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

(Question N° EFSA-Q-2003-044)

Adopted on 3 December 2003

SUMMARY

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

The active agent in Deccox is decoquinate, a 4-hydroxyquinoline, incorporated at 0.6 % by weight in the feed additive. Evidence generated at the time of its introduction suggested that a product delivering 20-40 mg of decoquinate per kg complete feed was effective as a coccidiostat for chickens. However, a full assessment of the present day efficacy was not possible as an insufficient number of trials had been carried out in the last ten or so years, reflecting current production.

Tolerance tests showed that Deccox is safe for the target animals. With the exception of an incompatibility with bentonite used as a binder in pelleting, no incompatibilities or interactions with feedingstuffs, carriers, other approved additives or medicinal drugs have been recognised.

Most strains of bacteria appear resistant to the effects of decoquinate at concentrations of \(> 64 \text{ mg l}^{-1}\), substantially higher than the concentration of decoquinate expected in the digestive tract. Consequently, any effects on the bacterial flora of chickens is likely to be minimal. In the absence of selective pressure, it is also very unlikely that the use of decoquinate would result in cross resistances with clinically important antimicrobial compounds.

Studies on the metabolism of decoquinate in chickens have shown that this lipophilic compound is absorbed, but is rapidly eliminated after absorption largely via bile and the faeces and to a much lesser extent in urine. The metabolic equilibrium in tissues is reached after three days. However, data showing that unchanged decoquinate represents by far the major fraction of the excreted compounds were obtained only in one experiment in which 20 times the dose proposed for decoquinate was used. This data cannot be safely extrapolated to cover the proposed use of Deccox at its recommended dose range.

Skin/fat appears as the target tissue. The discrepancies observed between studies concerning the proportion of unchanged decoquinate in tissue residues, the fact that metabolites represent more than 10 % of the total residues but have not been identified or individually quantified and the lack
of data on the distribution of metabolites along the withdrawal period, do not allow the identification of the marker-residue.

Toxicity studies generally were done to standards appropriate to the time but many were not in accordance with GLP and would not be considered satisfactory by present standards. A NOEL of 15 mg kg\(^{-1}\) bodyweight (bw) day\(^{-1}\) was identified from repeat dose dog studies based on reduced physical activity. No treatment-related adverse effects were seen in a three-generation rat study or in a developmental study in rabbits. Retarded skeletal development was observed at 300 mg kg\(^{-1}\) bw day\(^{-1}\) in a developmental study in rats. The NOEL for this study was 100 mg kg\(^{-1}\) bw day\(^{-1}\).

The results of the in vitro genotoxicity tests indicated that decoquinate was not genotoxic. The clearly negative results from bacterial tests and from cytogenetics studies gave sufficient reassurance of safety to overcome any doubts generated by the equivocal result from a mouse lymphoma assay. In the view of the FEEDAP Panel the adverse response seen with 2500 µg ml\(^{-1}\) decoquinate in the mouse lymphoma assay was a consequence of the general cytotoxicity of the compound.

No reports of carcinogenicity bioassays were available, but the results of a two-year rat study gave limited evidence of an absence of carcinogenicity. Furthermore, the results of repeated dose toxicity studies gave no suggestion of any effects of decoquinate that might be associated with a non-genotoxic mechanism of carcinogenicity. It was therefore concluded that decoquinate was unlikely to be a carcinogen either by a genotoxic or non-genotoxic mechanism.

The NOEL of 15 mg kg\(^{-1}\) bw day\(^{-1}\) may be used as the basis for setting an acceptable daily intake (ADI) for decoquinate. Applying a 200-fold uncertainty factor to this NOEL gives an ADI of 0.075 mg kg\(^{-1}\) bw. A higher than usual uncertainty factor was used to derive the ADI to compensate for concern about the precision of the results from the older studies not performed to present standards. The ADI of 0.075 mg kg\(^{-1}\) bw allows an adequate margin of safety for the other toxicological effects seen in the studies. The limited data available indicates that there are differences in the number and nature of the metabolites produced in the chickens compared to rats. As none of the metabolites have been identified or quantified (in either species) the risk for the consumer exposed to decoquinate residues in chicken tissues cannot be adequately assessed. As a consequence, the FEEDAP Panel is unable to establish an MRL for decoquinate.

Deccox has been shown to have a low dusting potential. Consequently, worker exposure will occur primarily by skin contact. The extremely low acute toxicity combined with lack of irritant or sensitisation potential of the product suggests that only minimal precautions would need to be taken during handling.

The use of Deccox at the recommended dose range does not pose a risk for the terrestrial or aquatic environment.

Key words: Coccidiostat, feed additive, Deccox, decoquinate, 4-hydroxyquinoline, anticoccidial efficacy, microbiological risks, target animal safety, consumer safety, ADI, MRL, worker safety, environmental safety
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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 1st 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 1st October 1998. Furthermore, a dossier, as referred to in Article 4 of Directive 70/524/EEC, had to be submitted not later than 1st 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 of the Panel on Additives and Products or Substances used in Animal Feed deals with the coccidiostat DECCOX.

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 each of the products on the basis of the dossiers presented, the Scientific Panel on Additives and Products or Substances used in Animal Feed is requested to answer the following questions. Under the conditions proposed for its use as additive in feed,
1) Is the efficacy of the product as described in the respective table (Annex inscriptions) demonstrated?

2) For the product considered can its use result in the development of resistance in bacteria to prophylactic or therapeutic preparations?

3) Is the product and its metabolites safe for
   - the target animals,
   - the user,
   - the consumers,
   - the environment?

4) Can the product be monitored?

**ANNEX INSRIPTIONS**

<table>
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<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</th>
<th>Maximum content</th>
<th>Other provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decoquinate (Deccox)</td>
<td>3-ethoxycarbonyl-4-hydroxy-6-decyloxy-7-ethoxyquinoline</td>
<td>Chickens for fattening</td>
<td>-</td>
<td>20</td>
<td>40</td>
<td>Use prohibited at least 3 days before slaughter</td>
</tr>
</tbody>
</table>
ASSESSMENT

1. INTRODUCTION

Deccox is an additive intended for incorporation into the feed of chickens for fattening for the control of coccidiosis caused by *Eimeria* spp. The product is approved for the production lifetime of the birds, with a withdrawal period of three days. The present approved dose range is 20–40 mg decoquinate kg\(^{-1}\) complete feed although the Notifier now recommends \(^{1}\) a minimum incorporation of 30 mg kg\(^{-1}\).

The product Deccox is formulated to contain 6.0 g kg\(^{-1}\) of the active substance decoquinate. The product Deccox is formulated to contain 6.0 g kg\(^{-1}\) of the active substance decoquinate.

![Decoquinate molecule](image)

Decoquinate \([I]\), a 4-hydroxyquinoline (ethyl-6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate, C\(_{25}\)H\(_{35}\)NO\(_5\), CAS Number 18507-89-6), is manufactured by a seven step chemical synthesis and then blended (micronised) with 1% by weight silica. Quality control analysis of 146 batches of micronised decoquinate gave a mean value of 989.2 ± 2.2 g kg\(^{-1}\). Three chemically closely related impurities may occur in the final product in concentrations not exceeding 10 g kg\(^{-1}\) product.

- 6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylic acid
- Methyl-6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylic acid
- Diethyl 6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylic acid

After micronising, decoquinate (6.06 g kg\(^{-1}\) of the micronised product) is mixed with refined soybean oil (2.8% by weight of final product), the remainder of the product Deccox being wheat middlings used as a carrier. The final product is a light-brown powder with less than 10% passing a 125 µm sieve and with a low dusting potential.

Decoquinate is authorised for use in the EU under Council Directive 70/524/EEC and is being re-evaluated in accordance with the requirements of Council Directive 96/51/EEC.

1.1. Physical and chemical properties of decoquinate

Decoquinate (MW 417.53) is poorly soluble in water (0.06 mg l\(^{-1}\) in purified water and < 0.01 mg l\(^{-1}\) in buffered water pH 4–9). An exact solubility cannot be given, since the method used was not applicable for substances with solubility < 0.01 mg l\(^{-1}\). The partition coefficient, determined by a HPLC method, indicates that decoquinate is highly lipophilic (Log \(K_{ow}\) 5.2-5.5). The vapour pressure of decoquinate is not cited, but for the purposes of the environmental risk assessment partitioning to air can be considered negligible.

\(^{1}\) Response to Questions on Application for Brand Specific Approval of Deccox. Vol. 1, p 6.
1.2. **Mode of action**

Based on data obtained from clinical studies, decoquinate is thought to act by arresting the development of sporozoites following their penetration of the gut epithelium. The degree of damage to the gut in terms of lesions is significantly reduced and oocyst output is also reduced. Decoquinate significantly inhibits mitochondrial respiration and electron transport in *Eimeria* (Fry and Williams, 1984).

