial activity (Ryley and Wilson, 1975).

2.1.1. Chickens for fattening

Four published studies on the efficiency of chickens for fattening of controlled battery trials were performed post 1990.

The first study (Watkins et al., 1990) investigated the effect of Elancoban (100 mg monensin sodium kg\(^{-1}\) diet) against experimentally produced infections of *Eimeria mitis*. Four chick trials were conducted using 13 replicates each for the uninfected, untreated chicks and for the infected, untreated chicks. There were 17 replicates for the infected chicks fed the basal containing 100 mg of monensin. Each replicate consisted of either 4 or 5 birds per pen. On day 7 after hatching the male chicks were randomly assigned to treatment group on basis of body weight. Two days after the initiation of each experiment, the chicks were crop-intubated with oocysts of *E. mitis*. At 7 days postinoculation the birds were killed. A reduction of body weight gain by 21 %, of feed efficiency by 7 % and relatively high intestinal scores are observed in infected untreated group. The infected birds fed diets containing Elancoban had gains and feed:gain ratios similar (P>0.1) to those of non infected birds. No differences (P>0.1) in the intestinal scores were detected between the infected birds fed Elancoban and the non infected, untreated birds.

In the second study (Williams, 1992) three experiments were carried out to investigate why the anticoccidial monensin is more potent against the coccidium *Eimeria tenella* in chickens fed on a maize-based diet than in chickens fed a wheat-based diet. The concentrations of monensin sodium were 0, 25, 50, 100, 200, 300 or 400 mg kg\(^{-1}\) feed in experiment 1, 0, 25, 50, 100, 200 or 300 mg kg\(^{-1}\) in experiment 2 and 0 mg kg\(^{-1}\) in experiment 3. Each concentration of monensin was administered to equal numbers of chicks (two groups of five each). Each chick was infected where appropriate with 50 000 sporulated oocysts of *Eimeria tenella*. Mortality and the presence of caecal lesions in the survivors were recorded on the fifth day after infection, when lesions are maximal, and the experiments were therefore terminated. Using mortality as a criterion in the treated birds, the potency of monensin was greater in the maize diet than in the diet wheat.

In the third study (Logan et al., 1993) a series of 39 trials employing monospecific infections of *E. acervulina*, *E. mitis/E. mivati*, *E. maxima*, *E. brunetti* or *E. tenella* evaluated the effect of a supplementation with 100 mg monensin sodium kg\(^{-1}\) feed but also of a supplementation with another coccidiostat. The comparison was
performed between infected/treated, infected/untreated and non infected/untreated treatments. Broiler chickens were housed in battery units or tier brooders. Treatments consisted of five or four pens each containing 8 or 10 chicks per pen. Monensin significantly improved body weight gain and feed conversion compared with the infected untreated group.

In the fourth study (Varga et al., 1994) seven battery tests were conducted in order to evaluate the effects of 6.3, 12.5, 25, 50 and 100 mg monensin sodium kg\(^{-1}\) feed, but also of three other anticoccidials. The comparison was performed between infected/treated, infected/untreated and non infected/untreated treatments. At the age of 7 days the chickens were orally infected with sporulated oocysts of coccidian, and the surviving animals were killed 8 days later. 100 mg monensin kg\(^{-1}\) feed improved body weight gain and reduced number of birds showing lesions in the caeca as well as the number of oocysts per gram of faeces compared with infected untreated group.

### 2.1.2. Turkeys

Two trials were conducted by Cabel et al. (1991) to compare the efficacy of monensin (60 and 100 mg kg\(^{-1}\) feed) with that of different anticoccidials (SYN 1, SYN 2, SYN 3, SYN 4) for turkeys against a challenge using a field isolate of mixed *Eimeria* species (*E. adenoides, E. gallopavonis, E. meleagrimitis*). Day old male turkey poults (Nicholas Large White) were placed in wire-floored batteries and fed an untreated diet. At day 18 (trial 1) or 21 (trial 2) days of age, the poults were randomly distributed among 42 batteries (5 birds per pen) and fed the test diets. Each diet was fed to 6 replicate pens. The poults were challenged at day 23 or 26 of age for trial 1 and 2, respectively. Three of the six replicate pens from each treatment were inoculated with 30 000 oocysts and the other three pens with 60 000 oocysts per bird. Total faecal collections were obtained for days 1 to 5 and 6 to 10 post challenge for oocyst output determination. The birds were weighed at day of challenge and at 5 and 10 days post-challenge and killed at 10 days post-challenge to determine severity of intestinal lesions (score: 0, non visible lesions to 4, most severe lesions). In comparison with the untreated control, all anticoccidials supported significantly greater body weight gain in challenged turkeys in both trials (Table 4). In trial 1, birds fed monensin (60-100 mg kg\(^{-1}\)) showed the least severe intestinal lesion score. In trial 1, from day-1 to day-5 post challenge, oocyst counts were lower in turkeys fed SYN 3 and monensin (100 mg kg\(^{-1}\)). In trial 2, there were no significant differences in oocyst counts among birds fed any of the anticoccidials. The effects of the anticoccidials on faecal oocyst counts 6 to 10 days post challenge were more variable. In trial 1, none of the anticoccidials significantly reduced fecal oocyst counts as compared with the control, however there were significant differences between anticoccidials. In trial 2, faecal oocyst counts of turkeys fed monensin (100 mg kg\(^{-1}\)) were significantly lower than those fed the control diet. No data on mortality are given.
Table 4. Effect of different anticoccidials on body weight gain, lesion scores and oocyst counts in challenged turkeys\(^1\) (Cabel et al., 1991)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight gain (g)(^2)</th>
<th>Lesion score</th>
<th>Oocyst excreted per bird (x 10(^6))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Trial 1</td>
</tr>
<tr>
<td>IUC(^4)</td>
<td>367(^e)</td>
<td>552(^d)</td>
<td>1.30</td>
</tr>
<tr>
<td>Monensin (60)</td>
<td>751(^b)</td>
<td>-</td>
<td>0.13</td>
</tr>
<tr>
<td>Monensin (100)</td>
<td>736(^ab)</td>
<td>909(^bc)</td>
<td>0.20</td>
</tr>
<tr>
<td>SYN 1</td>
<td>635(^c)</td>
<td>830(^cd)</td>
<td>0.40</td>
</tr>
<tr>
<td>SYN 2</td>
<td>651(^bc)</td>
<td>941(^ab)</td>
<td>0.73</td>
</tr>
<tr>
<td>SYN 3</td>
<td>667(^abc)</td>
<td>991(^a)</td>
<td>0.40</td>
</tr>
<tr>
<td>SYN 4</td>
<td>485(^d)</td>
<td>796(^c)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Values in columns with different superscripts differ significantly (P<0.05)

1. mixed field isolates (E. adenoides, E. gallopavonis, E. meleagrimitis)
2. mean body weight gain from d 23 to d 28 (trial 1), d 26 to d 31 (trial 2)
3. PC: post challenge
4. IUC: Infected untreated control
SYN: synthetic anticoccidial drug

2.2. Controlled floor pen studies

2.2.1. Chickens for fattening

Two floor pen studies (McDougald et al., 1996) were conducted in broiler chickens using the same facility and protocol to evaluate Elancoban (110 mg monensin sodium kg\(^{-1}\) feed) and of two other ionophores on performance and coccidial lesion scores of the birds, in comparison with infected untreated treatment. In each trial treatments were assigned to 12 pens (30 female and 30 male birds per pen) each in a randomized complete block design. On day 24 of each test, birds in each treatment were inoculated via the feed with a mixture of recent field isolates of *Eimeria* spp. at a dose rate calculated to provide 2x10\(^5\) E. acervulina, 3x10\(^4\) E. maxima, and 2x10\(^4\) E. tenella sporulated oocysts per bird. Standard diets were fed during the starter (days 0-21), grower (days 22-38), and finisher (days 39-43) periods in each trial. Results from the two trials were pooled for statistical analysis.

Feed analysis data (coccidiostats content) were not presented. The experimental data basis was small and the results from both experiments were pooled, which weakens the analysis.

Total mortality for all causes was low across treatments. No coccidiosis-related mortality occurred (Table 5). Body weight gain and feed conversion were significantly improved in the monensin-treated broilers and in the two other anticoccidial treatments in comparison with the untreated broilers.

Table 5. Broiler performance in two floor pen studies, pooled data

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality %</th>
<th>Body weight gain, kg</th>
<th>Feed gain ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUC</td>
<td>2.71</td>
<td>1.642(^a)</td>
<td>2.105(^a)</td>
</tr>
<tr>
<td>Monensin sodium</td>
<td>2.71</td>
<td>1.707(^b)</td>
<td>2.044(^b)</td>
</tr>
</tbody>
</table>

Values in columns with different superscripts differ significantly (P<0.05)

IUC: infected untreated control
Opinion on Elancoban

Monensin was effective in controlling lesions in the middle and cecal zone (Table 6).

Table 6. Mean intestinal lesion scores (%) at day 31

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Upper zone</th>
<th>Middle zone</th>
<th>Cecal zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUC</td>
<td>2.17</td>
<td>1.19 a</td>
<td>1.53 b</td>
</tr>
<tr>
<td>Monensin sodium</td>
<td>1.80</td>
<td>1.74 b</td>
<td>0.53 a</td>
</tr>
</tbody>
</table>

Values in columns with different superscripts differ significantly (P<0.05)
IUC: infected untreated control

2.2.2. Turkeys

Four post 1990 floor pen studies are included in the dossier.

Two floor pen trials involving a total of 560 males (trial 1) and 560 females (trial 2) have been performed to evaluate the effect of a continuous monensin program from one day-of-age to 16 weeks age (hens) and 18 weeks age (males). From one day-of-age to 10 weeks birds were fed a diet containing 90 mg monensin sodium kg\(^{-1}\) feed. From week 10 until market age one-forth of the turkeys were fed a diet containing either 0, 60, 80 or 100 mg monensin sodium kg\(^{-1}\). Live weight, feed:gain and mortality were recorded during this period. Feeding of monensin to turkeys at 60-100 mg kg\(^{-1}\) to market age under conditions of low coccidial challenge resulted in no significant effect on body weight gain, feed conversion or mortality when fed to hens, but males fed 60 mg monensin kg\(^{-1}\) were significantly heavier than the control birds and males fed monensin at 80 mg kg\(^{-1}\) had significantly improved feed:gain ratio. Mortality was not affected. There was an inverse linear relationship between oocyst numbers and dose of monensin.

Two trials were conducted in the USA (Cabel and Waldrup, 1991) with young turkeys (Nicholas Large White) fed diets treated with different anticoccidials from day-old to 8 weeks of age. Sixteen females or 12 males were placed in each pen with 24 pens of each sex. For both trials, each of the dietary treatment was assigned to four pens of each sex. Body weight and feed:gain ratio on week-8 in both trials of birds fed monensin sodium (60 and 100 mg kg\(^{-1}\)) were within the middle of the range, noted with the anticoccidials tested. Overall deaths were normally distributed among treatments and not related to dietary treatments. Only occasionally were minor coccidial lesions found in birds that died during the trials. No untreated control group was included in the experiment.

Watkins et al. (1993) evaluated graded levels of monensin sodium (0, 60, 100, 140 mg kg\(^{-1}\) feed) on the health and growth of Nicholas males 5 to 10 weeks of age. A grower ration was fed from day-35 to day-55 and a developer ration from day-56 to trial termination on day-71. All diets were based on corn (40-60 %) and soybean meal (25-45 %). Each treatment group was replicated 8 times with 24 birds per replicate. Gain was determined from initial (day-34, average initial weight was 1.55 kg) and final bird weights. Feed:gain ratios were calculated. As no oocysts were found during the study, it can be assumed that the birds were not exposed to a coccidial challenge. Increasing the level of monensin linearly decreased feed:gain ratio (Table 7). Feed intake was significantly decreased with 100 mg monensin kg\(^{-1}\). No significant effect on mortality was observed.

---

Table 7. Effect of monensin on the performance and mortality of males from 5 to 10 weeks of age (Watkins et al., 1993)

<table>
<thead>
<tr>
<th>Treatment mg kg⁻¹</th>
<th>Body weight gain kg</th>
<th>Feed intake kg</th>
<th>Feed:gain</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.89ᵇ</td>
<td>8.35ᵃ</td>
<td>2.14ᵃ</td>
<td>3.65</td>
</tr>
<tr>
<td>60</td>
<td>3.95ᵃᵇ</td>
<td>8.04ᵃᵇ</td>
<td>2.03ᵇ</td>
<td>2.08</td>
</tr>
<tr>
<td>100</td>
<td>3.96ᵃᵇ</td>
<td>7.92ᵇ</td>
<td>2.00ᵇ</td>
<td>1.04</td>
</tr>
<tr>
<td>140</td>
<td>4.08ᵃ</td>
<td>7.99ᵃᵇ</td>
<td>1.95ᶜ</td>
<td>3.65</td>
</tr>
</tbody>
</table>

Chapman and Saleh (1999) conducted in the USA a floor pen trial with different concentrations of Elancoban (0, 60, 80, 100 mg monensin sodium kg⁻¹ feed). One day old males (Nicholas) were randomly allocated to 56 pens (12 poults per pen) on concrete floors. Each treatment contained 16 replicate pens (except for treatment 0 mg kg⁻¹, eight pens). Poults were weighed when they were 3 and 10 weeks old. All poults were infected at 2 weeks of age with a mixture of three species of Eimeria (100 000 oocysts per bird) (E. adenoides, E. gallopavonis, E. meleagrimitis). Samples of litter were collected from four randomly selected pens from each treatment every 7 days until the birds were 10 weeks old, for oocyst counts. Poults given 60 mg kg⁻¹ monensin were heavier and had a lower feed conversion at three weeks than poults given 80 or 100 mg monensin kg⁻¹ feed, but at ten weeks no differences in body weight or feed conversion of poults given different concentrations of drug were apparent (Table 8). Best suppression of parasite multiplication was achieved with the higher concentrations of monensin. Lesion scores were not performed. Monensin significantly suppressed mortality.

Table 8. Effect of monensin on body weight, feed intake, oocyst number in the litter and mortality of male turkeys (Chapman and Saleh, 1999)

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Feed intake (kg)</th>
<th>Oocyst (per gram, x 100)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>3</td>
<td>10</td>
<td>1-3</td>
</tr>
<tr>
<td>Treatment mg kg⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>539ᵇ</td>
<td>5 835</td>
<td>0.882</td>
</tr>
<tr>
<td>60</td>
<td>573ᵃ</td>
<td>6 070</td>
<td>0.852</td>
</tr>
<tr>
<td>80</td>
<td>543ᵇ</td>
<td>5 981</td>
<td>0.873</td>
</tr>
<tr>
<td>100</td>
<td>521ᵇ</td>
<td>5 923</td>
<td>0.890</td>
</tr>
</tbody>
</table>

2.2.3. Chickens reared for laying

Studies on the efficiency for this target species of controlled floor pen were not performed.