1.3. **Stability**

Decoquinate tested in the form of Deccox is degraded over a period of four days under strong acidic (0.1 M HCl) and alkaline (0.1 M NaOH) conditions, but is resistant to photodegradation, thermal degradation (< 60°C) and to oxidative conditions. When stored as supplied by the manufacturer at temperature not exceeding 25°C and protected from moisture, the product has a shelf life of at least three years. Deccox survives pelleting. When incorporated in a premix or a complete feed it has an expected shelf life of at least three months when stored below 25°C at ambient humidity. During the claimed shelf life of the product there is no evidence of breakdown products.

1.4. **Control methods**

1.4.1. **Determination of decoquinate in poultry feed**

An HPLC method for the determination of decoquinate in premixes and complete feed has been developed for quality control purposes and validated. The limits of detection (LOD) established as the absolute amount required to generate a signal-to-noise ratio of 3 are 900 mg kg\(^{-1}\) and 300 mg kg\(^{-1}\) for the 6% and 2.5% premixes respectively. The corresponding limits of quantification (LOQ) are 3000 mg kg\(^{-1}\) and 1000 mg kg\(^{-1}\). For feed the respective LOD and LOQ are 0.2 and 1.0 mg kg\(^{-1}\).

1.4.2. **Determination of decoquinate in tissues**

An HPLC method for the determination of decoquinate in tissues has been described and validated. The chromatographic system proved to be satisfactory in terms of specificity (no interference of the matrices), linearity of response, precision and accuracy. The LOD, established as the absolute amount required to generate a signal-to-noise ratio of 3, was determined to be 0.005, 0.005, 0.004, 0.006 and 0.003 mg kg\(^{-1}\) for the liver, kidney, muscle, fat and skin/fat respectively. The LOQ has been validated at 0.05 mg kg\(^{-1}\) for the whole tissues.

2. **Efficacy**

Commission Directive 2001/79/EC requires efficacy data derived from:

a. controlled battery-cage experiments;

b. controlled floor pen studies;

c. controlled field trials (actual use conditions).

The continuous use of coccidiostats coupled with changes to breeds and production methods may have changed the response of present day *Eimeria* populations to the application of coccidiostats. Directive 2001/79/EC states that "dossiers must enable an assessment to be made of the additive based on the present state of knowledge". Consequently, in its assessment of the efficacy of Deccox, FEEDAP Panel took only those
efficacy studies completed no earlier than about 1990 into consideration. An exception, however, was made for those battery-cage experiments which served for the principal discovery or confirmation of anticoccidial properties of an additive against single or mixed infections or were used for initial dose response studies.

2.1. **Dose titration**

Battery trials reported in 1967 showed that in controlled conditions using 3.75 to 40 mg decoquinate kg⁻¹ feed that all dosages except the lowest were effective against *E. necatrix*, *E. maxima* and *E. tenella*. The recommended dose range derived from these studies was 20 – 40 mg decoquinate kg⁻¹ complete feed.

2.2. **Studies concerning the efficacy of the additive on the target species**

2.2.1. **Controlled floor pen study**

One study only, reported in 1999², was considered in this assessment. This was intended to establish the efficacy of decoquinate supplied in the form of Deccox in controlling a virulent challenge infection of field isolates of *Eimeria* species in broiler chicks (challenge dose of oocysts given to each bird: 50969 *E. acervulina*, 9267 *E. maxima*, 19306 *E. tenella*, 8258 *E. mitis* and 1521 *E. praecox*).

Two concentrations of decoquinate (intended dose 20 and 40 mg decoquinate kg⁻¹ complete feed, measured dose 18 and 32 decoquinate kg⁻¹ complete feed) and for comparison, two other anticoccidial products used at a single recommended concentration were included in the study.

Female broiler chicks (250, 13 day old) were randomised by weight into 18 pens of ten birds (three pens per treatment: uninfected control, infected control, treatments). Birds from the median weight range were selected for use in the trial and surplus birds discarded at this stage. At 18 days old the birds in one untreated group were "sham dosed" with distilled water and the rest of the birds were dosed with the mixture of oocysts. The birds were weighed on days –5, 0 and 7, feed consumption over the pre-challenge period (day –5 to 0) and over the challenge period (day 0 to day 7) was recorded and feed conversion calculated.

Birds were observed daily for signs of coccidial infection (diarrhoea, blood in faeces, depression, loss of appetite), faecal state scores were assessed daily for each group on days 4 to 7, mortality was recorded and all birds examined for obvious causes of death. Birds judged to have died as a result of coccidial infection were assessed for lesion score and included in the weight data analysis. A complete collection of faeces produced by each group was made on days 6 and 7 post challenge (Table 1). The numbers of oocysts were determined and a percentage differential count of species made by direct microscopical examination. The study was terminated seven days after challenge, when birds were killed by cervical dislocation and examined for coccidial lesions.

A total of nine birds died as a result of the coccidial challenge, four in the infected control groups and five in the groups treated with one of the alternative products. Mortality rate was not statistically evaluated. The bodyweights of these birds were included in the bodyweight analysis. Lesion scores indicated a severe *E. tenella* infection.

Opinion on the additive Deccox

No clinical signs were observed in any of the control non-infected non-medicated birds or in any birds notionally receiving 40 mg decoquinate kg\(^{-1}\) feed. The infected control birds had a droopy huddled appearance and looked poor on days 5 and 6 post infection and several birds died. By day 7 the survivors were slightly better in appearance. A few birds in two groups given 20 mg decoquinate kg\(^{-1}\) feed were droopy or quiet on day 6, but had recovered by day 7.

The faeces from non-infected control and birds given one of the alternative products remained normal throughout the study period. Copious blood was seen in the faeces of the infected controls and birds given the second alternative product from day 5 onwards. With 20 mg decoquinate kg\(^{-1}\) feed, blood was seen in one group of day 5 and in all groups by day 6. In birds receiving 40 mg decoquinate kg\(^{-1}\) feed, blood was only seen on day 7.

### Table 1. Effect of treatments on Mean body weight (BWG) gains (as a % of the uninfected control-UIC), feed:gain ratio from day 0 to 7 and mean faecal state score at day 7

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BWG (g) (% of UIC)</th>
<th>Feed:Gain</th>
<th>Faecal score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected control</td>
<td>272a(100)</td>
<td>4.2</td>
<td>1</td>
</tr>
<tr>
<td>Infected control</td>
<td>98b(36)</td>
<td>8.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Decoquinate 20 mg kg(^{-1})</td>
<td>128c(47)</td>
<td>8.8</td>
<td>4</td>
</tr>
<tr>
<td>Decoquinate 40 mg kg(^{-1})</td>
<td>141e(52)</td>
<td>6.8</td>
<td>3.3</td>
</tr>
<tr>
<td>Coccidiostat 1</td>
<td>93b(34)</td>
<td>12.6</td>
<td>1</td>
</tr>
<tr>
<td>Coccidiostat 2</td>
<td>124c(45)</td>
<td>8.6</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Different superscripts within the column indicate significant differences (P<0.05).

Faecal score:  

1 = normal faeces  
2 = presence of slimy white stuff amongst the diarrhoea  
3 = slight blood  
4 = very bloody diarrhoea and presence of caecal cores

Statistical analysis showed evidence for an increased body weight gain when decoquinate was used as shown by the significant positive trend with concentration (Table 1). The body weight gain for all dead birds was included in the calculation of feed conversion.

An estimate was made of the number of lesions in each of three areas of the intestine (upper small intestine, mid small intestine and caecum). A maximum score of 4 could be given for the most severe condition which when summed over the three sites gave a possible total score of 12 (Table 2).

### Table 2. Mean lesion scores in the upper small intestine (USI), mid small intestine (MSI) and caecum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>USI</th>
<th>MSI</th>
<th>Caecum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infected control</td>
<td>2.3</td>
<td>1.4</td>
<td>3.6</td>
<td>7.3</td>
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<tr>
<td>Decoquinate 20 mg kg(^{-1})</td>
<td>1.4</td>
<td>0.8</td>
<td>2.8</td>
<td>5.1</td>
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<tr>
<td>Decoquinate 40 mg kg(^{-1})</td>
<td>1.2</td>
<td>0.5</td>
<td>1.1</td>
<td>2.9</td>
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<tr>
<td>Coccidiostat 1</td>
<td>0.9</td>
<td>0.7</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Coccidiostat 2</td>
<td>1.2</td>
<td>0.7</td>
<td>3.2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

All the medicated birds had lower total lesions scores than the infected non-medicated birds, with 40 mg decoquinate kg\(^{-1}\) feed giving better control of lesions than the lower dose. No oocysts were detected in faeces of birds in the uninfected control group. Birds in all other groups were positive but there was no appreciable difference in the total number of oocysts.
produced by any of the groups on day 6 (range 900-1100 oocyst output per group x 10^6) or day 7 (range 400-800 oocyst output per group x 10^6).

2.2.2. Field trial

One field trial\(^3\) was performed to determine the utility and safety of 30 mg decoquinate kg\(^{-1}\) feed supplied in the form of Deccox under commercial use conditions in comparison with another commercial synthetic coccidiostat used at its recommended dose. This study was carried out to the principles of good clinical practice for the conduct of clinical trials for veterinary medicinal products.

Two identical buildings were used, one used for the Deccox-treated birds and one for birds treated with the alternative coccidiostat. Each building had the same type of feeding and watering systems. Birds were placed in the buildings immediately upon arrival from the hatchery (day 0). The breed used was ISA (broilers) with a stocking density of 24480 birds in 1200 m\(^2\) (20.4 birds m\(^2\)). Birds were as-hatched and were not individually identified or sexed.

The two feeds supplied differed only in nature of the coccidiostat. The concentrations of the additives were measured (24 mg decoquinate kg\(^{-1}\) feed for Deccox).

Samples of litter containing faeces were collected on days 7, 14, 22, 28 and 35. In each building 20 fresh caecal faeces and 20 fresh intestinal contents were collected from each of four predefined areas for determination of the oocyst counts. Litter samples (depth of about 10 cm) were collected for determination of the dry matter content on day 38. Data from the manual weighing of approximately 100 birds were collected once a week from day 0.

At days 29 and 35, 50 birds from each building were sent for necropsy. Lesions related to \textit{E. acervulina} and \textit{E. tenella} were quantified. Farm staff recorded daily bird health, state of the litter (bloody and/or unusual droppings), mortality and water consumption. Feed intake was recorded (the feed bins were emptied before and after the study).

No significant increase in the number of oocysts combined with increased mortality was observed during the study.

No clinical signs of coccidiosis outbreak were observed in either group at any time during the study. However, a necrotic enteritis outbreak was encountered in the building of the group given the alternative coccidiostat. Necropsy revealed intestinal ulcers, necrosis and enteritis. Bacteriology showed the presence of \textit{Clostridium perfringens}. Therefore, amoxicillin was administered for four days in both buildings.

The overall mortality for the entire study period was 2.82% in the Deccox group and 3.67% in the group given the alternative product. Mean body weights of 100 individual records are shown in Table 3. Statistical analysis did not show a significant difference between the two groups. Feed:gain ratios were calculated as 1.77 for the Deccox group and 1.79 for the group given the alternative treatment.

\(^3\) Study 00D1, 2000. Vol. 5, pp 261-299.
Table 3. Mean body weights (g) (n = 100 per treatment group)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>22</th>
<th>28</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deccox</td>
<td></td>
<td>41a</td>
<td>162a</td>
<td>418a</td>
<td>880a</td>
<td>1252a</td>
<td>1733a</td>
</tr>
<tr>
<td>Coccidiostat 1</td>
<td></td>
<td>42a</td>
<td>155b</td>
<td>413a</td>
<td>892a</td>
<td>1238a</td>
<td>1780b</td>
</tr>
</tbody>
</table>

Different superscripts within the column indicate significant differences (P<0.05).

The oocyst counts remained low throughout the study, but were significantly higher in the group given the alternative product on day 28. At days 28 and 35, no chicken presented *E. tenella* lesions. At day 28, four chickens out of 50 showed *E. acervulina* lesions in the group receiving the alternative treatment versus one chicken out of 50 in the Deccox group (non-significant).

Dry matter content of the litter was 22.4% in the group receiving the alternative coccidiostat and 27.1% in the Deccox group.

2.3. Studies on the development of resistance in *Eimeria*

Included in the dossier are four published studies dating between 1971 and 1986 on the development of resistance in *Eimeria* spp.

Resistance gradually developed in *E. tenella* during nine passages, five passages with 15 mg kg⁻¹ decoquinate followed by four passages at 30 mg (Mcloughlin and Chute, 1971). Similar results, again with *E. tenella* were obtained in an experiment where the decoquinate concentration was increased from 5 to 100 mg kg⁻¹ during nine passages (Chapman, 1985). However, in another study a multiresistant but decoquinate sensitive strain of *E. necatrix* remained sensitive to decoquinate at the recommended level after 51 passages exposed to a sub-optimal level of 2 mg kg⁻¹ (Saitoh et al., 1986). In an experiment with a resistant *E. tenella*, which was passaged ten times in non-medicated chickens, the resistance remained unaffected (Chapman, 1986).

Taken in totality, the results demonstrate a tendency to the development of stable decoquinate resistance in *Eimeria* spp.

2.4. Quality of animal produce

No data was submitted on the effects of Deccox (or decoquinate) on the sensory qualities of meat from chickens given feed containing the additive.

2.5. Conclusions

In the floor pen study, decoquinate at 40 mg kg⁻¹ feed (measured value 32 mg kg⁻¹) gave better control of coccidial infection than at 20 mg kg⁻¹ feed (measured value 18 mg kg⁻¹) although no birds died in either dose group. Both concentrations of decoquinate demonstrated protection when compared to the infected controls. From this study, decoquinate would appear to be at least as efficacious in protecting chickens against a virulent coccidial challenge as the two other commercial coccidiostats used. Coccidiosis was well controlled by Deccox (24 mg decoquinate kg⁻¹ feed) under field conditions in comparison with another synthetic coccidiostat. However, these results derive from only
two trials and provide insufficient evidence of the present value of Deccox in the control of *Eimeria* infections.

That resistance may develop is suggested by some laboratory studies in which *Eimeria* spp. repeatedly exposed to sub-lethal concentration develop a tolerance and by the Notifiers recognition that the minimum dose of 20 mg decoquinate kg\(^{-1}\) feed previously claimed may no longer be sufficient. No studies consider the use of Deccox in a shuttle programme, although the need to develop such programmes is further evidence of a growing resistance amongst some *Eimeria* populations to the use of single cocccidiostats.

# 3. Safety Studies on Target Species

## 3.1. Tolerance Test on Broiler Chicks

Two early studies on the tolerance of chickens for fattening were reported in 1967\(^4\) and 1968 (Lucas, 1968) and a more recent study in 2000\(^5\). FEEDAP Panel acknowledges that both of the earlier studies demonstrated that, at ten times the maximum recommended concentration, no adverse effects were seen in the birds. However, more weight is given to the later study as this more closely reflects current commercial practice. This study was designed to investigate the tolerance of the target species to decoquinate at two dose levels. These were the recommended maximum incorporation rate of 40 mg kg\(^{-1}\) complete feed and the ten times the recommended dose (400 mg kg\(^{-1}\) complete feed).

Groups of 10 (5 male and 5 female) chickens received either no coccidiostat in their diet for 42 consecutive days or received feed containing Deccox at the recommended dose or ten times the recommended dose. Feed and water intake per group were recorded daily and body weight was recorded weekly throughout the duration of the study.

The animals were observed for any signs of intolerance during the dosing period and up to the time of sacrifice. Animals were sacrificed immediately following completion of dosing with the exception of two animals from Group 3 which were terminated prematurely. These animals were subjected to necropsy and gross pathological investigation.

Immediately prior to sacrifice a sample of blood was collected from each bird for the measurement of clinical chemistry and haematological parameters.

Two females from the high dose group and one from the low dose group died from non-treatment related causes. The health status of the remaining birds receiving Deccox in their diet at both dose levels was comparable to that of the control group. No abnormal observations were noted in any of the remaining birds at necropsy.

The feed consumption was slightly increased in the low dose group by 12.8% and substantially increased in the high dose group by 56.9% compared with the control group. Water intake in the high dose group was also higher than in controls (14% increase) due to the higher feed intake. The higher feed intake resulted in an increased body weight gain in the Deccox treated birds (Table 4).

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Table 4. Weekly body weight gain (g; mean ± SD) and feed:gain ratio in birds given no coccidiostat or Deccox at x1 or x10 the recommended dose

<table>
<thead>
<tr>
<th>Weeks/Dose</th>
<th>Bodyweight</th>
<th>Feed:gain ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>x1</td>
</tr>
<tr>
<td>0</td>
<td>39 ± 2.9</td>
<td>38 ± 9.9</td>
</tr>
<tr>
<td>1</td>
<td>83 ±10</td>
<td>84 ± 9.9</td>
</tr>
<tr>
<td>2</td>
<td>134 ±24</td>
<td>140 ± 22</td>
</tr>
<tr>
<td>3</td>
<td>209 ±49</td>
<td>227 ± 40</td>
</tr>
<tr>
<td>4</td>
<td>305 ±84</td>
<td>347 ± 67</td>
</tr>
<tr>
<td>5</td>
<td>454 ±130</td>
<td>502 ± 101</td>
</tr>
<tr>
<td>6</td>
<td>624 ±85</td>
<td>698 ± 135</td>
</tr>
<tr>
<td>0 – 6</td>
<td>1846</td>
<td>2036</td>
</tr>
</tbody>
</table>

Some small but statistically significant changes were observed in the biochemical parameters measured but all values remained within physiological ranges (Na, K, Cl, total protein and albumin for males from the high dose group were significantly different from the control group and in the females from both Deccox-treated groups the albumin and albumin/globulin ratio results were significantly different from the control group). All other clinical chemistry and haematology parameters measured in birds from the Deccox-treated groups did not differ significantly from birds in the control group.