2.3. Controlled field trials

2.3.1. Chickens for fattening

One field study (Daugschies et al., 1998) was performed over three periods in a commercial broiler farm with a total number of approximately 100 000 animals
housed in a complex of seven separate stables. During periods 1 and 2, shuttle programs were used for anticoccidial treatment in which two synthetic coccidiostats were used for 15 days then replaced by monensin sodium (100 mg kg\(^{-1}\) feed). Another ionophore was the only anticoccidial drug applied in period 3. In these studies, a control group were missing. Moreover, feed analysis revealed that monensin was distinctly below (measured 19 mg kg\(^{-1}\) feed) the recommended dose (100 mg kg\(^{-1}\)) in the fattening feed in period 1. Therefore this field experiment can not be considered further by FEEDAP Panel.

### 2.3.2. Turkeys

A series of trials was conducted in different locations in the UK\(^4\) (1976) to compare the effects of monensin at 100 mg kg\(^{-1}\) feed with a synthetic coccidiostat on the performances of turkeys. Both treatments were included in the feed from week 0 to week 8 of age. Birds were slaughtered between 12 and 23 weeks of age. A total of 229,882 birds were placed in 16 houses on 4 separate sites, giving 8 house replicates on each treatment. The turkeys given monensin showed a significant (\(p<0.01\)) improvement in final live weight of 3.6 % and consumed significantly more feed. There was no difference in mortality or feed:gain.

Commercial field trials (no date) involving 115,903 turkeys in the USA\(^4\) indicate that monensin (60-75 mg kg\(^{-1}\) feed) is an efficient drug (growth, feed:gain, mortality) for turkeys when compared with other anticoccidials.

Even if there is a proof of the efficacy of monensin sodium in a dose range of 75-100 mg kg\(^{-1}\) and monensin is at least as efficient as other control anticoccidials under field conditions, at least one additional, recent, complete and significant field trial should be available, recognizing the efficacy at present in the recommended dose (90-100 mg kg\(^{-1}\) feed).

### 2.3.3. Chickens reared for laying

No data on field trial has been presented.

### 2.4. Studies on the development of resistance in *Eimeria*

#### 2.4.1. Published studies for chickens

Resistance to anticoccidials is a widespread phenomenon, which occurs even with the more recent additives. To describe the resistance situation clinically important of *Eimeria* spp. 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

\(^4\) Vol 1, Section 1, p 53-118
differences between the ionophores tested confirm – according to authors - earlier data of incomplete cross-resistance to polyether ionophorous drugs.

Two studies published in 1997 and 1998 describe the situation in Germany. Stephan et al. (1997) studied the sensitivity of 10 *Eimeria* field isolates. Nine 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, meticlorpindol 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) studies the *Eimeria* resistance situation in Dutch poultry production. Four isolates from 1996, selected from farms with clinical coccidiosis problems, four isolates from 1999 and seven isolates from 2001, both originating 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 coccididostats tested in 1996 (diclurazil, halofuginone, lasalocid, meticlorpindol 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 meticlorpindol/methylbenzoquate (7/7), salinomycin and narasin (4/7), followed by nicarbazin (3/7) and monensin (2/7). Resistance has 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.

### 2.4.2. Turkeys

No specific studies on resistance of monensin against *Eimeria* in turkeys are included in the dossier. Jeffers and Bentley (1980) stated that although it is possible to experimentally induce resistance in *Eimeria* species from the turkey and resistant field strains of *Eimeria* have been isolated, more recent studies have demonstrated
that field isolates obtained from turkey farms in the USA (E. adenoides, E. gallopavonis, E. meleagrimitis) were sensitive to monensin (Schildknecht et al., 1995; Chapman and Saleh, 1999).

A reduction in clinical efficacy has not been reported from the USA (undated) where monensin sodium has been used at 60 mg kg\(^{-1}\) in turkey feed for many years without evidence of selection for resistant strains. A report on 8 years experience in the UK with an 80/60 mg monensin kg\(^{-1}\) feed program with some 50,000,000 commercial turkeys has shown no outbreaks of clinical coccidiosis and no perceived slippage in performance related to subclinical challenge or resistance/tolerance problems.

2.5. Study on the quality of animal produce
One study (Metzler et al., 1987) with four experiments was conducted to evaluate the effect of Elancoban (100/120 mg monensin kg\(^{-1}\) diet) feeding (49 days of age) and withdrawal (5–10 days) on broiler performance and carcass characteristics. Whole body composition of protein, water, lipid, and ash were not significantly affected by monensin feeding or withdrawal.

One study (Izat et al., 1990) was conducted to compare the effects of monensin sodium (99 mg kg\(^{-1}\) feed) and two other anticoccidials (one synthetic and one ionophore) treated broiler diets on carcass characteristics and parts yield. In this study, a control group was missing and results were not comparable.

No data on the effect of feeding monensin on the quality of turkey meat are presented in the dossier.

2.6. Conclusion on efficacy
Three dose titration and confirmation studies performed post nineties indicate that monensin sodium at a concentration of 100 mg kg\(^{-1}\) feed is effective in the control of coccidiosis in challenged chickens. Two studies performed in 1991 allow an identical conclusion for the turkey.

The combined results of two floor pen trials indicate that 110 mg monensin from Elancoban kg\(^{-1}\) feed is effective in controlling coccidiosis of challenged chickens for fattening. However, this does not fulfill the present requirements of the Commission Directive 2001/79/EC for at least three studies indicating positive results.

The FEEDAP Panel considers also that at least one floor pen trial should be presented to assess the efficacy of monensin for chickens reared for laying. Due to the inadequate study design of the floor pen studies supplied, the FEEDAP Panel is not in a position to conclude on the efficacy of monensin for turkeys in controlled floor pen conditions.

Only one recent efficacy field trial test has been presented for chickens for fattening. This field experiment can not be considered further by the FEEDAP Panel as measured contents of monensin in feed were substantially below the expected value and the recommended range of dosage. No recent field studies were included in the dossier concerning turkey. This does not fulfill the present requirements of the Commission Directive 2001/79/EC for controlled field studies where three significant results on every target species should be presented. When chickens reared for laying are concerned, FEEDAP considers that the results of at least one field trial should be available.
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 development of resistance can successfully be counteracted in practice by rotation (the alternation of coccidiostats from run to run) or by shuttle programmes. No specific experiments on resistance with turkey specific *Eimeria* spp resistance are reported in the dossier. Only indirect evidence of the absence of resistance in the USA and the UK is given. Due to the uncertainty on the dates these studies were carried out, conclusive evidence on the absence of resistance with monensin in the actual whole turkey commercial production cannot be given.

As no data on the effect of feeding monensin on the nutritional, organoleptic and technological quality of chicken and turkey products are presented in the dossier, this matter could not be evaluated. It is noteworthy that the so far known history of the product never reported negative effects of monensin sodium on product quality.

3. Safety – studies on target species

3.1. Tolerance test

3.1.1. Chickens for fattening

In a first test\(^5\) (seven-week), monensin sodium was fed at levels of 0, 134, 402 and 670 mg kg\(^{-1}\) complete feed. One-day-old sexed broiler chicks were randomly assigned to floor pens. Each treatment had at least two replicate pens of males and two replicate pens of females. Each floor pen contained 50 chicks. All levels depressed body weight gain compared to untreated controls. The 134 mg level improved the feed conversion. The higher levels (402 mg, 670 mg) had adverse effects on growth and feed conversion. The highest dose (670 mg) increased mortality.

In the second study\(^6\) (eight-week) chickens were fed four different forms of monensin sodium (mycelial, crystalline, recrystallized, crystalline working standard) at levels of 0, 121, 363 and 605 mg kg\(^{-1}\) feed. One-day-old sexed broiler chicks were randomly assigned to brooder batteries and at age of 3 weeks were transferred to finisher batteries. Each treatment had at least two replicate pens of males and two replicate pens of females. Each battery cage contained 12 chicks. The effect of feeding monensin to chickens was a significant reduction of feed consumption at the 363 and 605 mg kg\(^{-1}\) levels which resulted in a significant depression of body weight gain. Feed conversion was only significantly depressed at the 605 mg kg\(^{-1}\) level. At four weeks treatment control comparisons showed significant dose associated changes for the 363 and 605 mg groups for hematocrit (Hc), total protein (TP), serum glutamic oxalacetic transaminase (SGOT) and alkaline phosphatase (AP) values. There were no significant differences for the clinical parameters at the 121 mg level. The clinical laboratory data at eight weeks showed significant elevation of haemoglobin (Hb) for the 121 mg level and significant decrease for AP and SGOT values. The four-week organ weight data showed significant dose associated changes for the 363 and 605 mg levels which generally consisted of decreases in total organ weights and increase in organ to body weight ratios. Values for the 121 mg level were

\(^5\) Vol 6, Ref. 12
\(^6\) Vol. 6, ref. 11
similar to control values. The monensin effects on the eight week organ weights were very similar to those effects recorded at four weeks. At eight weeks heart, liver and kidney weights decreased with an increase in organ to body weight ratios for all but the kidney for the 363 and 605 mg groups. Histopathologically there was no tissue changes observed with any form of monensin.

In the third study\(^7\) (eight-week) monensin sodium (in crystalline and mycelial form) was fed at 0, 121, 363 and 605 mg kg\(^{-1}\) feed. One-day-old sexed broiler chicks were randomly assigned to brooder batteries and at age of 3 weeks were transferred to finisher batteries. The nontreated treatment had at least four replicate pens of males and four replicate pens of females and the medicated treatments had at least two replicate pens of males and two replicate pens of females. Each battery cage contained 12 chicks. At 363 and 605 mg kg\(^{-1}\), both feed intake and body weight gain were reduced. Although not statistically significant, an increase in mortality among chickens fed 605 mg kg\(^{-1}\) is regarded as biologically important. All other monensin effects on haematology and serum chemistry (Hb, Hct, Glu, TP, SGOT, Na, and K) were regarded as not indicative for toxicity but were considered as the result of a decrease in feed consumption. These effects occurred only at the higher doses and were probably a result of the effect of monensin on feed consumption, except for possibly the slight increases in SGOT which agreed with the histopathological report of slight muscle degeneration. Microscopic lesions consisting of light degeneration and regeneration of skeletal muscles from the abdominal wall and legs occurred at 363 and 605 mg kg\(^{-1}\). No monensin effects were found for any of the studied parameters for the 121 mg concentration.

In the fourth test (seven-week)\(^8\) monensin sodium was fed at levels, 0, 110, 330 and 550 mg kg\(^{-1}\) feed. One-day-old sexed broiler chicks were randomly assigned to floor pens. Each treatment was replicated five times as follows: two pens of 100 males each, two pens of 100 female each and one pen of 50 males and 50 females. There were no significant differences in body weight gain, feed consumption and feed conversion between the chickens fed 110 mg kg\(^{-1}\) and those on the nontreated control diet. The performance parameters (body weight gain, feed consumption, and feed conversion) were significantly depressed by both the 330 and 550 mg kg\(^{-1}\) levels compared to the nontreated controls and the birds fed 110 mg kg\(^{-1}\). The 550 mg kg\(^{-1}\) level impaired all factors (body weight gain, feed consumption, and feed conversion) compared to the 330 mg kg\(^{-1}\) level. Mortality was significantly greater for 550 mg monensin kg\(^{-1}\) treatment compared to the other treatments.

### 3.1.2. Turkeys

Several studies were presented in the dossier to evaluate the toxicity of monensin for turkeys, showing toxicity, when overdosed; some of them however are off-station tests and without date.

A floor pen trial was conducted with 1650 day old poults fed graded levels of monensin (0, 110, 330 and 550 mg kg\(^{-1}\) feed; assay values: 113, 355, 573 mg kg\(^{-1}\) feed) for 98 days. Feed intakes of both 330 and 550 mg kg\(^{-1}\) groups was substantially less (P<0.05) than in the control or 110 mg kg\(^{-1}\) groups. Body weight gains in the low dose were 3 % greater than in controls, while in the mid and high

\(^7\) Vol. 6, ref. 14
\(^8\) Vol. 6, ref. 13
dose group the weight was severely depressed (P<0.05). Mean mortality rates over the entire period amounted to 20 % and 55 % (P<0.05) for the mid and high dose. Mortality at the low dose was less than in the control.

Ficken et al. (1989) reported high mortality in two flocks of 1900 turkey hens accidentally fed monensin at 280 mg kg\(^{-1}\) feed (analysed value). Mortality attributed to poisoning was 76 % in flock 1 and 18 % in flock 2. Microscopic analysis revealed myofiber degeneration and necrosis of skeletal and myocardial muscles. The reason that flock 2 had considerably less mortality than flock 1 was based on the estimate that flock 2 had enough feed from previous delivery to last 18 to 24 hours and flock 1 was out of feed at the time of delivery of new feed.

Cardona et al. (1993) reported skeletal myopathy produced with experimental dosing of turkeys (5-6 weeks old) with monensin (analysed dose, suspended in oil) by gavage feeding at 8.8, 17.6 mg kg\(^{-1}\) bw per day for 4 days, equivalent to the amount of monensin a turkey of that age would consume in a day, given feed containing 150 and 300 mg monensin kg\(^{-1}\). Turkeys receiving 8.8 mg kg\(^{-1}\) bw were paralyzed after the fourth dose, while those receiving 17.6 mg kg\(^{-1}\) bw were paralyzed 8 hours after the first dose. Only turkeys given a dose of 4.7 mg kg\(^{-1}\) bw (= 75 mg kg\(^{-1}\) feed) showed no clinical signs of myopathy, although they were ataxic after the third dose.

3.2. Interactions

Clinically important interactions between the ionophore anticoccidials and the antibiotic tiamulin are well known phenomena in chickens, turkeys and other species. The interaction depends on dose (Meingassner et al., 1979; Lehel et al., 1995; Weisman et al., 1983a, 1983b) and the ionophore itself (co-administration of tiamulin and lasalocid being without adverse effects (Comben, 1984). It was assumed that tiamulin reduces metabolic degradation and excretion of monensin (Meingassner et al., 1979). Later, also further toxic interactions with polyethers (mainly monensin) became known for sulphonamides (Frigg et al., 1983), chloramphenicol (Broz and Frigg, 1987), erythromycin, oleandomycin and furazolidone (Anadon and Reeves-Johnson, 1999).

Recent data allow to relate this interaction to the inhibition of cytochrome P-450, 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 this (iso)enzyme 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

Monensin is mainly active against Gram-positive bacteria (Table 9), In general, the activity against Gram-negative organisms is low including Escherichia coli Salmonella spp. and Pseudomonas spp.\(^9\).