3.2. Interactions

Since its introduction decoquinate has been extensively used in combination with a variety of other medicinal compounds and ingredients in animal feed with only one incompatibility reported. The United States FDA record an incompatibility with bentonite used as a binder in pelleting. In the only documented study in which compatibility was a consideration, no incompatibility between decoquinate and the antibiotics chlorotetracycline, oxytetracycline or spiramycin was found (Hodgson, 1968).

3.3. Microbiological safety of the additive

MIC values for decoquinate against a range of bacterial species isolated either from healthy or infected chicken were determined using the agar-dilution method. The total number of strains tested was 104. The results are summarised in Table 5.

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Table 5. The MIC-ranges of different bacterial genera of chicken origin (10 strains of each genus except Bacteroides, where n=4)

<table>
<thead>
<tr>
<th>Genus</th>
<th>MIC-range (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides</td>
<td>64 - &gt; 128</td>
</tr>
<tr>
<td>Clostridium</td>
<td>64 - &gt; 128</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>128 - &gt; 128</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>64 - &gt; 128</td>
</tr>
<tr>
<td>Escherichia</td>
<td>32 - &gt;128</td>
</tr>
<tr>
<td>Salmonella</td>
<td>64 - &gt; 128</td>
</tr>
<tr>
<td>Pasteurella</td>
<td>16 - 64</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>128 - &gt; 128</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>&gt; 128</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>32 - &gt; 128</td>
</tr>
</tbody>
</table>

Decoquinate does not have marked antibacterial properties against 92 % of the strains examined (all have MICs of 64 mg l⁻¹ or greater). Pasteurella spp. were the most sensitive species tested.

3.4. Metabolism and residues

3.4.1. Metabolism

Chicken

Thirteen non-GLP studies carried out in 1967-1968 aimed at investigating the metabolic fate of decoquinate in the chicken⁸. Some of these results have been published (Filer et al., 1969; Button et al., 1969; Craine et al., 1971). They all used [¹⁴C]-decoquinate labelled on carbon 3 of the quinoline ring administrated by gavage twice daily to animals at doses of 4 mg or 40 mg day⁻¹ that correspond to twice and 20 times respectively the quantity ingested daily from the maximum proposed dosage of 40 mg kg⁻¹ feed. The main results were the following:

- the balance study indicated that 90 % and 100 % of the administered dose were excreted after 24 and 48 hours respectively. The urinary excretion was very limited (about 1 %). The fact that significant amounts of radioactivity were measured in the bile and tissues indicated that the decoquinate was absorbed to a certain extent.

- the metabolic equilibrium in tissues was reached within three days.

- on the basis of the comparative chromatographic behavior (TLC) of excreta radioactive extracts and standard decoquinate, as well as the specific fluorimetric determination of decoquinate, it was shown that unchanged decoquinate represented the major part (94 %) of the radioactivity excreted. Urine analysis indicated that 85 % of the radioactivity corresponded to decoquinate and 15 % to non-decoquinate compounds. In the bile, 23 % corresponded to decoquinate and 53 and 23 % to two metabolites or groups of unresolved metabolites. However, these

⁸ Craine, E.M. Vol. 8, pp. 21-115
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figures correspond to an oral dosage 20 times higher than the proposed dosage; this may modify quantitatively and even qualitatively the metabolic profile.

- after a 4 h withdrawal period (in effect zero withdrawal) the highest residue levels in tissues were found either in the fat followed by the liver, kidney and skin (decreasing order) or liver and kidney then fat and skin, depending on the study. The longer retention of residues in skin/fat observed for longer withdrawal periods leads the FEEDAP Panel to consider the skin/fat as the target tissue.

- using the same technical approach used to identify excreta metabolites it was established that unchanged decoquinate represented 85 and 59 % respectively of the total radioactivity measured at 4 h withdrawal in the liver and kidney of animals dosed at twice the proposed dosage. The remaining 14 and 42 % of total activity corresponded to one metabolite or to a group of unresolved metabolites. The corresponding figures for the muscle and skin/fat indicated values of 100 and 87 %. No information was available concerning the incidence of longer withdrawal periods on these figures. The identify of the metabolite(s) was not reported.

A GLP-compliant study of the metabolism of decoquinate in chickens reported in 2000 was carried out using $^{14}$C-decoquinate administered to groups of male and female animals (nine each) in capsules twice daily for six days at a dose equivalent to 40 mg kg$^{-1}$ in feed. Excreta were collected and groups were slaughtered after 1, 3 and 5 days withdrawal and tissues collected. The results concerning the balance study confirmed the former ones while tissue distribution again indicated that the skin/fat was the target tissue at zero withdrawal time and onwards. The ratio between decoquinate concentrations measured using a validated HPLC method and the total radioactivity expressed as decoquinate in tissues indicated values ranging from 0.28 for the liver to 0.24, 0.28, 0.42 and 0.44 for the kidney, liver, muscle and skin/fat. These figures appeared to be similar after 1 and 3 days withdrawal but much lower than those obtained previously under similar conditions but after a shorter withdrawal period (4 hours). This discrepancy may be explained by the technical limitations of the separation methods already mentioned. Consequently, further analysis and identification of the extractable tissue metabolites that represent more than 10 % of the total residues would be necessary before identifying the marker-residue.

Laboratory animals

A non-GLP metabolic study has been carried in the rat with $^{14}$C-decoquinate. Groups of male and female animals were administered for 3, 5, 7, 9 and 11 days daily doses of about 0.5 and 0.4 mg respectively, and were killed after the last treatment. The urinary excretion of males represented 12 % and for females 6 % of the administered dose. The metabolic plateau was reached in the tissues after three days. No sex related difference was observed. The thin-layer chromatographic analysis of all tissue extracts showed the presence of unchanged decoquinate and three metabolites. Considering the limited capacity of the technique to separate it cannot be excluded that these metabolites may constitute groups of unresolved metabolites. Decoquinate accounted for 43 % of the total radioactivity in the liver and kidney, and 65 % in the muscle and skin. One of the metabolites/group of

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metabolites represented 20% (skin) and 30-50% (liver and kidney). No identification was provided.

A comparison of the tissue metabolic profile of the chicken and rat was performed using the same analytical technique and based on the retention time values. The retention time of one rat metabolite was found to correspond to one chicken metabolite present in the liver and kidney. Another rat metabolite was found in the chicken bile, but a third apparently was not present in the chicken. However, due to the limitations of the method already mentioned it cannot be concluded unambiguously on the identity of the metabolites in both species.

### 3.4.2. Residues

The study analysed previously reported residue levels in tissues measured as the total radioactivity but also as decoquinate (considered by the Notifier as the marker-residue). The determination of decoquinate was performed using a validated HPLC method with a limit of detection of 0.05 mg kg⁻¹. The results are shown on Table 6.

**Table 6. Kinetics of tissue residues resulting from [¹⁴C]-decoquinate administration to chickens for six consecutive days at a dose equivalent to 40 mg kg⁻¹ feed followed by a variable withdrawal period.**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Withdrawal period (day)</th>
<th>1</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TRR¹</td>
<td>Dec²</td>
<td>TRR</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>0.51</td>
<td>0.14</td>
<td>0.24</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>0.63</td>
<td>0.15</td>
<td>0.22</td>
</tr>
<tr>
<td>Muscle (thigh)</td>
<td></td>
<td>0.30</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>Skin/fat</td>
<td></td>
<td>0.75</td>
<td>0.33</td>
<td>0.30</td>
</tr>
</tbody>
</table>

¹TRR = total residual radioactivity expressed as mg decoquinate kg⁻¹
²Dec = decoquinate residue mg kg⁻¹
³LOQ = limit of quantification (0.05 mg kg⁻¹)

Other residue studies performed in the chicken under field conditions have been carried out in 1967 using a non-validated spectrofluorimetric method of analysis with a limit of detection of 0.1 mg kg⁻¹. The results of the most complete one that investigated different withdrawal times showed similar values for the muscle and skin/fat but higher ones for liver (x3) and kidney (x2) after 1 and 3 days, most values being below the detection limit after five days.

### 3.5. Conclusions

There were no adverse effect on the health and growth detected in birds administered Deccox in their diet for 42 consecutive days at the recommended incorporation rate of 40 mg kg⁻¹ complete feedingstuffs or at ten times the recommended incorporation rate (400 mg kg⁻¹ complete feedingstuffs).

The only incompatibility to be recognised is with bentonite and, given the use of this technological additive in feed preparation, this should be noted in the Annex description.