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Table 9. MICs for strains of anaerobic bacteria isolated from the digestive tract of poultry and pigs. The number of isolates examined is shown in parenthesis (Benno et al., 1988)

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>MIC range (mg l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides fragilis group (27)</td>
<td>0.2 – 50.0</td>
</tr>
<tr>
<td>Fusobacterium spp. (3)</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td>Eubacterium spp. (12)</td>
<td>3 – 50.0</td>
</tr>
<tr>
<td>Bifidobacterium spp. (8)</td>
<td>3 – 12.5</td>
</tr>
<tr>
<td>Clostridium perfringens (3)</td>
<td>3 – 12.5</td>
</tr>
<tr>
<td>Peptostreptococcus spp. (9)</td>
<td>0.1 – 1.6</td>
</tr>
<tr>
<td>Megasphasera spp. (1)</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td>Lactobacillus spp. (14)</td>
<td>3 – 12.5</td>
</tr>
</tbody>
</table>

The chicken isolates of B. fragilis (17/27) and Eubacterium (5/12) were more sensitive than those of pig origin, the growth of all being inhibited in the presence of 12.5 mg monensin l\(^{-1}\). The sensitivity of Cl. perfringens to monensin has been confirmed in two published studies. In the first (Dutta et al., 1983), the growth of 68 strains isolated from a variety of livestock species including chickens were inhibited by monensin in the range 0.25 - 4 mg l\(^{-1}\). This result was confirmed in a second study\(^{10}\) in which 48 strains from turkeys and broiler chicks were inhibited by 2 mg l\(^{-1}\), and the majority by 1 mg l\(^{-1}\) (Watkins et al., 1997). Enterococcus faecium (200 strain of poultry origin from four EU Member States) was also shown to be sensitive to Monensin within the range 1 – 4 mg l\(^{-1}\).

3.3.2. Cross-resistance with other antibiotics

A number of studies\(^{11}\) to determine whether bacteria repeatedly grown in the presence of monensin in the laboratory develop resistance to monensin and to other antibiotics are reported. Four of the organisms selected in the main study (Staphylococcus spp., Enterococcus spp., bifidus and Clostridium perfringens) were susceptible to monensin and so were grown in the presence of the highest concentration that did not suppress growth. For Bacteroides fragilis the MIC of monensin increased markedly after the second passage and thereafter was treated like the two naturally resistant strains studied (Salmonella Typhimurium and Escherichia coli) and exposed to 50 mg monensin l\(^{-1}\) at each transfer. The susceptibility of each bacterial strain to a total of 13 antibiotics was measured at the start of the experiment and after 40 passages through medium containing monensin. No significant changes in the MICs were observed and it was concluded that exposure to monensin of these and the other intestinal microorganisms tested did not result in the development of resistance to antibiotics commonly used for therapy in human or veterinary medicine.

3.3.3. Effects on colonisation and shedding of enteropathogens

A number of studies consider the effect of monensin on the carriage and excretion of human enteric pathogens, notably Salmonella spp., in chickens.

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\(^{11}\) Various studies, Vol 11, part 7.
An experiment was designed to determine the effect of monensin at 110 mg kg\(^{-1}\) feed on the colonisation and shedding of *Salmonella* Typhimurium in experimentally infected chickens\(^{12}\). The 30-day exposure of *Salmonella*-challenged chickens to monensin did not affect the colonisation of *Salmonella* as measured by numbers of organisms excreted, number of shedding days or the results of a post-mortem examination.

Studies by Smith and Tucker (1978), Linton *et al.* (1985), Hinton *et al.* (1986) and Manning *et al.* (1994) similarly concluded that *Salmonella* carriage in broiler chickens was not apparently influenced by the use of monensin in the diet. The observations also indicated that monensin did not influence the shedding of *Salmonella* in naturally infected chickens. In chickens challenged with *E. coli* O 157, monensin reduced the number of this strain in caecum and colon, to the extent that by day 21 no *E. coli* O 157:H7 were detected (Stanley, *et al.*, 1996). However, it should be noted that the dose used (227 mg kg\(^{-1}\) feed) was substantially above the maximum dose recommended.

### 3.3.4. Conclusion

Monensin sodium is safe when fed to chickens for fattening and chickens reared for laying at dietary levels not exceeding the maximum recommended dosage of 125 mg monensin sodium kg\(^{-1}\) complete feed.

Monensin sodium is safe when fed to turkeys at dietary levels not exceeding the highest recommended dose of 100 mg kg\(^{-1}\) complete feed.

Gram-positive bacteria present in the digestive tract of poultry and other livestock are generally susceptible to monensin. While laboratory studies have shown that Gram positive strains can develop resistance to monensin, there was no evidence to suggest that exposure of Gram-positive bacteria to monensin results in the development of cross-resistance to other antibiotics used for therapy in human and veterinary medicine.

Incompatibilities or interactions with feedingstuffs, carriers or other approved additives are not expected by means of the known history of monensin. However, it is well known from the literature that severe interactions between the ionophore coccidiostats and the antibiotic tiamulin but also other antibiotics (mainly macrolides) may occur. Therefore the simultaneous use of Elancoban and certain antibiotic drugs (i.e. tiamulin) is contra-indicated. Feed containing Elancoban 100 or Elancoban 200 should therefore be labelled with a warning statement: *Avoid simultaneous administration with tiamulin and monitor for possible adverse reactions when used concurrently with other medicinal substances.*

The use of monensin as a coccidiostat in chickens did not affect the colonization or shedding of *Salmonella* in the gastro-intestinal tract of broilers.

### 3.4. Metabolism

The metabolic fate of monensin sodium has been studied in the chicken and turkey using a molecule \([^{14}\text{C}]\)-labelled at seven different positions distributed over the whole carbon skeleton, including four of the five cyclic ether rings. This labelling was adequate to follow metabolites that would result from an eventual breaking-down of

\(^{12}\) Section IV, Vol. 11, p. 1024-30-207
the molecule. However, the labelling at C-1 corresponding to the carboxyle function 
may lead to the loss of radioactivity in case de-carboxylation of the molecule would 
occur and to the labelling of endogeneous compounds through biosynthetic 
processes.

A metabolic balance established in colostomized and bile-cannulated chickens (3 
and 6 animals respectively) following a single dose administration of labelled 
monensin sodium indicated a fairly rapid excretion of the radioactivity (61-83 % in 24 
hours and 85-94 % after 5 days) mainly in the faeces (about 96 %) and to a very 
limited extent in the urine (about 1 %). However, the cumulated biliary excretion over 
a 72-hour period represented 11 to 31 % of the administered dose which indicates 
monensin sodium is absorbed at a considerable extent. Excretion of [14C]-labelled 
carbon dioxide accounted for 1.6 % (Davison, 1984). The metabolic steady state of 
monensin sodium was reached after a 4-day supplementation of the chicken13.

Extensive metabolic studies of monensin in the rat, steer, chicken and turkey have 
been conducted in order to identify the nature of monensin metabolites in the faeces, 
bile and tissues14 (and Donoho et al., 1978). These non-GLP studies were conducted 
using the up to date analytical methods and techniques available at that time and 
involved the use of [14C]-monensin, separative techniques associating HPLC and TLC, 
and mass-spectrometry identification. Most the identification work was performed on 
steers and rat faeces, bile and tissue extracts, while data concerning chicken and 
turkey were obtained on the basis of the comparative chromatographic behaviour of 
the corresponding sample extracts or comparison with metabolite standards. More 
recently the metabolic fate of [14C]-monensin in the chicken has been re-visited using 
more advanced techniques of separation, identification and quantification 
(HPLC/ESI-MS) of the metabolites15. These last results confirm and complete the 
former with the identification of an additional metabolite. Taken all together these 
metabolic data indicate that monensin sodium is metabolized extensively and gives 
rise to nine metabolites quoted M-1 to M-9 resulting from: 1) the demethylation of 
the methoxy- group at R and the subsequent oxidation of the resulting hydroxy group 
(keto derivative), 2) decarboxylation, 3) the concomitant mono- or di-hydroxylation at 
different but undertermined positions of the E, D, B and C rings which correspond 
essentially to the O-demethylation at R and simultaneous hydroxylation (mono- or di-) 
at different positions of the different rings of the molecule (Table 10).

13 Section IV, Vol. 12, Ref. 5
14 Section IV, Vol. 12, Ref. 3 ; Section IV, Vol. 12, Ref. 4
15 Section IV, Vol. 12, Ref. 3
Table 10. Chemical structure of monensin sodium metabolites identified in chicken (C), turkey (T) and rat (R) excreta

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>R</th>
<th>Other modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monensin sodium</td>
<td>O-CH3</td>
<td>-</td>
</tr>
<tr>
<td>M-1 CT,R</td>
<td>OH</td>
<td>-</td>
</tr>
<tr>
<td>M-2 CT,R</td>
<td>OH</td>
<td>monohydroxylation on ring E</td>
</tr>
<tr>
<td>M-3 CT,R</td>
<td>OH</td>
<td>monohydroxylation on ring E (M-2 epimer)</td>
</tr>
<tr>
<td>M-4 T,R</td>
<td>OH</td>
<td>monohydroxylation on ring D</td>
</tr>
<tr>
<td>M-5 T</td>
<td>O-CH3</td>
<td>monohydroxylation on ring D</td>
</tr>
<tr>
<td>M-6 CT,R</td>
<td>=CO</td>
<td>decarboxylation</td>
</tr>
<tr>
<td>M-7 CR</td>
<td>OH</td>
<td>monohydroxylation on rings B,C or D</td>
</tr>
<tr>
<td>M-8 CT,R</td>
<td>OH</td>
<td>dihydroxylation on rings B,C or D</td>
</tr>
<tr>
<td>M-9 C</td>
<td>=CO</td>
<td>decarboxylation and monohydroxylation on ring E</td>
</tr>
</tbody>
</table>

These data indicate also that the metabolic pathways are very similar in chicken, turkey and rat.

From a quantitative point of view unchanged monensin and metabolite M-2 represented each at least 10 % (but less than 20 % - FEEDAP estimation) of the total radioactivity in the chicken excreta while the other metabolites accounted each less than 10 %. A similar figure was found in the turkey where unchanged monensin represented 8 % of the radioactivity in the excreta. All metabolites identified in the chicken liver represented each less than 10 % of the labelling and were accompanied by a great number of very minor ones, unchanged monensin contributing to about 5 % only. It can be reasonably assessed that these conclusions apply to the turkey also. When the other tissues are concerned the very low amounts of residual radioactivity found in the kidney and muscle did not allow to separate and identify metabolites. No radioactivity that would correspond to monensin and main metabolites was extractable from the abdominal fat.

It must be noted that 88 % of the radioactivity of the faeces but only 57 and 45 % from the liver and 60 and 49 % from the kidney was extractable after 0 and 1-day

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\(^{16}\) Section IV, Vol. 12, Ref. 3.
withdrawal respectively. In the abdominal fat 95% of the labelling was not extractable and shown to be associated to fatty acids that indicates the occurrence of an active metabolic de-carboxylation process\textsuperscript{17}. Similar results concerning the extractability of the metabolites were obtained for liver (62% at 0 withdrawal and 31% at 5-day) (Donoho et al., 1982).

No data have been submitted concerning the eventual passage of monensin and/or metabolites in the first eggs layed (around week-20 of age) hens that received Elancoban until week-16 of age.

A study of the biological properties of metabolite M-1, one of the six O-demethylated metabolites, has been performed. It appeared that in a variety of biological systems, i.e. antimicrobial activity against \textit{Bacillus subtilis} and \textit{Streptococcus faecalis}, anticoccidial activity, cytotoxicity against murine leukemia lymphoblasts, inotropic activity and ionophorous properties, the metabolite exhibited only 5% of the activity of monensin sodium\textsuperscript{18}. It is reasonable to assume that any metabolic modification of the chemical structure of monensin sodium would modify the polarity or the complexing ability of the molecule towards sodium and therefore impair the biological properties that characterize ionophores.

### 3.5. Residues

Two studies\textsuperscript{19} (and Donoho et al., 1982) were performed in order to establish the kinetics of the elimination of tissue residues consecutive to the withdrawal of [\textsuperscript{14}C]-monensin administered for 6 consecutive days at a 125 mg kg\textsuperscript{-1} feed to chickens (3 males and 3 females per time point). The results were very similar concerning the relative distribution of the radioactivity in the different tissues and organs at 0, 1, 2, 3 and 5 days withdrawal. However, radioactivity levels were generally higher in the second study, especially in skin/fat, and therefore the worse case scenario has been retained. Concerning the turkey, a similar study (animals fed 110 mg [\textsuperscript{14}C]-monensin kg\textsuperscript{-1} feed during five days then slaughtered 6 hours off feed, i.e. at practical 0-day withdrawal) was carried out\textsuperscript{20} that showed that the residual levels in the liver, kidney and muscle were quite similar to those found in the chicken with the exception of the fat and skin/fat where these concentrations were much lower. However, no kinetic data are available concerning the whole residues along the withdrawal period (Table 11).

\textsuperscript{17} Section IV, Vol. 12, Ref. 3  
\textsuperscript{18} Section IV, Vol. 12, Ref. 12  
\textsuperscript{19} Section IV, Vol. 12, Ref. 3  
\textsuperscript{20} Section IV, Vol. 12, Ref. 4
Table 11. Total tissue residues expressed as monensin equivalent in the chicken and turkey following the continuous (6 days) oral administration of 125 and 110 mg [14C]-monensin sodium kg⁻¹ feed respectively

<table>
<thead>
<tr>
<th>Withdrawal (day)</th>
<th>Liver (mg monensin equivalent kg⁻¹)</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Skin/fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.94</td>
<td>0.2</td>
<td>0.06</td>
<td>0.29</td>
</tr>
<tr>
<td>1</td>
<td>0.47</td>
<td>0.14</td>
<td>0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>3</td>
<td>0.27</td>
<td>0.09</td>
<td>0.05</td>
<td>0.23</td>
</tr>
<tr>
<td>5</td>
<td>0.14</td>
<td>0.05</td>
<td>0.04</td>
<td>0.17</td>
</tr>
<tr>
<td>Turkey</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.91</td>
<td>0.16</td>
<td>&lt;LD</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<LOQ = limit of quantification: 0.025 mg kg⁻¹

Two studies have been carried out in the chicken to determine monensin residues in tissues in the practical conditions of use. In the first study (Okada, 1980) four groups of 5 male chickens were administered Elancoban at the dose proposed, i.e. 120 mg monensin kg⁻¹ feed, for life time, then slaughtered after 0, 1, 2 and 3 days withdrawal of the supplemented feed. Using a quantitative thin layer chromatography with a limit of quantification of 0.01 and 0.0125 mg monensin kg⁻¹ for the fat and the other tissues (liver, kidneys and muscle) respectively it has been shown (Table 12) that the highest concentrations were measured in the fat then the liver, that no monensin was detectable in tissues except fat after a 1-day withdrawal period and in any tissue after 2 days.

Table 12. Monensin residues in tissues of chickens (5 animals) fed monensin at 120 mg kg⁻¹ feed in practical conditions of use (from Okada et al., 1980)

<table>
<thead>
<tr>
<th>Withdrawal time (day)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Fat</th>
<th>Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.039</td>
<td>0.014</td>
<td>0.029</td>
<td>0.110</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>LD</td>
<td>LD</td>
<td>LD</td>
<td>0.017</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>LD</td>
<td>LD</td>
<td>LD</td>
<td>LD</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>LD</td>
<td>LD</td>
<td>LD</td>
<td>LD</td>
<td>-</td>
</tr>
</tbody>
</table>

LD = limit of detection (0.01 mg kg⁻¹ in fat, 0.0125 mg kg⁻¹ in the other tissues)

In the second study (Godfrey et al., 1997) groups of 3 chickens (sex not indicated) received a starter feed supplemented with 100 mg monensin kg⁻¹ for 14 days then a grower/finisher feed with 120 mg monensin kg⁻¹, and were slaughtered after 0, 1, 2 and 3 days withdrawal. Using immunoaffinity chromatography and ELISA quantitative technique with a much lower limit of detection comprised between 0.0001 mg kg⁻¹ (liver) and 0.002 mg kg⁻¹ (skin), it was confirmed that at 0-day withdrawal the highest residual level was found in the fat (0.079 mg kg⁻¹) followed by the skin (0.033 mg kg⁻¹), muscle (0.026 mg kg⁻¹), kidney (0.019 mg kg⁻¹) and liver (0.015 mg kg⁻¹). Similar values were obtained after 1 day withdrawal whereas monensin contents declined rapidly the following days. Interestingly, monensin was still detectable at a
0.001 mg kg\(^{-1}\) level after a 26-day withdrawal period. Considering the limited number of animals and the fact that the numerical values were not supplied (only graphs), these results have not been taken into account for the assessment.

No specific data have been submitted concerning monensin residues in the first eggs laid by hens that had received Elancoban supplemented feed at the proposed dosage from 0 to 16 weeks of age as chickens reared for laying. However, two studies supplied data on monensin residue levels in eggs laid by hens fed a 80 mg monensin kg\(^{-1}\) diet. In the first one\(^{22}\), 316-day old hens were fed the monensin supplemented diet for 42 consecutive days and the eggs collected during the third week (15-21 days) were tested for monensin residue. All eggs contained residues but the concentrations were \(\leq 0.05\) mg kg\(^{-1}\) (LOQ of the method: 0.025 mg kg\(^{-1}\)) and no increase was observed along this period. In the second study\(^{23}\) 0-day old pullets received 120 mg monensin kg\(^{-1}\) feed for 40 days, 100 mg kg\(^{-1}\) feed for another 42 days and finally 80 mg kg\(^{-1}\) feed until 140 days of age (20\(^{th}\) week), i.e. when laying is established. The eggs were collected from one day prior to supplementation withdrawal and up to six days post-treatment (139-146 days of age). Only a limited number of eggs exhibited monensin residues but \(\leq 0.05\) mg kg\(^{-1}\) and until the 5\(^{th}\) day after withdrawal.

When the turkey is concerned, only one study conducted in the conditions of practice is available concerning monensin residues in tissues\(^{24}\). The animals (3 males and 3 females) were given 111 mg kg\(^{-1}\) unlabelled monensin in feed for 17 weeks and 0, 1, 2, 3 and 4-day withdrawal periods were applied before slaughter. Monensin was assayed using a semi-quantitative thin layer chromatography/bioautography method with limits of detection and determination of 0.05 and 0.025 mg kg\(^{-1}\) respectively. The results indicate that monensin residues are lower in turkey than in chicken tissues, with the exception of muscle where residues are higher (exact not specified) and detectable until 1-day withdrawal. The FEEDAP Panel considers that results obtained with a semi-quantitative method are not acceptable when more advanced quantitative methods are available (see chapter 1.3.).

### 3.6. Conclusion

Monensin sodium is absorbed and metabolized extensively by the chicken and turkey and its metabolic fate is similar in these two species but also the rat. A great number of metabolites has been isolated from the excreta and tissues of which eight major metabolites have been identified which each represent less than 10 % of the whole residues. They result from either single or combined demethylation, decarboxylation and hydroxylation of the molecule. The biological activity of one of the major metabolites, evaluated against different biological end-points, represents only 5 % of that of monensin sodium, and it can be reasonably assessed that this loss of activity applies to most metabolites. Unchanged monensin represents less than 20 % of the whole metabolites in chicken excreta and 8 % of turkey excreta. It represents less than 5 % of tissue residues.

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\(^{22}\) Section IV, Vol. 7, p. 1004-28-25  
\(^{23}\) Section IV, Vol. 7, p. 1004-28-213  
\(^{24}\) Section IV, Vol. 12, Ref. 11
Kinetic studies of the whole residues in tissues, established using the highest monensin recommended dosage, are available in the chicken but not the turkey (only 0-day withdrawal). The proportion of unextractable residues in tissues increases with the withdrawal time that corresponds to the labelling of endogenous compounds resulting from the decarboxylation of the radiolabelled monensin. Despite the inconsistency of the available studies and subsequent difficulty to choose between the liver and skin/fat the most pertinent target tissue, FEEDAP considers for practical reasons, i.e. control purposes, that skin/fat should be retained. Although monensin represents a small fraction of tissue residues, it is retained as the marker-residue.

No specific data have been submitted concerning monensin residues in the first eggs laid by hens that had received Elancoban supplemented feed at the proposed dosage from 0 to 16 weeks of age as chickens reared for laying. However, data obtained in hens that received 80 mg monensin kg\(^{-1}\) feed for at least three consecutive weeks indicate that monensin residues in eggs are present at very low level, do not accumulate and disappear after a 5-day withdrawal.

4. **Safety – studies on laboratory animals**

The studies provided had been performed in the 1960s, 1970s or early 1980s. No modern studies were available. Most of the studies did not conform to the principles of Good Laboratory Practice (GLP). The studies that were GLP-compliant are indicated in the text.

4.1. **Single dose toxicity**

The acute oral toxicity of monensin in a wide range of species was reviewed by Todd et al., (1984) – see the Table 13. Signs of toxicity in all animals were similar and comprised anorexia, hypoactivity, skeletal muscle weakness, ataxia, diarrhoea and decreased weight gain.
Table 13. Acute Oral LD$_{50}$ values for different animal species$^+$

<table>
<thead>
<tr>
<th>Species</th>
<th>sex</th>
<th>LD$_{50}$ (mg kg$^{-1}$ bodyweight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td></td>
<td>2-3</td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Dog</td>
<td>F</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Swine</td>
<td></td>
<td>17 – 50</td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
<td>22 – 80</td>
</tr>
<tr>
<td>Rat</td>
<td>F</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>40.1</td>
</tr>
<tr>
<td>Goat</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>Guinea fowl</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>Mouse</td>
<td>F</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>70-108</td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td>200 – 231</td>
</tr>
<tr>
<td>Turkey</td>
<td>F</td>
<td>416</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>347</td>
</tr>
<tr>
<td>Monkey</td>
<td></td>
<td>&gt;160</td>
</tr>
</tbody>
</table>

$^+$ Taken from Langston et al., 1985

RODENT

The original Lilly study data$^{25}$ were not found. The following information is taken from summary data$^{26}$.

The major signs of intoxication were anorexia, depression, ataxia, recumbency and increased heart rate. Rats were more sensitive than mice and female rats were more sensitive than males.

DOG

Single oral doses of 20 mg kg$^{-1}$ bw of monensin produced no mortalities$^{27}$. However, oral doses of 2 mg kg$^{-1}$ monensin have been reported to increase heart rate, arterial blood pressure and plasma glucose in conscious dogs from half an hour after dosing (Pressman and Fahim, 1983).

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$^{25}$ 1032-12
$^{26}$ 1024-13 pp.2 and IRD 140A p.48 and Mitema et al., 1988
$^{27}$ 1024-13 p.2
4.2. Repeated dose toxicity

**Mice**

In a 90-day study: groups of 15 male and 15 female B6C3F1 mice were fed dietary concentrations of monensin of 37.5, 75, 150 or 300 mg kg\(^{-1}\) for three months. A dose-related decrease in weight gain occurred in all groups but the only other sign of toxicity was a slight increase in serum creatine phosphokinase (indicative of muscle damage) in the two highest dose groups. Minimal cardiac muscle degeneration was found on histopathological examination of the highest dose group.

A NOEL cannot be derived because growth was affected in all groups.

In a 2-year GLP-compliant chronic toxicity/carcinogenicity assay, groups of 60 male and 60 female B6C3F1 mice were maintained for 2 years on diets containing mycelial monensin at concentrations that gave dietary levels of monensin of 0, 10, 25, 75 and 150 mg kg\(^{-1}\). These dietary levels were equal to monensin sodium dosages of 0, 1.2, 3.1, 10.2 and 22.6 mg kg\(^{-1}\) day\(^{-1}\) for males and 0, 1.4, 3.5, 11.7 and 25.6 mg kg\(^{-1}\) day\(^{-1}\) for females (estimated time-weighted average dosages). Bodyweight gain was decreased in both sexes given 25 mg monensin kg\(^{-1}\) feed or greater, and leukocyte counts were decreased in the males at these dose levels. No other adverse effects were seen on mortality, physical and behavioural signs of toxicity, haematology (other than leukocytes), clinical chemistry, gross pathology, organ weights, or histopathology (including tumour incidences).

The NOEL for this study was a dietary concentration of monensin sodium of 10 mg kg\(^{-1}\), which was equivalent to dosages of monensin of 1.2 mg kg\(^{-1}\) bw d\(^{-1}\) for males and 1.4 mg kg\(^{-1}\) bw d\(^{-1}\) for females.

**Rat**

A 90-day subacute rat toxicity study was performed in Harlan Wistar rats (15/sex/dose group) were fed monensin at dietary concentrations of 50, 150 or 500 mg kg\(^{-1}\) for 90 days. These dietary concentrations were equivalent to dosages of monensin of 3.5, 5-15 and 39-47 mg kg\(^{-1}\) d\(^{-1}\) respectively. Monensin in the feed was determined by bioassay; data on the stability throughout the trial appear satisfactory. Information on the chemical purity of the monensin was not given. There was a dose-related depression of body weight gain which was statistically significant at 150 and 500 mg kg\(^{-1}\) feed. Female rats were more severely affected than males. In the highest dose group, both food intake and efficiency of food utilisation were diminished. Mortality in the females was 80%, whereas that amongst males was 40%. Haematological studies showed a decrease in haematocrit and white blood cell count in male rats (too few female rats survived to make statistically valid analyses). Blood urea nitrogen, glucose and serum glutamate pyruvate transaminase (sALT) were unaffected. Changes in the relative organ weights were unremarkable and probably related to decreased overall growth. Histopathology revealed significant compound-related abnormalities in the high dose group only. Myositis of the skeletal muscle and myocarditis of a diffuse type with areas of degeneration and infiltration by histiocytes and some cases of diaphragm muscle fibre degeneration were found. There was no effect on the incidence of neoplasia. The NOEL, based on body weight

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28 Study M00281 & M00381 – TSR 71 & 72
29 R06378 & R06478 – TSR 67 & 68
gain, was considered to be a dietary level of monensin of 50 mg kg\(^{-1}\) in the feed or 3 mg kg\(^{-1}\) bw d\(^{-1}\).

A 90-day study of the oral toxicity of crystalline monensin sodium was performed in groups of 10 male and 10 female Harlan rats\(^{30}\). The groups were given diets containing monensin at concentrations of 0, 50, 100, 200 or 400 mg kg\(^{-1}\) in the feed. One male rat in the top dose group died during the study. At dietary concentrations of 200 or 400 mg kg\(^{-1}\) feed, there was reduced bodyweight gain. There were no treatment-related changes in feed intake, clinical signs of toxicity, haematology, clinical chemistry, gross pathology, organ weights or histopathology. The NOEL for this study was 50 mg kg\(^{-1}\) bw in the feed, which was roughly equivalent to a dosage of monensin of 5 mg kg\(^{-1}\) bw d\(^{-1}\).

A GLP-compliant 2-year chronic toxicity/carcinogenicity study\(^{31}\) of crystalline monensin sodium was reported. Two identical experiments with 40 rats/sex/dose group and 60 rats/sex in the control group were used. One half of the animals were treated for 2 years and the remaining animals sacrificed after one year. Harlan Wistar rats were used. Monensin was present in the feed at 0, 25, 56 or 125 mg kg\(^{-1}\) feed (equivalent to 0, 1.14, 2.57 and 5.91 mg kg\(^{-1}\) d\(^{-1}\) in males and 0, 1.46, 3.43 and 8.63 mg kg\(^{-1}\) d\(^{-1}\) in females). The parents of the rats had received similar concentrations of monensin in their diets prior to mating, during gestation and until the offspring were weaned. The two parallel experiments produced very similar results, so the results were combined so that the two experiments were considered as a single study. Bodyweight gain was decreased consistently at the top dose level and transiently during the initial 4 months for the 56 mg kg\(^{-1}\) group. There were no treatment-related effects on mortality, clinical signs, feed consumption, haematological, clinical chemistry, urinalysis, gross pathology, organ weights or histopathology (including tumour incidences). The NOEL for this study was 25 mg kg\(^{-1}\) bw in the feed (equivalent to a dosage of monensin of 1.14 mg kg\(^{-1}\) bw d\(^{-1}\) in males and 1.46 mg kg\(^{-1}\) bw d\(^{-1}\) in females), based on reduced bodyweight gain.

In a GLP-compliant 2-year chronic toxicity/carcinogenicity study, groups of 50 Wistar rats of either sex were given diets containing mycelial monensin. The diets contained monensin concentrations of 0, 33, 50 and 80 mg kg\(^{-1}\). This was equivalent to dosages of monensin of 0, 1.40, 2.18 and 3.60 mg kg\(^{-1}\) d\(^{-1}\) in males and 0, 1.72, 2.86 and 5.02 mg kg\(^{-1}\) d\(^{-1}\) in females. Bodyweight gain was reduced in both sexes at the top dose and in females at 50 mg kg\(^{-1}\). There were no treatment-related effects on mortality, clinical signs, feed consumption, haematological, clinical chemistry, urinalysis, gross pathology, organ weights or histopathology (including tumour incidences). The NOEL for this study was 33 mg monensin kg\(^{-1}\) feed (equivalent to 1.40 mg kg\(^{-1}\) bw d\(^{-1}\) in males and 1.72 mg kg\(^{-1}\) bw d\(^{-1}\) in females), based on reduced bodyweight gain.

**Dog**

A twelve week study in beagle dogs\(^{32}\), originally reported in 1974, is presented. Beagles of 12-18 months of age were used. Two dogs of each sex were given monensin at 0, 5, 15 or 50 mg kg\(^{-1}\) d\(^{-1}\) as oral doses in gelatin capsules. Animals were

\(^{30}\) Ref 35; studies R11478 and R11578

\(^{31}\) Study No. D-4753
not dosed on days they refused food; food refusal was high in all groups, including controls. Consequently, for the animals that survived to the end of the study, the number of doses given ranged from 35 to 81 out of a potential 91 doses. Even the lowest dose of 5 mg kg\(^{-1}\) d\(^{-1}\) produced emesis. The incidence of vomiting increased with the dose. Both male dogs receiving 50 mg kg\(^{-1}\) d\(^{-1}\) died in less than 2 weeks and the two female dogs receiving this level showed severe anorexia, vomiting and weight loss. Serum lactate dehydrogenase and glutamic oxaloacetate transaminase activities were increased. No adverse effects were seen in haematology or urinalysis results. Relative organ weights were increased in several cases but as total body weights were suppressed, these values have little meaning. Histopathology revealed no abnormalities in dogs receiving 5 mg kg\(^{-1}\) d\(^{-1}\). Male dogs receiving 15 mg kg\(^{-1}\) d\(^{-1}\) and all dogs receiving 50 mg kg\(^{-1}\) d\(^{-1}\) showed striated muscle pathology, represented by diffuse degeneration of fibres and infiltration of histiocytes. Degenerative muscle changes were present in the diaphragm and were also found in cardiac muscle. A NOEL could not be precisely identified because toxic effects were recorded at the lowest dose used (5 mg kg\(^{-1}\) bw d\(^{-1}\)). The NOEL was therefore less than 5 mg kg\(^{-1}\) bw d\(^{-1}\).