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Amongst the strains of bacteria investigated, most (92%) appear resistant to the effects of decoquinate at concentrations > 64 mg l⁻¹, a concentration substantially higher than that expected in the digestive tract of poultry. Consequently, effects on the bacterial flora of chickens are likely to be minimal.

Decoquinate is partially absorbed in the chicken and once absorbed excreted rapidly mainly through the faeces. The metabolic equilibrium in tissues is reached after three days. Data showing that unchanged decoquinate represents by far the major fraction of the excreted compounds were obtained at 20 times the dosage proposed for decoquinate and therefore cannot be extrapolated. Skin/fat appears as the target tissue. The discrepancies observed between studies concerning the proportion of unchanged decoquinate in tissue residues, the fact that some metabolites represent more than 10% of the total residues but have not been identified and the lack of data on the distribution of metabolites along the withdrawal period, do not allow the setting of a marker-residue.

4. **SAFETY – STUDIES ON LABORATORY ANIMALS**

4.1. **Toxicology**

4.1.1. **Acute Oral toxicity**

The acute oral toxicity of decoquinate has been investigated in rat studies that were performed before the introduction of GLP (see below). It also has been reported that decoquinate is of low acute oral toxicity in "a range of avian and mammalian species" with LD₅₀ values of 5000 mg decoquinate kg⁻¹ body weight or greater [CVMP, 2000].

Groups of five males and five female Wistar rats were given single oral gavage doses of 5000 mg kg⁻¹ body weight of an aqueous suspension of decoquinate. No signs of toxicity were observed in the following five days and no treatment-related gross pathology was seen at post-mortem.

In a similar study groups of five males and five female Sprague-Dawley rats were given single oral doses of 5000 mg kg⁻¹ body weight of suspensions of either micronised decoquinate (containing 1% silica by weight) or pure decoquinate. A third group was given the vehicle only and acted as a control. None of the rats died during a 7-day post-treatment observation period. There were no adverse effects on clinical signs or bodyweight gain. Post-mortems were performed on two males and two females from each decoquinate-treated group, but there were no effects on gross pathology evident.

4.1.2. **Repeat dose oral toxicity**

Decoquinate was tested in a number of repeat dose studies, all of which were performed before the introduction of Good Laboratory Practice (GLP). None of the studies conform to the standards laid down in current OECD methodological guidelines.

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Rats

Groups of ten male and ten female Carworth CFE rats were each given 12 daily gavage doses of 2000 mg kg\(^{-1}\) body weight of a suspension of micronised decoquinate\(^{13}\). A control group of five male and five female rats was given vehicle only. Daily clinical observations and body weight measurements until the 16\(^{th}\) day after the end of treatment did not reveal any adverse effects. Post-mortems performed at the end of the observation period did not reveal any gross pathology. An NOEL cannot be identified for this study because of the limited nature of the observations made.

In a second study\(^{12}\), groups of 20 male and 20 female Wistar rats were given daily oral doses of 0, 4, 15, 62.5, 250 and 1000 mg kg\(^{-1}\) body weight of an aqueous suspension of decoquinate, five times per week (weekdays) for 11 weeks. At weekends a similar dose was given in the diet. Pooled samples of urine were taken from five rats/sex/group at fortnightly intervals, and after 11 weeks, blood samples were taken from five rats/sex/group. All rats were killed for post-mortem, but tissues were taken for weighing and histopathology from only ten rats/sex/group. There was a high incidence of pneumonia (about 30%), affecting all groups. Rats with pneumonia were not chosen for histopathological examination. Testis weights were significantly increased in the groups given 4, 25 and 1000 mg kg\(^{-1}\) body weight, but there was no dose-response relationship. There were no treatment-related effects on mortality, bodyweight gain, haematology, blood biochemistry, urinalysis, gross pathology or histopathology (including tests). The results this study indicated no effect at the highest dose level of 1000 mg kg\(^{-1}\) body weight day\(^{-1}\).

In a third study\(^{12}\), groups of 50 (25 male and 25 female) Carworth CFE rats were fed concentrations of 0, 200, 2000 and 20 000 mg decoquinate kg\(^{-1}\) in their feed for 26 weeks. These dietary levels gave dosages roughly equivalent to 0, 25, 250 and 2500 mg kg\(^{-1}\) bw day\(^{-1}\) at the start of the experiment, decreasing to 0, 10, 100 and 1000 mg kg\(^{-1}\) bw day\(^{-1}\) at the end. All animals were necropsied, but histopathology was performed only on the top dose group and the controls. There was a high mortality (up to 60%) in all groups as a result of respiratory infections with associated haemorrhaging, which the authors suggested might indicate a vitamin K insufficiency. In the top dose group only, bodyweight gain was transiently decreased and there was a small depression of feed conversion efficiency in females. There were no treatment-related effects observed on clinical signs, mortality, haematology, blood chemistry, urinalysis, organ weights, gross pathology or histopathology (Wheldon et al., 1968). Because of the high mortality and probable deficiencies of diet no conclusions can be drawn from this study.

In the final study\(^{13}\), groups of 25 male and 25 female Sprague-Dawley rats were given decoquinate in their diet for two years at concentrations of 0, 200 or 1000 mg decoquinate kg\(^{-1}\) diet. Over the course of the study, these levels of dietary incorporation gave mean dosage ranges of 0, 7.4-12.2 and 37.7-62.0 mg kg\(^{-1}\) body weight day\(^{-1}\) for males and 0, 9.7-14.7 and 48.4-73 mg kg\(^{-1}\) body weight day\(^{-1}\) for females. Satellite groups of five rats/sex from each group were killed after 12 months. At the 12-month interim kill, relative liver weight was reduced at both dose levels. However, the absence of associated pathology suggested that this was not caused by the treatment with decoquinate.


There was no treatment-related effect on mortality, bodyweight gain, haematology, clinical chemistry, gross pathology or histopathology. The tumours that were found in the animals were those commonly found in old Sprague-Dawley rats. There was no treatment-related effect on the incidence of any type of tumour.

The NOEL for this study was 37.7 mg kg\(^{-1}\) body weight day\(^{-1}\), which was given to the highest dose group.

**Dogs**

Groups of two male and two female Beagle dogs were given daily doses of 0, 4, 15, 62.5, 250 and 1000 mg kg\(^{-1}\) body weight of decoquinate in aqueous suspension for 12 weeks\(^{16}\). The doses were administered by stomach tube each day except for the third weekend of the study, when the decoquinate was administered in capsules to all dogs, and several odd occasions when individual dogs refused the stomach tube and the dose had to be administered in a capsule. Urine and blood samples were collected pre-treatment and at two-weekly intervals during dosing. A urine concentration test and a combined bromosulphthalien retention/phenolsulphonephthalein excretion test were carried out pre-treatment and after two and three weeks of dosing. Groups given 62.5 mg kg\(^{-1}\) body weight or more were noted to be less active than controls. The bodyweights of most dogs fluctuated during the experiment, but there was no indication of a treatment-related effect on body weight. There were no treatment-related effects revealed by urinalysis or blood biochemistry, organ function tests and haematology showed only a slight increase in erythrocyte count in the dogs given the high mg kg\(^{-1}\) body weight test dose. There were no treatment-related effects on gross pathology, organ weights or histopathology. The NOEL for this study was set at 15 mg kg\(^{-1}\) bw day\(^{-1}\), based only on a subjective observation of "subdued behaviour".

In the second study considered for dogs\(^{14}\), groups of three male and three female Beagles were given decoquinate in their diet for two years at concentrations of 0, 200 and 1000 mg decoquinate kg\(^{-1}\) feed. This equated to dosages of 0, 4.6-5.0 and 25.8-28.5 mg kg\(^{-1}\) bw day\(^{-1}\). No clinical signs or effects on growth or food consumption were noted. Blood and urine samples were taken at three monthly intervals and revealed no treatment-related effects on haematology or urinalysis. Blood biochemistry showed slightly elevated serum activities of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in only the final blood sample taken from one female in the top dose group. A bromosulphthalien test showed no effect on liver function. No treatment-related effects were seen on ophthalmoscopy, gross pathology, organ weights and histopathology. As the elevated serum enzymes found in one dog were not reflected in the organ function or liver histology for that dog, this result was not considered to be indicative of hepatotoxicity of the test material. The NOEL for this study was set by the authors as 25.8 mg kg\(^{-1}\) bw day\(^{-1}\), the lowest dosage received by the top dose group.

There is an inconsistency between the results of the dog studies. The NOEL for the 12-week dog study was 15 mg kg\(^{-1}\) body weight day\(^{-1}\), based on the reduced activity that was seen at doses of 62.5 mg kg\(^{-1}\) body weight day\(^{-1}\) or greater. The reduced activity was not associated

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with any other toxicological effect in the 12-week study and was not seen at all in the two-year dog study. Although the dogs in the two-year study were observed for physical signs of toxicity, there is no mention in the report of them being examined for changes in their behaviour. Therefore the possibility cannot be excluded that the reduced activity in dogs is due to a toxicological effect of the treatment with decoquinate. Consequently, 15 mg kg\(^{-1}\) bodyweight day\(^{-1}\) is regarded as the NOEL for the combined results of the dog studies.