A 90-day study\(^{33}\) was performed using groups of 2 dogs/sex (a cross breed of Beagles and mongrels) using oral doses of monensin of 0, 2.5, 5, 11 and 25 mg kg\(^{-1}\) d\(^{-1}\) in capsules. No treatment related effects were produced at doses of 2.5 or 5 mg kg\(^{-1}\) d\(^{-1}\). Signs of toxicity at higher doses (11 or 25 mg kg\(^{-1}\) d\(^{-1}\)) were ataxia, loss of motor co-ordination, relaxation of the nictitating membrane, salivation, vomiting and tremors. Both males receiving 25 mg kg\(^{-1}\) bw d\(^{-1}\) and one female receiving 11 mg kg\(^{-1}\) bw d\(^{-1}\) died after 3 doses, and treatment of females in the 25 mg kg\(^{-1}\) bw d\(^{-1}\) group was stopped after dose 4 due to progressing morbidity. These dogs were observed but untreated for the remainder of the study, and haematuria was observed in one of them. Histopathology showed evidence of liver toxicity at 11 and 25 mg kg\(^{-1}\) bw d\(^{-1}\). A NOEL of 5 mg kg\(^{-1}\) bw d\(^{-1}\) for was identified, based on liver toxicity together with more generalised toxicity.

A year study\(^{34}\) was performed on Beagle dogs (4/sex/dose level) were given daily oral doses of mycelium that provided dosages of monensin of 1.25, 2.5, 5 or 7.5 mg kg\(^{-1}\) bw d\(^{-1}\) for a year. Transient anorexia, hypoactivity and weakness were observed in the two highest dose groups. Elevated serum alanine transaminase and creatine phosphokinase were found in some of the dogs in these dose groups during the first 4 weeks of treatment. Body weight gain was decreased in the highest dose group. Electrocardiogram evaluations gave normal results in all dose groups. There were no effects on ophthalmoscopy, haematology, urinalysis, gross pathology, organ weights or histopathology. A NOEL of 2.5 mg kg\(^{-1}\) bw d\(^{-1}\) was identified on the basis of various signs of toxicity.

4.3. Reproductive toxicity

Multigeneration studies, incorporating investigation of developmental toxicity, were performed on mycelial and crystalline grades of monensin using rats. A developmental study was performed on monensin sodium using rabbits.

\(^{33}\) Study No. R-363, R-1203, R-104

\(^{34}\) Ref 28
RAT

A 3-generation study\textsuperscript{35} of crystalline monensin sodium was performed using Harlan Wistar rats. The study incorporated an investigation of developmental toxicity. Purified monensin sodium (91.7 %) was used to make feed containing monensin concentrations of 0, 2.5, 12.5 or 25 mg kg\textsuperscript{-1} (equivalent to dosages of monensin of about 0, 0.25, 1.25 and 2.5 mg kg\textsuperscript{-1} d\textsuperscript{-1}). In the F0 generation, there were 45 rats of each sex in the control group and 30 rats of each sex in each of the dosed groups. For the F1 generation, the number of breeding pairs was 30 in the control and 20 in each of the treated groups. For the F2 generation, the numbers of breeding pairs were 50 and 40 in the control and treated groups, respectively. The F1a animals from this study were assigned to a 2-year chronic toxicity/carcinogenicity study\textsuperscript{36} that is referred to in sections on “repeat dose toxicity” and “Carcinogenicity”. Twenty pregnant females from each group were separated for teratogenicity investigations. There was no apparent effect on the reproductive capacity or health of offspring and no evidence of a teratogenic effect at any dose level. Mortality was high in the F\textsubscript{2} generation due to an outbreak on pneumonia. As no treatment-related adverse effects were seen, the NOEL for this study was the highest dose level, 2.5 mg kg\textsuperscript{-1} bw d\textsuperscript{-1}. There was no indication of any embryotoxicity, fetotoxicity or teratogenicity at the doses used.

A 3-generation study\textsuperscript{37} of mycelial monensin sodium was performed using Wistar rats. The study incorporated an investigation of developmental toxicity. Groups of rats were given feed containing monensin concentrations of 0, 33, 50 or 80 mg kg\textsuperscript{-1} (equivalent to dosages of monensin of about 0, 3.3, 5 and 8 mg kg\textsuperscript{-1} d\textsuperscript{-1}). The group size for the F\textsubscript{0} parents was 30 rats/sex/group and for the F1 and F2 parents was 25 rats/sex/group. The F1a animals were assigned to a 2-year chronic toxicity/carcinogenicity study\textsuperscript{38} that is referred to in sections on “repeat dose toxicity” and “Carcinogenicity”. Bodyweight gain was decreased in pregnant and lactating rats at the mid and high dose levels. There were no clinical signs of toxicity and adult mortality was unaffected by treatment. Gross pathology and histopathology of the adults showed no treatment-related adverse effects. Mean litter weight was reduced in all generations at the high dose level and in the F\textsubscript{2}-generation mid dose group. However mean fetal weight was higher in treated groups than in controls, especially at the highest dose level. Bodyweight gain was depressed during the growth phase at even the lowest dose level in F\textsubscript{0} males and F\textsubscript{2} females and in both sexes at the mid and high dose for most generations. There were no effects on numbers of live and dead pups, sex ratio of pups, numbers of corpora lutea and implantation sites, fetal viability, and incidence of external, skeletal and soft tissue abnormalities. The increased mean pup weight but decreased litter weight with no effect on the number of live pups appears contradictory and may not be indicative a real toxic effect of monensin. The LOEL was 33 mg kg\textsuperscript{-1}, which is equivalent to 3.3 mg kg\textsuperscript{-1} bw d\textsuperscript{-1}, based on maternal toxicity (reduced bodyweight). There was no evidence of any embryotoxicity or teratogenicity and the evidence to suggest fetotoxicity was unconvincing.

\textsuperscript{35} Ref 29; studies R-363, R-1203 & R-104
\textsuperscript{36} Ref 35; studies R11478 & R11578
\textsuperscript{37} Ref 30; studies R-78, R-958 & R29
\textsuperscript{38} Ref 34; studies R06378 and R06478
**RABBIT**

A teratology study\(^{39}\) was performed using Dutch belted rabbits. Monensin given at doses of 0, 0.076, 0.38 or 0.76 mg kg\(^{-1}\) d\(^{-1}\) by oral gavage on gestation days 6-18 inclusive. There were 25 rabbits in the vehicle-only and 15 in each of the treatment groups. A dose-dependent decrease in maternal food intake was observed but maternal bodyweight gain was not affected. Post-mortem examination of the mothers, with emphasis on the reproductive system, showed no adverse effects. There was no effect on the number, uterine location, sex or weight of fetuses and there were no effects on the numbers of corpus luteum and resorptions. The incidence of external, skeletal and soft tissue abnormalities of the fetuses was not affected by treatment. The dose levels chosen were very low, presumably because monensin is toxic to the mothers at higher doses. There was no indication of any embryotoxicity, fetotoxicity or teratogenicity at the doses used. As no treatment-related adverse effects were seen, the NOEL for this study was the highest dose level, 0.76 mg kg\(^{-1}\) d\(^{-1}\).

**4.4. Mutagenicity**

Three bacterial tests were available: bacterial reverse mutation assays (modified Ames test) of monensin A and B sodium salts, an *in vitro* cytogenetics assay (metaphase analysis in CHO cells) and an *in vivo* micronucleus test. The latter two tests were GLP-compliant but the bacterial tests were performed before the introduction of GLP requirements.

**Bacterial test\(^{40}\)**

In modified Ames tests, monensin B sodium salt and monensin A sodium salt were tested for reverse mutation in various histidine auxotrophs of *Salmonella* Typhimurium and tryptophan auxotrophs of *Escherichia coli* at concentrations from 0.1 to 1000 µg ml\(^{-1}\) with and without metabolic activation with S9 from the livers of male Fischer rats that had been treated with Aroclor 1254. The positive controls, streptozotocin and 2-acetylaminofluorene, gave the expected results. Monensin sodium showed no mutagenic nor growth inhibitory activity in any of the strains (listed below) that were tested using either monensin A sodium or monensin B sodium, i.e. *Salmonella* Typhimurium G 46, TA 1535, TA 100, TA 1537, TA 1538, TA 98, D 3052 and C 3076, and *Escherichia coli* WP2 and WP2 uvrA-.

**In vitro cytogenetics\(^{41}\)**

Monensin sodium (93.8 % monensin) was dissolved in dimethyl sulfoxide (DMSO) and tested for cytogenetic effects in CHO cells (subline WB\(_L\)) in the presence and absence of metabolic activation with S9 from the livers of Aroclor 1254 treated rats. The CHO cells were exposed for 4 hrs to concentrations of 25, 50 or 100 µg ml\(^{-1}\) in the absence of S9 or 50, 80 or 100 µg ml\(^{-1}\) in the presence of S9. CHO cells were also exposed for 19 hrs to concentrations of 5, 10 or 25 µg ml\(^{-1}\) in the absence of S9. All cells were examined at metaphase. Mitomycin C and cyclophosphamide were used as positive controls and confirmed that the study was performing as required. The 4

\(^{39}\) Ref 31; study B-7293

\(^{40}\) Ref 32, Vol 13, Part 5 and Ref 33, Vol 13, Part 6

\(^{41}\) Supplementary data submission, July 2002, Vol 4, Tab 5; studies 010926CAB0692, 011003CAB0692 & 011024CAB0692
hrs caused an increase in the incidence of cells with diplochromosomes at the low dose in the absence of S9 and at the mid and high doses in the presence of S9. No diplochromosomes were seen following the extended exposure period in the absence of S9. The authors suggested that the diplochromosomes were an indication of the induction of endoreplication. However it did appear to induce endoreduplication to cause a doubling of the chromosome complement of the affected cell. Extra complete sets of chromosomes (polyploidy) do not appear to be associated with the production of cancer, unlike imbalanced numbers of chromosomes (aneuploidy) which is associated with increased cancer risk. None of the treatments produced any increase in the numbers of chromosome aberrations. It was concluded that this study showed that monensin sodium did not cause damage to chromosomes and that the increased number of diplochromosomes had no relevance to processes that may lead to cancer.

**In vivo micronucleus test**

Monensin sodium (93.8 % monensin) was given by oral gavage to groups of 5 male and 5 female ICR mice on 2 consecutive days. The doses of monensin given each day were 0, 181, 363 and 725 mg kg\(^{-1}\) bw. The high dose was 80 % of the maximum tolerated dose identified in dose range finding studies. Positive control animals were given a single gavage dose of 20 mg kg\(^{-1}\) of cyclophosphamide. Bone marrow was harvested at 24 hrs after the final dose. Mortality was high, with 2 males and 1 female dying in the mid dose group and 4 males and 5 females dying at the high dose. There was no increased incidence of micronucleated polychromatic erythrocytes in the bone marrow of any of the animals given monensin. There was a clear increase in the positive controls, as expected. There was no indication from the results of this study to suggest that monensin sodium might be mutagenic. However, the value of this negative result is compromised by the small group sizes resulting from the high mortality in some groups.

4.5. **Carcinogenicity**

**MICE**

In a GLP-compliant chronic toxicity/carcinogenicity assay (that is also reported under “Repeat dose toxicity”), groups of 60 male and 60 female B6C3F\(_1\) mice were maintained for 2 years on diets containing mycelial monensin at 0, 10, 25, 75 and 150 mg monensin kg\(^{-1}\) feed. These dietary levels gave doses of 0, 1.2, 3.1, 10.2 and 22.6 mg monensin kg\(^{-1}\) bw day\(^{-1}\) for males and 0, 1.4, 3.5, 11.7 and 25.6 mg monensin kg\(^{-1}\) bw day\(^{-1}\) for females (estimated time-weighted average dosages). The type and incidence of neoplasms found were typical of ageing mice. There was no treatment-related effect on the incidence of benign or malignant tumours. It was concluded that the mycelial monensin was not carcinogenic in mice.

**RAT**

A GLP-compliant two-year chronic toxicity/carcinogenicity study of crystalline monensin sodium was performed. This study is also reported under “Repeat dose toxicity”. Two identical experiments with 40 rats/sex/dose group and 60 rats/sex in

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42 Supplementary data submission, July 2002, Vol 4, Tab 6; study 020123MNT0692
43 Ref 36; M00281 & M00381
44 Ref 35; studies R11478 & R11578
the control group were used. One half of the animals were treated for 2 years and the remaining animals sacrificed after one year. Harlan Wistar rats were used. Monensin was present in the feed at 0, 25, 56 or 125 mg monensin kg\(^{-1}\) feed (equivalent to 0, 1.14, 2.57 and 5.91 mg kg\(^{-1}\) bw day\(^{-1}\) in males and 0, 1.46, 3.43 and 8.63 mg kg\(^{-1}\) bw day\(^{-1}\) in females). The parents of the rats had received similar concentrations of monensin in their diets prior to mating, during gestation and until the offspring were weaned. There was no apparent sex effect and the two studies produced very similar results, so the results can be combined. The incidences of benign and malignant tumours were unaffected by monensin treatment. It was concluded that crystalline monensin sodium was not carcinogenic to rats.

In a GLP-compliant chronic toxicity/carcinogenicity study\(^45\), groups of 50 Wistar rats of either sex were given diets containing mycelial monensin for 2 years. This study is also reported under “Repeat dose toxicity”. The diets contained 0, 33, 50 and 80 mg monensin kg\(^{-1}\) feed. This was equivalent to 0, 1.40, 2.18 and 3.60 mg kg\(^{-1}\) bw day\(^{-1}\) in males and 0, 1.72, 2.86 and 5.02 mg kg\(^{-1}\) bw day\(^{-1}\) in females. There were no treatment-related effects on the incidences of either benign or malignant tumours. It was concluded that the mycelial monensin used in this study was not carcinogenic to rats.

4.6. Special studies

Horse toxicity

Toxicity of ionophores to horses is worldwide known since decades. Different authors (Amend et al., 1981; Kamphues et al., 1990) described anamnesis and clinical signs, mostly severe myocardial degeneration.

The Notifier has submitted three sets of studies\(^46\). The median lethal dose was estimated to be between 2 and 3 mg kg\(^{-1}\) body weight for crystalline monensin and 1.38 mg kg\(^{-1}\) for mycelial monensin. In a subacute feeding study (28 days) horses given a dose of 33 mg monensin (as mycelial monensin) kg\(^{-1}\) feed reduced the feed intake without clinical signs of toxicity, while 1 of 3 horses fed with a 121 mg kg\(^{-1}\) dosage and all 3 horses fed a 330 mg kg\(^{-1}\) dosage died. In another 28-day study horses fed 121 mg monensin (as mycelial monensin) kg\(^{-1}\) consumed less than one-half that consumed by healthy control horses. Increases in serum levels of muscle origin enzymes peaked by 16 days and 2 horses died. Clinical signs generally included anorexia, weakness and ataxia.

Cardiac Effects

The pharmacological effects of ionophores result from their interaction with intracellular Ca\(^{2+}\) exchanges, namely in mitochondria, that explain skeletal muscles and the heart are the main target tissues. Indirect effects through the release of catecholamines cause coronary vasodilatation. The cardiovascular toxicity of ionophores but especially monensin has been investigated (Pressman and Fahim, 1983). The notifier presents also specific studies performed in the dog and cat.

\(45\) Ref 34; studies R06378 & R06478

\(46\) Section IV, 4.2.8.
**DOG**

The effects of monensin sodium (purity not stated) on blood pressure and blood flow in the left anterior descending coronary artery were measured in Beagle dogs\(^47\). Oral doses of 0, 0.138, 0.345, 0.69 and 1.38 mg kg\(^{-1}\) bw were given. Oral doses of 0.69 mg kg\(^{-1}\) bw or more increased blood flow in the coronary artery, but blood pressure and heart rate were not affected at any of the oral doses tested. The pharmacological NOEL for increased coronary blood flow was 0.345 mg kg\(^{-1}\) bw.

In another study (Pressman and Fahim, 1983) the authors showed an increase in heart rate and arterial pressure in the period up to 2 h following a single oral dose of 2 mg kg\(^{-1}\) bodyweight. Coronary blood flow was not measured in this study. An increase in plasma glucose was also reported but this effect has not been observed in other studies on monensin. An NOEL was not precisely identified in this study, but it must be less than 2 mg kg\(^{-1}\).

**CAT**

An oral dose of 30 mg kg\(^{-1}\) bw had no effect on the spinal reflex, blood pressure, heart rate, respiration or electrocardiogram of an anaesthetised cat. The same dose to an anaesthetised cat also had no effect on the blood pressure responses to intravenous injections of epinephrine, acetylcholine and histamine. As no effects were produced, the NOEL must be greater than 30 mg kg\(^{-1}\) in cats\(^48\).

### 4.7. Conclusion

Monensin sodium is not genotoxic.

The results of chronic toxicity/carcinogenicity studies performed in rats and mice with either the mycelial or the crystalline form suggest that monensin sodium is not carcinogenic. In oral subchronic toxicity tests the dog appears to be more sensitive than the rodents but without evidence of a specific target tissue or organ.

No indication of embryotoxicity, fetotoxicity or teratogenicity was found at the doses tested in the rat and the rabbit.

Monensin exhibits inotropic activity, i.e. acute pharmacological effects on the cardiovascular system. Monensin sodium is highly toxic for horses. A NOEL has been established in the dog, the most sensitive of the experimental animals tested.

### 5. Safety evaluation for the human consumer

#### 5.1. Microbiology of the human GI tract

The susceptibility of a range of human enteric bacteria to monensin was determined, excluding representatives of those Gram-negative aerobic bacteria known to be resistant\(^49\). MIC\(_{50}\) of <4.0 mg monensin l\(^{-1}\) (at pH 6.6 and a concentration of 10\(^6\)-7 organisms ml\(^{-1}\)) were recorded for all of the strains of bifidobacteria, *Clostridium bifermentum*, *Cl. perfringens*, lactobacilli and *Peptostreptococcus* spp. tested. *B. fragilis* and the enterococci were more resistant with MIC\(_{50}\) values of 8.0 mg l\(^{-1}\). When the pH and concentration of organisms was raised to 7.7 and 10\(^8\) respectively, the

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\(^{47}\) Holland, 1981  
MIC values were increased one to four fold. This data was used by the Company to generate a microbiological ADI.

5.2. Proposal for an acceptable daily intake

The lowest NOEL identified from the toxicological studies was $1.1 \text{ mg kg}^{-1} \text{ bw d}^{-1}$ based on the 2-year chronic toxicity/carcinogenicity assay in rat.

An NOEL of $0.345 \text{ mg monensin sodium kg}^{-1} \text{ bw d}^{-1}$ was identified in the dog for acute pharmacological effects on the cardiovascular system. This is the lowest NOEL identified in any of the toxicological or pharmacological studies. As no data on the pharmacological effect of monensin sodium on human was available a safety factor of 100 has been retained by the FEEDAP Panel that leads to an ADI of $0.003 \text{ mg kg}^{-1} \text{ bw d}^{-1}$.

5.3. Proposal for a provisional maximum residue limit

Considering the very low contribution of monensin to the whole tissue residues and the great number of minor metabolites, the evaluation of the consumer exposure must consider the most conservative situation in term of toxicological risk. As most chicken metabolites are also produced in the rat and therefore have been evaluated from a toxicological point of view, the FEEDAP Panel considers that the all metabolites represent a risk which is at most equal to an equivalent quantity of monensin. An additional margin of safety factor is related to the significant proportion of tissue residues which are non-extractable but non-drug related and therefore of no toxicological concern.

The exposure of the consumer has been calculated according to daily human food consumption values set by the SCAN (Directive 2001/79/CE\textsuperscript{50}) and the highest residue levels in tissues measured at different withdrawal times. Considering that the residue levels are similar in chicken and turkey tissues, chickens values which cover a longer withdrawal period have been retained. It must be noted that the consumption figures of both commodities are substitutive and not cumulative. The results are presented on Table 14 at the same time as the percentage of the ADI taken up.

Table 14. Daily exposure of human consumer to monensin sodium residues from chicken tissues along the withdrawal period and corresponding percentage of the acceptable daily intake (ADI)

<table>
<thead>
<tr>
<th>Withdrawal time (day)</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total daily exposure</td>
<td>0.140</td>
<td>0.079</td>
<td>0.063</td>
<td>0.042</td>
</tr>
<tr>
<td>(mg d\textsuperscript{-1} equivalent monensin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of the ADI\textsuperscript{1}</td>
<td>78</td>
<td>44</td>
<td>35</td>
<td>23</td>
</tr>
</tbody>
</table>

\textsuperscript{1} ADI value: $0.180 \text{ mg d}^{-1}$

Considering the marker residue levels found at O-day withdrawal time but also the LOQ of the method of analysis of monensin in tissues (see 1.3.), a uniform MRL value of $0.05 \text{ mg kg}^{-1}$ has been used by the FEEDAP Panel as a first approach for all tissues.

\textsuperscript{50} \url{http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_267/l_26720011006en00010026.pdf}
According to the calculation described in Annex 1, the maximized exposure of the consumer corresponding to this MRL would be 0.171 mg d\(^{-1}\) which is within the limits of the ADI.

Considering the interindividual variability of the residual values which represent about 50% of the mean values retained for the calculations, the FEEDAP Panel proposes to increase the margin of safety in retaining a 3-day withdrawal period after which the consumer exposure has declined to about 50% of the ADI.

The LOQ of the validated analytical method does not allow to follow the marker residue in the different tissues beyond 0-day withdrawal. This uncertainty on the data leads the FEEDAP Panel to consider the uniform 0.05 mg kg\(^{-1}\) MRL as provisional. The deduction of tissue specific MRL’s would necessitate a refinement of the data.

When the turkey is concerned, tissue residue values at 0-day withdrawal time are in the same range as those found in the chicken and consequently the resulting consumer exposure would be similar. Therefore the FEEDAP Panel proposes to retain the same provisional MRL of 0.05 mg kg\(^{-1}\). Considering the interindividual variability of the residual values but also the lack of data on residues beyond 0-day withdrawal time, the FEEDAP Panel proposes to increase the margin of safety in setting a 3-day withdrawal period. The setting of tissue specific MRL’s would also necessitate a refinement of the data.

6. **Worker Safety**

The studies related to worker safety were not GLP-compliant and most were poorly reported. The absence of full details of the protocols of some studies made it impossible to comprehensively evaluate those doses.

6.1. **Observations in humans in the work place**

The petitioner has performed extensive studies in various areas of feed mills producing and packaging a variety of monensin-treated feeds\(^{51}\). The mean air concentrations during mixing of a supplement at 1320 mg kg\(^{-1}\) in feed was about a fourth of the NOEC from the dog inhalation study; exposure during mixing a supplement at 33 mg kg\(^{-1}\) in feed was essentially zero. The highest level of air contamination was in bagging areas, where geometric mean levels over 6 years varied from 0.07-0.17 mg m\(^{-3}\) air (0.07–0.17 µg l\(^{-1}\)). Wearing a dust mask would reduce the exposure by a factor of 10. Studies in packaging areas indicated that 10-50% of the monensin was in the respirable fraction (<10 µm aerodynamic diameter).

Records of physical examinations given annually to feed mill and factory employees over a period of more than 30 years have been reviewed\(^{52}\). The data considered included a health questionnaire, chest x-ray, blood pressure, bodyweight, haematology, urinalysis, test for occult faecal blood, audiometry, ophthalmology and electrocardiogram. There was no evidence of blood dyskrasias, renal or hepatic dysfunction, cardiac disease, neuromuscular disease, pulmonary disease, neoplasm, chronic skin disease or other chronic illnesses in these employees that could be attributed to monensin exposure. Six cases of IgE-mediated allergic response to monensin were identified. Several cases of acute irritative conjunctivitis and one

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\(^{51}\) Brandon et al., 1983; 980/THUMAN/AM/36  
\(^{52}\) Scruby, 1994, Lilly Research Labs; Twenty, 2001
cases of skin irritation were reported following splashes. All resolved completely following symptomatic treatment.

Suspected adverse reactions in humans that have been reported to the petitioner following sale of Elancoban have included occasional cases of contact dermatitis and local respiratory irritation.

6.2. Dermal toxicity/irritancy

Single applications of 500 mg kg\(^{-1}\) (not clear if this refers to a concentration or a dose in mg kg\(^{-1}\) bw) of a dried production lot of mycelial monensin (containing 13.4% monensin activity) were applied to the clipped and abraded skin of 3 male and 3 female New Zealand White rabbits. The application site was covered with an occlusive dressing for 24 h, after which it was then washed thoroughly. The animals were observed for 14 days. Bodyweight was not measured. There were no signs of toxicity or dermal irritation, except for slight erythema in one animal\(^\text{53}\).

Granular monensin (2000 mg of a 9.9% formulation) was applied to the clipped skin of 3 male and 3 female New Zealand White rabbits. Three of the animals had abraded skin. The application site was kept under occlusive dressing for 24 hours and then rinsed. Bodyweight loss was seen in all of the animals and one died during the study. Erythema with skin cracking and crusting was observed in only one animal and it was followed by normal healing\(^\text{54}\).

The above study was repeated to see if the bodyweight loss was due to systemic toxicity. This time the animals were collared to stop them licking the application site. Once again all of the animals lost bodyweight. One animal died of bronchopneumonia. None of the rabbits displayed any irritancy\(^\text{55}\).

Absorption via the skin would be expected to be high given the lipophilic nature of monensin. The finding of systemic toxicity following dermal exposure of rabbits confirms that transdermal absorption of monensin can occur.

6.3. Eye irritancy

Dry mycelial monensin sodium (79 mg, 13.4% monensin activity, i.e. 10.6 mg monensin sodium) was placed into the conjunctival sacs of New Zealand White rabbits. The eyes of 3 rabbits were rinsed for 2 minutes after a 2 minutes exposure time; however, the rinsing was unsuccessful in one of these rabbits. A further 6 rabbits were left with unrinsed eyes. In the two completely rinsed eyes no corneal irritation was observed, slight iris hyperaemia observed was cleared by 24 h and a slight to moderate palpebral conjunctivitis was healed by 7 days. Severe ocular irritation was evident in the unrinsed and inadequately rinsed eyes. Severe corneal opacity, iris hyperaemia and oedema, conjunctival inflammation and oedema were observed within 24 h. There was no healing and two of the rabbits died. Monensin is clearly very toxic to eyes and rapid and extensive rinsing must be carried out in the event of accidental ocular exposure\(^\text{56}\).

Dried production lot of mycelial monensin 59 mg was instilled into one eye of each of 9 New Zealand White rabbits. The treated eyes of three of the rabbits were rinsed

\(^{53}\) Study B-D-43-79

\(^{54}\) Study B-D-113-79

\(^{55}\) Study B-D-20-79

\(^{56}\) Study B-E-124-75, 1032-12.973-978
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after 2 min. The irritancy was mild (corneal dullness, mild iritis and slight conjunctivitis) and the signs of irritancy disappeared after 7 days. However, more severe effects were seen in the unrinsed eyes: corneal opacity, marked iritis and moderate conjunctivitis developed within 1 hour. One unrinsed eye exhibited staphyloma with corneal perforation after 7 days on test and, as healing occurred, extensive vascularisation of the cornea developed\(^{57}\).

Elancoban (53 mg) was instilled into one eye of six New Zealand White rabbits (3 male and 3 female). The eyes were not rinsed. Corneal dullness/mild corneal opacity, marked iritis and moderate conjunctivitis developed in all treated eyes within 1 hour, and within 2 hours well-defined to severe corneal opacity and severe conjunctivitis developed. The corneal opacity was not reversible\(^{58}\).

Elancoban (53 mg) was distilled into one eye of three New Zealand White rabbits, followed by rinsing the eyes 2 min later. After 1 hour there was slight conjunctivitis in all of the treated eyes and corneal dullness and slight iritis was noted in one animal. The eyes returned to normal within 72 hours of treatment\(^{59}\).

6.4. Skin Sensitisation

A modified Buehler test, mycelial monensin was applied to the skin of a group of 12 albino guinea-pigs at a dose of 2 g monensin kg\(^{-1}\) bw. (5-7.5 mg cm\(^{-2}\)) for 4 hours day\(^{-1}\) and 5 days week\(^{-1}\) for 15 applications (OECD Guideline 406 for the Buehler test recommends 6 hrs day\(^{-1}\) for 13-15 consecutive days to produce induction). Upon topical challenge of the same area of skin of half of the animals (6) with 2 g monensin kg\(^{-1}\) bw 17 days later, no evidence of skin irritation or sensitisation was found. However, the dosing period was shorter than that recommended in OECD guidelines.

Monensin sodium (93.8 % monensin) was evaluated for skin sensitising potential in mice using a local lymph node assay in accordance with OECD Guidelines. Concentrations of 0, 5, 10, 25, 50 and 100 mg l\(^{-1}\) of monensin were applied over the ears of groups of 4 female CBA/J mice for three consecutive days. A positive control group was given \(\alpha\)-hexylcinnamaldehyde at a concentration of 250 mg l\(^{-1}\). Ear thickness was measured on days 1, 2, 3 and 4 following dosing as a measure of irritancy. On day 6 the proliferation of lymph node cells was measured in the lymph node draining the application site by incorporation of tritiated methyl thymidine. The positive control material caused a clear lymphoproliferative response in the absence of local irritation. The test material caused no local irritation. There was however a positive lymphoproliferative response at 50 mg l\(^{-1}\), which was attributed to delayed contact hypersensitivity. The size of the response was small but significant indicating a weak sensitiser of skin.