**Genotoxicity**

The genotoxicity of decoquinate has been investigated in a series of *in vitro* assays, which are summarised in Table 7. The assays performed by Ohta *et al.*, 1989, were published in a peer-reviewed journal; the other studies were performed in accordance with GLP and reported in the Dossier provided. All of the tests used appropriate positive controls, which gave the expected results.

The results of the bacterial tests and the *in vitro* cytogenetics tests were clearly negative for genotoxicity. However the results of the mouse lymphoma assay were more difficult to interpret. The results of this test were negative in the absence of metabolic activation. However, high doses (3000 µg ml\(^{-1}\) or greater) were highly toxic to mouse lymphoma cells in the presence of S9. There was also a dose-related increase in this cytotoxicity from 50 to 2500 µg ml\(^{-1}\), with growth being 5% and relative growth (relative suspension growth x relative cloning efficiency/100) being 1% of that of untreated cells. Nevertheless there were sufficient cells surviving at 2500 µg ml\(^{-1}\) to allow a count of mutated cells. There was a slight dose-related rise in the mutant frequency from 1600 to 2500 µg ml\(^{-1}\), but only the highest concentration was statistically significantly greater than the concurrent negative control. The mutant frequency was also greater than the historical range of control results at 2500 µg ml\(^{-1}\) and in one of two duplicate tests at 1600 µg ml\(^{-1}\), as shown in Table 8.
Table 7. A summary of the results of *in vitro* genotoxicity tests

<table>
<thead>
<tr>
<th>Test Performed</th>
<th>System</th>
<th>Concentration ranges used</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial gene mutation (Ames test)(^{15})</td>
<td><em>Salmonella typhimurium</em> strains TA1535, TA1537, TA1538, TA98 &amp; TA 100</td>
<td>Two duplicate tests using 0.35 – 5.7 and 0.31 – 5.0 µg per plate (in DMSO)</td>
<td>Negative in all strains in the presence and absence of S9.</td>
</tr>
<tr>
<td>Bacterial gene mutation (Ames test)(^{16})</td>
<td><em>Salmonella typhimurium</em> strains TA1535, TA1537, TA1538, TA98 &amp; TA 100</td>
<td>Two duplicate tests using 333 – 10 000 µg per plate (in DMSO)</td>
<td>Negative in all strains in the presence and absence of S9.</td>
</tr>
<tr>
<td>Bacterial gene mutation (Ames test) Ohta <em>et al.</em>, 1980</td>
<td><em>Salmonella typhimurium</em> strains TA1535, TA1537, TA1538, TA98 &amp; TA 100 and <em>Escherichia coli</em> strain WP2 hcr trp</td>
<td>Unstated concentrations dissolved in DMSO</td>
<td>Negative in all strains in the presence and absence of S9.</td>
</tr>
<tr>
<td>“Rec-assay” for DNA repair Ohta <em>et al.</em>, 1980</td>
<td><em>Bacillus subtilis</em> strains H17 (rec+) and M45 (rec-)</td>
<td>Not reported.</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse lymphoma assay for gene mutations at the TK locus(^{17})</td>
<td>L5178Y cells</td>
<td>50 – 2500 µg ml(^{-1}) with S9; 50 – 5000 without S9 (in water but emulsified with Pluronic F-68)</td>
<td>Increased proportion of mutant cells in the presence of S9 only at 2500 µg ml(^{-1}), but 98% or more of the cells were killed at this concentration (see table 8). Negative in the absence of S9.</td>
</tr>
<tr>
<td>Mammalian cell cytogenetics assay(^{18}) (metaphase analysis)</td>
<td>CHO-WBL cells</td>
<td>75.1 – 300 µg ml(^{-1}) (in DMSO)</td>
<td>Negative in the presence and absence of S9 at a harvest time of 10.1 hrs.</td>
</tr>
<tr>
<td>Mammalian cell cytogenetics assay(^{19}) (metaphase analysis)</td>
<td>CHO-K1 cells</td>
<td>0.05 – 0.25 µg ml(^{-1}) (in DMSO)</td>
<td>Negative in the presence and absence of S9 at harvest times of 18 &amp; 42 hrs.</td>
</tr>
</tbody>
</table>

Table 8. Results of the mouse lymphoma assay in the presence of S9

<table>
<thead>
<tr>
<th>Concentration (µg m⁻¹)</th>
<th>Suspension growth relative to negative controls (%)</th>
<th>Number of mutant colonies</th>
<th>Relative growth (%)</th>
<th>Mutant frequency (10⁶ units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0</td>
<td>169</td>
<td>47.3</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100.0</td>
<td>165</td>
<td>43.4</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>95.4</td>
<td>152</td>
<td>96.4</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>90.8</td>
<td>135</td>
<td>87.9</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>79.4</td>
<td>115</td>
<td>63.7</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>71.7</td>
<td>125</td>
<td>72.5</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>57.8</td>
<td>142</td>
<td>51.3</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>54.0</td>
<td>136</td>
<td>50.4</td>
<td></td>
</tr>
<tr>
<td>1300</td>
<td>41.0</td>
<td>143</td>
<td>37.6</td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td>44.4</td>
<td>158</td>
<td>42.7</td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td>34.8</td>
<td>234</td>
<td>34.6</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>39.5</td>
<td>182</td>
<td>36.5</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>30.2</td>
<td>249</td>
<td>29.1</td>
<td></td>
</tr>
<tr>
<td>2500</td>
<td>25.3</td>
<td>267</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>2500</td>
<td>4.3</td>
<td>185</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>2500</td>
<td>5.5</td>
<td>354</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>

Historic pooled negative and solvent controls
Mean = 38.8
Range = 15.0 - 86.8

<table>
<thead>
<tr>
<th>Positive control (MCA) 2.5</th>
<th>47</th>
<th>1150</th>
<th>29.9</th>
<th>506.6*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control (MCA) 4.0</td>
<td>19</td>
<td>900</td>
<td>7.7</td>
<td>622.8*</td>
</tr>
</tbody>
</table>

* Statistically significantly different to the results for concurrent negative controls (0 µg m⁻¹)

According to OECD Guideline 476, the criteria for determining a positive result in an in vitro mammalian cell gene mutation test are a concentration-related or reproducible increase in mutation frequency that is biologically relevant. Statistical significance may be used as an aid in evaluating test results but should not be the only determining factor for a positive result. In this case there is statistical significance and a slight dose-response relationship in mutation frequency. It is not known if the result was reproducible or an artefact as the study has not been repeated.

It is questionable whether decoquinate was truly positive in the mouse lymphoma assay, as it gave reliably negative results in the Ames test and the in vitro mammalian cell cytogenetics assay. An extensive scientific literature shows no cases of any substance giving a clear positive in the mouse lymphoma assay whilst being clearly negative in the Ames test and the in vitro mammalian cell cytogenetics assay.

4.2. Carcinogenicity

The dossier contained no reports of carcinogenicity bioassays. There was however a report of a two-year chronic toxicity study in rats. Limited results from this study indicated that there was no treatment-related effect on tumour incidence. However, the reliability of this result was questioned as there were methodological shortcomings in the protocol of the study.
Some features associated with electrophilic (DNA-reactive) carcinogens and mutagens were noted in the chemical structure of decoquinate. The structural alert for mutagenicity was the quinoline structure and for carcinogenicity, the presence of a two ring heterocyclic hydrocarbon (I). However, substitution at the 3-position greatly reduces the likelihood of either outcome in the case of decoquinate. It is considered unlikely that decoquinate is a genotoxic carcinogen. The results of repeated dose toxicity studies gave no indication of non-genotoxic mechanisms relevant to carcinogenicity.

4.3. Reproductive Toxicity Studies, including developmental toxicity

Decoquinate was tested in a rat multigeneration study and developmental studies in rats and rabbits, all of which were undertaken before the introduction of GLP. As with the sub-chronic oral toxicity studies, none conform to the standards laid down in current OECD methodological guidelines.