Elancoban 200 was evaluated for skin sensitising potential in mice using a local lymph node assay using concentrations of Elancoban of 0, 50, 100, 250, 500 and 1000 g l\(^{-1}\). The same protocol was used as is described in the previous paragraph. The positive control acted as expected. The Elancoban 200 caused a dose-related lymphoproliferative response in the absence of local irritation. The effect was clearly

\(^{57}\) Study B-E-55-79 \\
\(^{58}\) Study B-E-100-79 \\
\(^{59}\) Study B-E-112-79
positive at 500 mg l\(^{-1}\) or more. The size of the response indicated that Elancoban 200 would need to be classified as a weak sensitiser of skin.

6.5. Dustiness and particle size

The specification for granulated monensin states that “not more than 10 shall pass a 125 µm sieve”. Antidusting oil is added to the formulated product at concentrations of 10-30 mg kg\(^{-1}\). Elancoban 100 and Elancoban 200 were tested for dusting potential using the Stauber-Heubach method in two studies and the particle size distribution of the dust was analysed using a laser. The dust production was shown to be low with very few particles of respirable size (0.05 % of Elancoban 100 and 0.1 % of Elancoban 200 was of less than 9.1 µm diameter).

6.6. Inhalation studies

Rat

Several inhalation studies were performed on rats using Elancoban, mycelial monensin or purified monensin. Most were uninterpretable, as the particle size of the dusts used was either not reported or was too large for the test material to be respired into the lungs. One study used respirable doses. In this study, 5 male and 5 female F344 rats were exposed head-only for one hour to a respirable aerosol of mycelial monensin sodium (mean particle size = 5.9 µm) at a mean measured concentration of 0.37 mg of mycelial monensin sodium per liter of air. All animals survived the exposure, but three had chromodacryorrhoea during the exposure period. The clinical appearance of the rats was normal during a 14-day observation period. There was a reduction in the mean female bodyweight gain, which was mainly due to severely reduced bodyweight in one animal. At necropsy, 5 males and 4 females had enlarged caeca\(^{60}\).

An NOEC was not identified for rats.

Dog\(^{61}\)

A GLP compliant study used groups of 2 male and 2 female Beagle dogs, which were exposed to a sub-80 sieve fraction (particle size <80 µm) of mycelial monensin as aerosols at concentrations equivalent to 0.8 \(10^8\), 1.5 \(10^7\) and 8.4 \(10^7\) mg monensin activity l\(^{-1}\) of air, for 6 hours day\(^{-1}\) on 5 day week\(^{-1}\) and for 90 days. The activity median aerodynamic diameters for the low, medium and high level groups were 6.24, 9.61 and 12.01 µm respectively. No effects on body weight were observed at any dose. At the highest dose level, there were transient increases in serum alanine transaminase, creatine phosphokinase (muscle isoform) and lactate dehydrogenase early in the exposure period. These effects were more marked in female dogs. Intermittent ocular irritation, bloody diarrhoea, salivation and hypoactivity were observed. Electrocardiograph recordings revealed various abnormalities in the top dose group only. These abnormalities included tachycardia, R wave suppression, alterations in T-waves and premature ventricular repolarisation. No other abnormalities (in blood parameters, organ weights or histopathological lesions) were found.

\(^{60}\) Study R-H-11-79

\(^{61}\) Study 3730, 1988\(^{2}\); 980/TBDOGS/AM/88
A NOEC of 0.15 ng monensin activity l⁻¹ of inspired air, equivalent to a dose of 0.151 mg kg⁻¹ bw day⁻¹ (assuming total absorption of the inspired dose) was found in this study. That dose is to compare to the corresponding NOEL of 2.5 mg kg⁻¹ bw day⁻¹ after oral dosing.

6.7. Conclusions on Worker Safety

Both mycelial monensin and Elancoban are very irritant for the eye. Neither mycelial monensin nor Elancoban cause skin irritancy but systemic toxicity may occur following skin exposure. Elancoban is a weak sensitisier by skin exposure and should be regarded as a potential skin sensitisier in humans. Inhalation exposure of the dog and the rat to a high concentration of a respirable fraction of dust from Elancoban caused systemic adverse effects on the heart. However, Elancoban is presented in a granulated form that should form little respirable dust during normal handling in the feed industry and in the farm.

The observations of exposed workers in the feed industry confirm that monensin can cause irritancy to eyes, contact dermatitis and local respiratory irritation.

Advice on personal protective measures (e.g. gloves, ventilation, dust mask, face shield and eye bath) in the feed industry is consistant with the occupational profile of Elancoban.

7. Environment

The active ingredient is no 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 continued 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 and the proposed recommended dose. Distribution to other compartments is also based on parent substance properties, as long as no data on relevant metabolites are submitted.

7.1. Exposure assessment

7.1.1. Fate and behaviour

7.1.1.1. Fate in manure

Experiments on monensin degradation in chicken faeces were performed on one month old chicken fed with 127 mg monensin kg⁻¹ feed. Fresh faeces contained 4 g monensin kg⁻¹ on a dry matter basis. This concentration seems very low in comparison with content in feed; justification or explanation are not provided. Faeces (dry matter 26-28 %) were incubated at 27 and 37°C. Levels remaining after 6 days ranged from 7 to 31 % of initial amount, but data showed a large variability. In one experiment, where observation was prolonged up to 12 days, residual amounts were 7 and 8 % after 6 days, and 14 (with a peak of 37 %) and 32 % after 12 days for incubation at 27 and 37°C respectively. To explain variability, contamination with

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feed or moisture losses were hypothesized. Anyway, the rapid decrease in the first 6 days cannot be confirmed in the longer time.

7.1.1.2. **Fate in soil**

**Adsorption**

The Koc value of monensin was experimentally determined using HPLC at pH 4.5 and 6.0 according to OECD 121. Using the mobile phase methanol: 0.01 M citrate buffer: 65:45, v/v, at both pH’s two peaks associated with monensin sodium were eluted. The first peak was considered to be a degradant and/or related substance of monensin sodium. This component was estimated, by interpolation to have a log Koc of 5.28 at pH 6 and 5.03 at pH 4.5. The second peak was monensin, which eluted at both pH 4.5 and 6 after the standard DDT and therefore was determined to have a log Koc of > 5.6.

Since the pKa of monensin is 6.6, the log Koc is only determined for the non-ionised form. At pH above 6.6 the adsorption of monensin to soil will probably be less. Since the field pH of arable land is more likely to be in the range of pH 8, the log Koc determined at pH 4.5 and 6 can not be used for the risk assessment. Moreover, considering the high water solubility and physical chemical properties of monensin, the HPLC method is not considered appropriate to determine the Koc value. A study with soil, according OECD 106, is considered more appropriate.

**Degradation**

The biodegradation rate of monensin (purity 98 %) was investigated in sandy loam, silty loam and clay loam soils (pH 7.3 – 7.5) under aerobic conditions at a application rate of 1.5 mg a.i. kg⁻¹ soil wwt., pF 2-3 and 20 ± 2 °C for up to 84 days. In addition the route of degradation was investigated in sandy loam soil. Degradation of monensin in all soils was rapid with DT₅₀ values of 18, 13 and 15 days for sandy loam, silty loam and clay loam soils, respectively. After 84 days, monensin was mineralised for 81, 43 and 63 % in the tree soil types, respectively. Several unidentified components (up to 26) were formed. One component, formed in clay loam, was higher than 20 % (36 % at day 14).

7.1.1.3. **Fate in water**

The stability of monensin in aqueous solution was determined turbidimetrically and radiochemically with sterile buffer solutions stored in the dark at 25°C. Monensin was stable in water at pH 5.0, 7.0 and 9.0 for at least 30 days. Using the same methodology the stability of monensin was determined after exposure to a laboratory irradiation apparatus which simulated natural summer sunlight. Data from microbiological assays showed a gradual decline of approximately 40 % after 30 days, which indicate that monensin photodegraded in water at pH 7.0 with a half-life of more than 30 days.

7.1.2. **Predicted environmental concentrations (PEC)**

The methodology for the calculation of the maximum PEC soil, groundwater and surface water is shown in Annex 2. The PECs in soil related to the use of Elancoban in

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63 Response to questions from MS, Vol 5, section IV, part 4, July 2002
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chicken are 0.59 and 1.21 mg monensin kg\(^{-1}\) for vulnerable and non vulnerable areas, respectively. Since the nitrogen concentration in manure of turkeys is higher than that in manure of chicken, the PEC related to the use in turkey will be lower. Therefore, the PECs related to the use of Elancoban in chicken are also considered to cover the potential environmental risk related to the use of Elancoban in turkey. The PECs in groundwater and surface water could not be determined, since no reliable Koc value for monensin is available.

7.1.3. Conclusion

The Phase I PEC trigger value for soil of 10 µg kg\(^{-1}\) is 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

7.2.1.1. Effects on plants

Effect of monensin sodium on the emergence and growth of seedlings of winter oat (Avena sativa), radish (Raphanus sativus) and mung bean (Phaseolus aureus) was studied in sandy loam soil mixed with horticultural grade sand at measured exposure concentration of 0.31, 4.35 and 35.97 mg kg\(^{-1}\) soil\(^{-1}\). The test period was 14 days after at least 50 % emergence.

Table 15. LC\(_{50}\) of monensin sodium for emergence and the EC\(_{50}\) for growth of the three plant species

<table>
<thead>
<tr>
<th>Species</th>
<th>Emergence LC(_{50}) (mg kg(^{-1}))</th>
<th>NOEC</th>
<th>Growth EC(_{50})</th>
<th>NOEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter oat</td>
<td>&gt; 36.0</td>
<td>4.3</td>
<td>12.9</td>
<td>4.3</td>
</tr>
<tr>
<td>Radish</td>
<td>9.8</td>
<td>4.3</td>
<td>&gt; 4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Mung bean</td>
<td>24.1</td>
<td>4.3</td>
<td>32.9</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Radish appeared to be the most sensitive species (Table 15) and at 36 mg kg\(^{-1}\) no plants emerged. The other species showed a significant reduction in growth at this concentration. For these species the NOEC was 4.3 mg kg\(^{-1}\) soil.

7.2.1.2. Effects on earthworms

The acute toxicity of monensin to Eisenia fetida andrei was determined in exposure rate ranging from 47 – 1063 mg monensin kg\(^{-1}\) soil for 14 days at 20\(^\circ\) C, according to OECD 207\(^{66}\). An artificial soil was used composed of 70% sand, 20% kaolinite clay and 10% sphagnum moss peat (pH 7.1-7.3). The LC\(_{50}\) was 264 mg kg\(^{-1}\) dw. The

\(^{65}\) Response to questions from MS, Vol 5, section IV, part 7  
\(^{66}\) Response to questions from MS, Vol 5, section IV, part 6
mortality at 125 mg kg\(^{-1}\) was higher than at 250 mg kg\(^{-1}\), which reduced the homogeneity of the expected and observed % mortality and therefore mortality showed a non-linear response. For this reason the LC\(_{50}\) as to be treated with caution. For the risk assessment the LC\(_{50}\) is normalised to 5 % organic carbon given a value of 132 mg kg\(^{-1}\).

### 7.2.1.3. Effects on soil micro-organisms

Effect of monensin sodium on respiration and nitrification was studied in sandy loam according OECD 216 and 217\(^{67}\). Soil samples were fortified at a rate of 3 and 15 mg monensin 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 activity and nitrate content was determined. After 7 days, the mean respiration rate at 3 and 15 mg a.i. kg\(^{-1}\) was significantly lower than the control. However, after 28 days no significant difference was observed. From day 7, the nitrate content was even significantly higher than the control. At 28 days the difference was less than 10 %. Therefore, the NOEC for effect on soil respiration and soil nitrification is established at > 15 mg a.i. kg\(^{-1}\) soil.

### 7.2.2. Toxicity to aquatic organisms

#### 7.2.2.1. Effects on algae

The acute toxicity of monensin-sodium to *Selenastrum subspicatus* was determined at a nominal concentration range of 0.05-6 mg l\(^{-1}\) according to OECD 201 (Kelly and Clayton, 2002\(^{68}\)). The actual concentration deviated less than 15 % from the nominal values and remains stable until the end of the test. This also suggests that monensin does not bind to algal cells. All the other test conditions were within the acceptable limits. The 72 h-NOEC based on both growth rate and biomass was 0.055 and 0.32 mg l\(^{-1}\), respectively. The EC\(_{50}\) was 0.98 and 4.3 mg l\(^{-1}\), respectively. The effect on growth rate is considered to be most ecologically relevant.

#### 7.2.2.2. Effects on crustaceans and fish

In the risk assessment report\(^{69}\) of the applicant an EC\(_{50}\) value of 10.7 mg l\(^{-1}\) for *Daphnia magna* and a LC\(_{50}\) value of 9 mg l\(^{-1}\) for rainbow trout is reported. Both studies are, however, not submitted in the dossier and can therefore not be validated.

#### 7.2.2.3. Effects on sediment dwelling organism

No data on the toxicity of monensin to sediment dwelling organisms is submitted (nor is its absence justified).

#### 7.2.2.4. Bioaccumulation

No data on bioaccumulation have been submitted. The LogKow is between 3 and 4 which indicates that monensin has a potential for bioaccumulation.

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\(^{67}\) Response to questions from MS, Vol 5, section IV, part 8  
\(^{68}\) Response to questions from MS, Vol 5, section IV, part 9  
\(^{69}\) Response to questions from MS, Vol 5, section IV, part 2
7.2.3. Conclusion

The lowest acute toxic dose for the soil compartment is an EC50 of 9.8 mg kg\(^{-1}\) for plants. Considering the low toxicity of monensin for earthworms and despite the lack of chronic toxicity study, a PNEC of 0.1 mg kg\(^{-1}\) has been retained for the risk assessment for soil, based on the EC50 for plants and applying a safety factor of 100.

As the toxicity data for daphnids and fish cannot be validated, no PNEC for the aquatic compartment can be determined.

7.3. Risk Characterisation

7.3.1. Risk for soil

Based on the assumption that 100 % of the proposed recommended dose is excreted as parent compound, the PEC/PNEC ratio for vulnerable and non-vulnerable areas are above 1, suggesting a risk for soil organisms. However, the metabolism data showed that not more than 20 % of the dose is excreted as parent compound and that each metabolite represents not more than 10 %. Moreover, the biological activity of one of the most representative metabolite evaluated against different end-points represents only 5 % of that of monensin sodium and it is reasonable to assume that the other metabolites would lose at least partly the biological properties that characterize ionophores (see 3.4.). For these reasons it is justified to assess the risk on the basis of 20 % the proposed recommended dose. In that case the PEC/PNEC ratio for vulnerable and non-vulnerable areas is still higher than 1 (Table 16). However, considering that the withdrawal period is not weighted in the PEC calculation and evidence is provided that monensin is degraded in chicken manure to some extent, a risk for the soil compartment is not expected.