4.3.1. Multigeneration Study

In a three-generation study\textsuperscript{20}, decoquinate was administered in the feed to groups of Sprague-Dawley rats at concentrations of 0, 200 and 1000 mg kg\textsuperscript{-1} throughout all generations. These dietary concentrations delivered dosages of 0, 12.1-17.8 and 60.6-87.1 mg kg\textsuperscript{-1} body weight day\textsuperscript{-1} respectively for males and 0, 14.4-18.9 and 70.7-97.6 mg kg\textsuperscript{-1} body weight day\textsuperscript{-1} for females. In the F0 generation, each dose group consisted of 15 males and 30 females. The F\textsubscript{0} rats were fed decoquinate for two months prior to breeding and then throughout two breeding cycles, which produced the F1a and F1b rats. The F1b rats were bred to produce two litters: the F2a and F2b rats, and the F2b rats were bred to produce F3a and F3b rats. The rats from the first litter of each generation (F1a, F2a & F3a) were killed and examined at weaning, whereas the second litters were killed as adults after weaning the subsequent generation. There were no treatment-related effects on libido, conception rate, duration of gestation, embryotoxicity, litter size, pup survival, pup weight (at 2 and 21 days of age), lactation, sex ratio or number of grossly malformed pups. The NOEL for this study was considered to be the lowest dosage received by the top dose group: 60.6 mg kg\textsuperscript{-1} bw day\textsuperscript{-1}.

4.3.2. Developmental Studies

Rats

Groups of 16 pregnant Sprague-Dawley rats were given daily oral gavage doses of 0, 100 and 300 mg kg\textsuperscript{-1} body weight of decoquinate suspended in aqueous gum tragacanth on days 6 to 16 of gestation\textsuperscript{21}. There were no effects on maternal bodyweight gain and food intake. The treatment also had no effects on litter size, foetal weight or the numbers of dead or resorbed embryos. Gross examination of the foetuses revealed no treatment related-effect on development. Microscopic examination of the foetuses, after staining with alizarin red, showed a doubling in the top dose group of the number of pups with retarded skeletal development. It is concluded that, in this study, decoquinate was not maternally toxic, embryotoxic or teratogenic at doses of up to 300 mg kg\textsuperscript{-1} body weight day\textsuperscript{-1}, but that there was some fetotoxicity at 300 mg kg\textsuperscript{-1} body weight day\textsuperscript{-1} as indicated by retarded


skeletal development. On this basis the NOEL for this study was 100 mg kg\(^{-1}\) bodyweight day\(^{-1}\).

**Rabbits**

Two experiments were performed on groups of pregnant New Zealand White does\(^{22}\). In the first decoquinate was administered to groups of 15 does at dosages of 0, 100 and 300 mg kg\(^{-1}\) body weight day\(^{-1}\). In the other experiment, dosages of 0, 30, 60 and 120 mg kg\(^{-1}\) body weight day\(^{-1}\) were given to groups of 11 does. In all cases the decoquinate was given orally in gelatine capsules on days 6 to 16 of gestation. Fetuses were examined externally and by dissection, then they were stained with alizarin red for skeletal examination.

In the first experiment, there were reduced numbers of *corpora lutea*, of implantations per corpus luteum and of live foetuses per litter, but no corresponding increase in dead or resorbed foetuses in the 100 and 300 mg kg\(^{-1}\) body weight day\(^{-1}\) groups as compared with controls. Increased foetal weight was also seen in these groups, and was probably due to the smaller sizes of litters. In the 120 mg kg\(^{-1}\) body weight day\(^{-1}\) group, there was a reduction in the number of live foetuses per litter, with no effect on the number of *corpora lutea*. The treatment had no effect on the number or type of foetal abnormalities found.

It was concluded that decoquinate was not fetotoxic or teratogenic at up to 300 mg kg\(^{-1}\) body weight day\(^{-1}\), but that it was embryotoxic at 100 mg kg\(^{-1}\) body weight day\(^{-1}\) or more. The NOEL for this study was 60 mg kg\(^{-1}\) body weight day\(^{-1}\).

**4.4. Conclusions**

The various toxicity studies reported were done to standards appropriate to the time but many were not in accordance with GLP and would not be considered satisfactory by present standards.

A NOEL of 15 mg kg\(^{-1}\) bodyweight day\(^{-1}\) was identified from repeat dose dog studies. This was derived from the 12-week dog study in which a behavioural effect (reduced activity) was reported to occur at doses of 62.5, 250 and 1000 mg kg\(^{-1}\) bodyweight day\(^{-1}\).

No treatment-related adverse effects were seen in a three-generation rat study or in a developmental study in rabbits. NOELs were identified at the top doses given in each of these studies: about 60 mg kg\(^{-1}\) bodyweight day\(^{-1}\) in both instances. Retarded skeletal development was observed at 300 mg kg\(^{-1}\) bodyweight day\(^{-1}\) in a developmental study in rats. The NOEL for this study was 100 mg kg\(^{-1}\) bodyweight day\(^{-1}\).

The results of the *in vitro* genotoxicity tests indicated that decoquinate was not genotoxic. The clearly negative results from bacterial tests and from cytogenetics studies gave sufficient reassurance of safety to overcome any doubts generated by the equivocal result from the mouse lymphoma assay. In the view of FEEDAP Panel the adverse response seen with 2500 µg ml\(^{-1}\) in the mouse lymphoma assay was a consequence of the general cytotoxicity of decoquinate.

No reports of carcinogenicity bioassays were available, but the results of a two-year rat study gave limited evidence of an absence of carcinogenicity. Furthermore, the results of

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repeated dose toxicity studies gave no suggestion of any effects of decoquinate that might be associated with a non-genotoxic mechanism of carcinogenicity. It was therefore concluded that decoquinate was unlikely to be a carcinogen either by a genotoxic or non-genotoxic mechanism.

The NOEL of 15 mg kg\(^{-1}\) bodyweight day\(^{-1}\) may be used as the basis for setting an acceptable daily intake (ADI) for decoquinate.

5. **SAFETY EVALUATION FOR THE HUMAN CONSUMER**

5.1. **Microbiology**

It has been shown that decoquinate itself has very limited action against only a few bacterial strains. Although the strains tested were of poultry origin, there is no reason to suppose a different response by related strains of human gut origin.

5.2. **Proposal for an acceptable daily intake**

The overall NOEL from toxicological studies of decoquinate was 15 mg decoquinate kg\(^{-1}\) bodyweight day\(^{-1}\), based on the results of a 12-week dog study. Applying a 200-fold uncertainty factor to this NOEL gave an ADI of 0.075 mg kg\(^{-1}\) bodyweight. A higher than usual uncertainty factor was used to derive the ADI to compensate for concern about the precision of the results from the older studies that were not performed to present standards.

The ADI of 0.075 mg kg\(^{-1}\) bodyweight allows an adequate margin of safety for the other toxicological effects seen in the studies (eg. safety margins equal 800 for embryotoxicity and 1333 for retarded skeletal development).

5.3. **Maximum residue limits**

The limited studies made in chickens and laboratory animals effectively preclude any comparison of the metabolic profile of the chicken with those of the rat. However, the limited data available indicates that there are differences in the number and nature of the metabolites produced in the two species. As none of the metabolites have been identified or quantified (in either species) the risk for the consumer exposed to decoquinate residues in chicken tissues cannot be adequately assessed on the basis of the existing data. As a consequence, the FEEDAP Panel is unable to set an MRL for decoquinate.

6. **WORKER SAFETY**

6.1. **Effects on the respiratory system**

Studies made with Deccox have shown that < 10 % (typically about 5 %) will pass a 125 µm sieve and consequently the fraction of respirable particles is low. The incorporation of
soybean oil in the product also reduces considerable its dusting potential, as shown by the value of 0.015 g m\(^{-3}\) obtained in a Stauber-Heubachs test\(^{23}\).

Five male and five female Sprague Dawley rats were exposed continuously (snout only) for four hours to a test atmosphere, generated using a Rotating Brush Generator, and containing decoquinate at concentrations of 0.95 or 4.19 mg l\(^{-1}\). The highest dose was the maximum concentration technically achievable. Approximately 25% of the aerosol mass for both concentrations had a particle size < 4.2 \(\mu\)m. The study was conducted in compliance with GLP. The control group received product free air for four hours. The highest dose resulted in slight transient signs of toxicity such as piloerection and hunched posture but no other adverse effects were seen\(^{27}\).

A second, earlier study\(^{24}\) in which eight Carworth CFE rats (four male and four female) were exposed continuously for six hours to a concentration of 4 g m\(^2\) showed no signs of irritation or other adverse effects.

6.2. Effects on eyes and skin

A study\(^{29}\) to assess eye irritancy of decoquinate in three male rabbits showed no responses at all in any animals up to 72 hr after instillation of approximately 34 mg as a powder decoquinate is concluded to be non-irritant to the eye.

A study\(^{30}\) to assess the skin irritation potential of decoquinate following a single occluded application of 0.5 g of wetted powder to the skin of three New Zealand White rabbits there was no evidence of erythema or oedema. Decoquinate is thus concluded to be non-irritant to the skin.