Table 16. The PEC/PNEC comparison based on 100 and 210 % of the proposed recommended dose

<table>
<thead>
<tr>
<th>Location</th>
<th>PEC(\text{Soil}) mg kg(^{-1})</th>
<th>PNEC mg kg(^{-1})</th>
<th>PEC/PNEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 %</td>
<td>20 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Vulnerable areas</td>
<td>0.59</td>
<td>0.12</td>
<td>0.1</td>
</tr>
<tr>
<td>Non vulnerable areas</td>
<td>1.21</td>
<td>0.24</td>
<td>0.1</td>
</tr>
</tbody>
</table>

7.3.2. Risk for groundwater and surface water

The provided data is insufficient to determine the PEC and PNEC in groundwater and surface water (see section 7.1.2 and 7.2.2.2). Consequently the risk for groundwater and surface water can not be assessed.

7.3.3. Risk for secondary poisoning

Since the log Kow is between 3 and 4 monensin has a potential for bioaccumulation. The provided data is however insufficient to assess the risk for secondary poisoning.

7.4. Conclusion on the safety for environment

The use of Elancoban at the recommended dose range is not considered to pose a risk for soil organisms. However, insufficient data was provided to allow the FEEDAP
Panel to assess the risk for the aquatic environment (groundwater and surface water) and secondary poisoning.

**CONCLUSIONS AND RECOMMENDATIONS**

From the assessment of the data submitted for Elancoban, FEEDAP Panel draws the following main conclusions:

**PRESENT EFFICACY**

Dose titration and confirmation studies demonstrate that Elancoban at a dose of 100 mg monensin sodium kg\(^{-1}\) is efficacious against coccidiosis of chickens and turkeys challenged with recent *Eimeria* strains. However, the data basis for an actual assessment of the practical efficacy considering only trials which were conducted earlier than 1990, does not fulfil the present requirements of Commission Directive 2001/79/EC. For chickens for fattening only two floor pen studies (instead of three) were considered, while none of the corresponding studies conducted in turkeys were considered as acceptable on the basis of the experimental protocols retained. No data was submitted concerning floor pen trials in chicken reared for laying. When field trials are concerned, the only study (instead of three) concerning chickens for fattening used monensin concentrations substantially below the expected value and the recommended dosage, while no recent studies were presented concerning turkeys and chickens reared for laying.

The sensitivity profiles of recent *Eimeria* spp. isolates from chickens against monensin sodium, tested in battery trials, indicate partial resistance that could be overcome by the use of rotation or shuttle programs. No specific experiments on resistance with turkeys are reported, only indirect evidence of the absence of being reported from the USA and the UK.

In the absence of specific data no assessment of any potential influence of monensin sodium on the quality of chicken and turkey produce is possible. However, no relevant findings became known from field experience with the additive over decades.

**RESISTANCE**

Gram positive bacteria, namely those present in the digestive tract of poultry and other livestock, are more susceptible to monensin sodium than Gram negative strains. However, no proliferation of the latter has been observed in the gastrointestinal tract of chickens treated with monensin at the recommended dosage. Laboratory studies have shown that some Gram positive strains can develop resistance to the additive.

However, no evidence was found that exposure to monensin sodium would induce the development of cross-resistance to other antibiotics of human clinical and veterinary importance.

It has been shown that the colonisation and shedding of enteropathogens, namely *Salmonella*, are not influenced by monensin sodium treatment in chickens.

**SAFETY FOR TARGET SPECIES**

Monensin sodium is safe when fed to chickens for fattening and chickens reared for laying at dietary levels not exceeding the maximum recommended dosage of 125 mg kg\(^{-1}\) complete feed. It can be considered as safe for turkeys at 120 mg kg\(^{-1}\) level but the highest recommended dose (100 mg kg\(^{-1}\)) should preferably not be exceeded.
Incompatibilities or interactions with feedingstuffs, carriers or other approved additives are not expected by means of the known history of monensin. However, it is well known from the literature that severe interactions between the ionophore coccidiostats and the antibiotic tiamulin but also other antibiotics (mainly macrolides) may occur. Therefore the simultaneous use of Elancoban and certain antibiotic drugs (i.e. tiamulin) is contra-indicated. Feed containing Elancoban 100 or Elancoban 200 should therefore be labelled with a warning statement: **Avoid simultaneous administration with tiamulin and monitor for possible adverse reactions when used concurrently with other medicinal substances.**

SAFETY FOR OTHER NON TARGET ANIMAL SPECIES

Monensin sodium in doses proposed for feed supplementation in chickens is very toxic to horses. A corresponding reference in the instructions for use is recommended.

SAFETY FOR THE CONSUMER

Monensin sodium is absorbed and metabolized extensively by chicken and turkey and its metabolic fate is similar in these two species and also the rat. A great number of metabolites has been isolated from the excreta and tissues of which eight major metabolites have been identified each representing less than 10 % of the whole residues. They result from either single or combined demethylation, decarboxylation and hydroxylation of the molecule. The proportion of unextractable residues in tissues increases with the withdrawal time that corresponds to the labelling of endogenous compounds resulting from the decarboxylation of radiolabelled monensin. Considering the weak biological activity of one of the major metabolites it can be reasonably assumed that monensin metabolites would be much less active than the original compound.

Kinetic studies of the whole residues in tissues are available in the chicken but not the turkey (only 0-day withdrawal). Despite the inconsistency of the available studies and subsequent difficulty to choose between the liver and skin/fat the most pertinent target tissue, FEEDAP Panel considers for practical reasons, i.e. control purposes, that skin/fat should be retained. Although monensin represents a small fraction of tissue residues, it is retained as the marker-residue.

No specific data have been submitted concerning monensin residues in the first eggs laid by hens that had received Elancoban supplemented feed at the proposed dosage, from 0 to 16 weeks of age as chickens reared for laying. However, data obtained in hens that received 80 mg monensin kg\(^{-1}\) feed for at least three consecutive weeks indicate that monensin residues in eggs are present at very low level, do not accumulate and disappear after a 5-day withdrawal period. Considering the usual lag time between the end of the administration period (16 weeks) and the starting of laying, these data represent a worse case scenario. Therefore the FEEDAP Panel considers that the contribution of these eggs to the whole consumer exposure is negligible.

Monensin sodium is not genotoxic. The results of chronic toxicity/carcinogenicity studies performed in rats and mice with either the mycelial or the crystalline form suggest monensin sodium is not carcinogenic. In oral subchronic toxicity tests the dog appears to be more sensitive than the rodents but without evidence of a specific target tissue or organ.
No indication of embryotoxicity, fetotoxicity or teratogenicity was found at the doses tested in the rat and the rabbit. The lowest NOEL identified from the toxicological studies was 1.2 mg kg\(^{-1}\) bw d\(^{-1}\) based on the 2-year chronic toxicity/carcinogenicity assay in mice.

Acute pharmacological effects on the cardiovascular system of the dog lead the FEEDAP Panel to retain a lower NOEL for monensin sodium of 0.345 mg kg\(^{-1}\) bw d\(^{-1}\). Applying an uncertainty factor of 100 to this NOEL gives an ADI of 0.003 mg kg\(^{-1}\) bw.

SAFETY FOR THE USER

Elancoban is very irritant to the eye but not the skin. It is regarded as a weak sensitizer by skin exposure and a potential respiratory sensitizer. Moreover the dermal and respiratory exposure could lead to systemic pharmacological effects, namely to the heart. Although the granular formulation of Elancoban minimises release of monensin sodium dust from the product, further protective measures to limit worker exposure at the feed industry level need to be considered.

SAFETY FOR THE ENVIRONMENT

The use of Elancoban at the recommended dose range is not considered to pose a risk for soil organisms. However, insufficient data was provided to allow the FEEDAP Panel to assess the risk for the aquatic environment (groundwater and surface water) and secondary poisoning.

MONITORING

An adequate and fully validated method was described allowing monitoring of monensin in premixes and complete feedingstuffs supplemented with Elancoban.

Provisional uniform MRL’s of 0.05 mg kg\(^{-1}\) have been retained for the liver, kidney, muscle and skin/fat of the chicken and turkey. A method has been validated for the determination of monensin, the marker-residue, in the liver, kidney, muscle and skin/fat. Its limit of quantification (0.025 mg kg\(^{-1}\) for all tissues) complies with the subsequent analytical requirement.

RECOMMANDATION

Feed containing Elancoban 100 or Elancoban 200 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.
DOCUMENTATION PROVIDED TO EFSA


2. Supplementary dossier submitted by Eli Lilly in July 2002. Responses to questions from Member States

3. Supplementary dossier submitted by Eli Lilly in May 2003. Further responses to questions from Member States

4. Supplementary dossier submitted by Eli Lilly in February 2004. Responses to questions from Member States

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ANNEXES

Annex 1. Calculation of the maximized consumer dietary intake corresponding to the chosen MRL(s)

Definitions, abbreviations and formulas

\( i \) \quad \text{Tissues: } i=1: \text{ liver, } i=2: \text{ kidney, } i=3: \text{ muscle, } i=4: \text{ skin plus fat}

\( \text{MRL}_i \) \quad \text{Maximum residue limit in tissues (mg kg}^{-1}\text{) set by an authority}

\( \text{Qt}_i \) \quad \text{Daily human food consumption (kg) set by Directive 2001/79/CE}

\( \text{TRC}_i \) \quad \text{Total residue concentration (mg kg}^{-1}\text{)}

\( \text{MRC}_i \) \quad \text{Marker residue concentration (mg kg}^{-1}\text{)}

\( \text{RMTR}_i \) \quad \text{Ratio MRC}_i \text{ to TRC}_i

\( \text{DITR}_i \) \quad \text{Dietary intake calculated from total residues (mg)} = \text{Qt}_i \times \text{TRC}_i

\( \text{DITR}_{\text{MRL}_i} \) \quad \text{Dietary intake calculated from MRL's (mg)} = \text{Qt}_i \times \text{MRL}_i / \text{RMTR}_i

Figures to be used for Elancoban

<table>
<thead>
<tr>
<th>Liver (1)</th>
<th>Kidney (2)</th>
<th>Muscle (3)</th>
<th>Skin+fat (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRL(^\dagger)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Qt(_{1-4})</td>
<td>0.1</td>
<td>0.01</td>
<td>0.3</td>
</tr>
<tr>
<td>TRC(_{1-4})</td>
<td>0.94</td>
<td>0.2</td>
<td>0.06</td>
</tr>
<tr>
<td>MRC(_{1-4})</td>
<td>0.039</td>
<td>0.014</td>
<td>0.029</td>
</tr>
<tr>
<td>RMTR(_{1-4})</td>
<td>0.0415</td>
<td>0.0700</td>
<td>0.4833</td>
</tr>
</tbody>
</table>

First approximation of the maximized consumer dietary intake with a common MRL for all tissues

<table>
<thead>
<tr>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Skin+fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>DITR(_{1-4})</td>
<td>0.094</td>
<td>0.002</td>
<td>0.018</td>
</tr>
<tr>
<td>DITR(<em>{\text{MRL}</em>{1-4}})</td>
<td>0.0121</td>
<td>0.007</td>
<td>0.031</td>
</tr>
</tbody>
</table>

\(^\dagger\) Provisional uniform MRL set by the FEEDAP Panel
Annex 2. 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 (Scientific Committee for Animal Nutrition), 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:

\[
P_{\text{ECsoil}} = \frac{P_{\text{ECmanure}} \cdot Q}{\text{RHOsoil} \cdot \text{CONV}_{\text{area field}} \cdot \text{DEPTHfield}}
\]

<table>
<thead>
<tr>
<th>Input</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHOsoil</td>
<td>bulk density of soil</td>
</tr>
<tr>
<td>DEPTHfield</td>
<td>mixing depth with soil</td>
</tr>
<tr>
<td>CONV(_{\text{area field}})</td>
<td>conversion factor for the area of the agricultural field</td>
</tr>
<tr>
<td>Q</td>
<td>nitrogen immission standard</td>
</tr>
<tr>
<td>PEC(_{\text{manure}})</td>
<td>concentration in manure expressed per amount nitrogen</td>
</tr>
<tr>
<td>PECsoil</td>
<td>highest concentration in the soil</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500 kg m(^{-3})</td>
</tr>
<tr>
<td>0.2 m (arable land)</td>
</tr>
<tr>
<td>10000 m(^2) ha(^{-1})</td>
</tr>
<tr>
<td>[kg ha(^{-1}) yr(^{-1})]</td>
</tr>
<tr>
<td>[mg kg(^{-1})]</td>
</tr>
<tr>
<td>[mg kg(_{\text{soil}}^{-1})]</td>
</tr>
</tbody>
</table>
PEC groundwater

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

\[
\text{PEC}_{\text{gw}} = \text{PEC}_{\text{porewater}}
\]

\[
\text{PEC}_{\text{porewater}} = \frac{\text{PEC}_{\text{soil}} \cdot \text{RHO}_{\text{soil}}}{K_{\text{soil-water}} \cdot 1000}
\]

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

\[
K_{p_{\text{soil}}} = F_{\text{oC-soil}} \cdot K_{\text{oC}}
\]

\[
K_{\text{air-water}} = \frac{\text{VP} \cdot \text{MOLW}}{\text{SOL} \cdot R \cdot \text{TEMP}}
\]

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_{soil}</td>
<td>1700 kg m\textsuperscript{-3}</td>
</tr>
<tr>
<td>Density of soil solids</td>
<td>RHO_{solid soi}</td>
<td>2500 kg m\textsuperscript{-3}</td>
</tr>
<tr>
<td>Fraction air in soil</td>
<td>FA_{soil}</td>
<td>0.2 m\textsuperscript{3}</td>
</tr>
<tr>
<td>Fraction water in soil</td>
<td>F_{water-soil}</td>
<td>0.2 m\textsuperscript{3}</td>
</tr>
<tr>
<td>Fraction solids in soil</td>
<td>F_{solid-soil}</td>
<td>0.6 m\textsuperscript{3}</td>
</tr>
<tr>
<td>Weight fraction organic carbon in soil</td>
<td>F_{oC-soil}</td>
<td>0.02 kg kg\textsuperscript{-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\textsuperscript{3} mol\textsuperscript{-1} K\textsuperscript{-1}</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>VP</td>
<td>[Pa]</td>
</tr>
<tr>
<td>Molar mass</td>
<td>MOLW</td>
<td>[g mol\textsuperscript{-1}]</td>
</tr>
<tr>
<td>Water solubility</td>
<td>SOL</td>
<td>[mg l\textsuperscript{-1}]</td>
</tr>
<tr>
<td>Partition coefficient solids and water in soil (v/v)</td>
<td>K_{soil-water}</td>
<td>[m\textsuperscript{3} m\textsuperscript{-3}]</td>
</tr>
<tr>
<td>Partition coefficient solids and water in soil (v/w)</td>
<td>K_{p_{soil}}</td>
<td>[dm\textsuperscript{3} kg\textsuperscript{-1}]</td>
</tr>
<tr>
<td>Partition coefficient air and water in soil</td>
<td>K_{air-water}</td>
<td>[m\textsuperscript{3} m\textsuperscript{-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