Dunkin/Hartley guinea pigs (ten control and 20 test females) were used to assess the skin sensitisation potential of decoquinate.\(^{31}\) Induction was via a combination dermal injection of 4% decoquinate in maize oil and subsequent topical application of 60% decoquinate in maize oil. Challenge was with 60% decoquinate in maize oil. All treated animals gave negative responses. Thus it is concluded that decoquinate is not a sensitizer in guinea pigs. An earlier undated study\(^{32}\) used ten Guinea pigs that were induced using nine dermal applications of 10% aqueous suspension of decoquinate followed by a challenge with the same material. No irritation was seen at any stage and it was concluded that the material did not have sensitising properties.

6.3. Systemic toxicity

The acute oral effects of decoquinate in laboratory animals are summarised in section 4.1.1 of this document and are not reproduced here. From this data there is sufficient evidence that this substance has a very low acute oral toxicity (> 5000 mg kg\(^{-1}\) body weight).

6.4. Conclusions

The two studies on the effects on the respiratory system were both made with the active agent rather than the product Deccox and would of relevance mainly for those exposed during manufacturing process. However, the formulation of Deccox includes soya oil which significantly reduces the creation of dust when handling the final product. Consequently, in the view of FEEDAP Panel, exposure will therefore occur primarily by skin contact. The extremely low acute toxicity combined with lack of irritant or sensitisation potential of the product suggests that only minimal precautions would need to be taken during handling. The proposed material safety data sheet suggest to avoid direct contact with skin and to wash hands after use. This advice would be compatible with the safety data provided.

7. Environment

The active ingredient decoquinate is not a substance which occurs naturally in the environment. The additive is also not intended for companion animals. Consequently, a Phase I assessment has to be made to determine the predicted environmental concentration.

As insufficient data on the excretion of metabolites is available (see section 3.4), in Phase I and II a total residues approach is taken. The maximum initial PEC is calculated, based on the assumption that the additive is excreted 100% as the parent compound with the maximum recommended dose of 40 mg decoquinate kg\(^{-1}\) feed.

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

Fate in manure

No data on the fate of decoquinate in manure of the target animals have been submitted. Therefore, biodegradation in excreta is not considered in the risk assessment.

Fate in soil

**Adsorption:** the Koc value of decoquinate was experimentally determined using HPLC according OECD standards (OECD 121)\(^{33}\). At pH 4.4 and 8.5 decoquinate has a log K\(_{oc}\) > 5.6.

**Degradation:** the route and rate of degradation of \([^{14}C]\) decoquinate in soil under aerobic condition was investigated in three soil types (sandy loam, loamy sand and clay loam)\(^{34}\). Soil samples (moistened at pH 2.5) were fortified to a concentration of 1.28 mg kg\(^{-1}\). Test was performed in the dark at 20°C in a closed system and volatiles were trapped in ethandiol and ethanolamine. Soil samples were taken over a 120 day period. Decoquinate and its degradation products were separated by HPLC and TLC.

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In all soil types the freely extractable radioactivity was low: <6% in sandy loam, <5% in loamy sand and <9% in clay loam and was mainly associated with the parent compound. Extraction into NaOH ranged from 36-70% in sandy loam, 47-79% in loamy soil and 24-63% in clay loam with no correlation with time. The combined extracted residue showed that at the end of the study 14% in clay loam, 19% in sandy loam and 32% in loamy soil of the applied radioactivity was the parent compound. After 16 (loamy sand and clay loam) to 32 days (sandy loam) an unknown biodegradation product is formed increasing to 35% in sandy loam, 38% in loamy sand and 35% in clay loam after 120 days. Another unknown biodegradation product was formed up to 5%. In all soil types mineralisation was low; levels of $^{14}$CO$_2$ after 120 days were <2%. Based on % parent compound in the combined extracts DT$_{50}$ values were estimated as 96, 140 and 116 days for the three soil types.

**Fate in water**

No data is available on the fate of decoquinate in water.

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

The methodology to estimate PEC soil, groundwater and surface water is shown in Annex 1 and the calculated values in Table 9.

**Table 9. Predicted environmental concentration of decoquinate in soil, groundwater and surface water**

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Concentration</th>
<th>Trigger value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vulnerable area</td>
<td>Non vulnerable area</td>
</tr>
<tr>
<td>Soil</td>
<td>230 µg kg$^{-1}$</td>
<td>470 µg kg$^{-1}$</td>
</tr>
<tr>
<td>Groundwater</td>
<td>0.03 µg l$^{-1}$</td>
<td>0.07 µg l$^{-1}$</td>
</tr>
<tr>
<td>Surface water</td>
<td>0.03 µg l$^{-1}$</td>
<td>0.06 µg l$^{-1}$</td>
</tr>
</tbody>
</table>

* The fate data show that the DT$_{90}$ of decoquinate > 1 year, which indicates a potential for accumulation in soil. For this reason a plateau concentration is calculated based on the highest a DT$_{50}$ value of 140 days. This concentration is 1.2 times higher than PEC value after 1 year.

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

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

7.2.1. Toxicity to soil organisms

Effects on soil micro-organisms

Effect of decoquinate on respiration and nitrification was studied in sandy loam at pH 2.5. Samples were fortified at a rate of 1.28 mg decoquinate kg\(^{-1}\) soil\(^{35}\). The samples were amended with 0.5% lucerne meal and incubated for 28 days under aerobic conditions. Samples for analysis of CO\(_2\) and NH\(_4\)-N, NO\(_2\)-N, and NO\(_3\)-N were taken after 0, 14 and 28 days. No significant effect was measured on respiration and nitrification.

In an additional study with the same soil type the effect on respiration and nitrification was investigated at higher rates of 1.8 and 9.2 mg decoquinate kg\(^{-1}\), according OECD standards (OECD 216 and 217). No effects were observed on the nitrification after 28 days. At this time point, the rates of respiration deviated from those of controls by more than 25%. However, after 56 days differences were less than 25%.

Effects on plants

Effect of decoquinate on the emergence and growth of seedlings of wheat (*Triticum aestivum*), mustard (*Brassica alba*) and mung bean (*Phaseolus aureus*) was studied in sandy loam soil according to OECD standards (OECD 208)\(^{36}\). At nominal concentrations up to 100 mg decoquinate kg\(^{-1}\) no effects on emergence or growth of the three species tested were observed.

Effects on earthworms

The acute toxicity of decoquinate to *Eisenia foetida andrei* was determined in a limit test at a nominal rate of 1000 mg decoquinate kg\(^{-1}\) soil for 14 days at 20°C, according to OECD standards (OECD 207)\(^{25}\). An artificial soil was used composed of 70% sand, 20% kaolinite clay and 10% sphagnum moss peat. No mortality or abnormal behaviour was observed. Though the weight was significantly reduced by 12%. The LC\(_{50}\) normalised to 5% organic matter is > 500 mg decoquinate kg\(^{-1}\) soil.

The effect of decoquinate on the reproduction and growth of *Eisenia foetida foetida* was assessed for 56 days at nominal concentrations of 62.5-1000 mg decoquinate kg\(^{-1}\) dry weight\(^{38}\). There was no significant effect on body weight. A significant difference was noted between the total number of juvenile worms at 250 mg decoquinate kg\(^{-1}\) dry weight, however, at higher concentrations no effect was observed. Therefore, the NOEC was considered to be > 1000 mg decoquinate kg\(^{-1}\) dry weight.

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7.2.2. Toxicity to aquatic organisms

**Effect on algae**

The acute toxicity of decoquinate to the algal species *Selenastrum capricornutum* was determined at a nominal concentration of 100 mg l\(^{-1}\) according OECD 201\(^{39}\). Test solutions were ultrasonicated for 2-4 min to achieve the maximum soluble concentration. At the start a concentration (after filtration) was measured of 0.234 mg l\(^{-1}\), which is far above the reported water solubility. After 72 h the concentration was below LOQ of 0.039 mg l\(^{-1}\). Excess undissolved material was retained in the test system. After 72 h no significant effect on growth rate and biomass was found.

**Effect on crustaceans**

The acute toxicity of decoquinate to the crustacean *Daphnia magna* was determined under semi-static conditions at a nominal concentration of 100 mg decoquinate l\(^{-1}\) according OECD standard (OECD 202)\(^{26}\). Test solutions were ultrasonicated for two minutes in order to achieve maximum solubility. Test solutions were replaced after 24 h. The test solutions appeared opaque and off-white colour with small quantities at the surface. At the start a concentration (after filtration) was measured of 0.054 mg decoquinate l\(^{-1}\), which exceeds the reported water solubility. After 24 and 48 h the concentration was below the LOQ (0.049 mg decoquinate l\(^{-1}\)). Excess undissolved material was retained in the test system. After 48 h no mortality was observed.

**Effect on fish**

The acute toxicity of decoquinate to rainbow trout *Oncorhynchus mykiss* was determined under semi-static conditions at a nominal concentration of 100 mg decoquinate l\(^{-1}\) according OECD standard (OECD 203)\(^{41}\). Test solutions were ultrasonicated for 10 minutes to achieve the maximum soluble concentration. Test solutions were replaced after 24 h. The test solutions appeared opaque and off-white colour with small quantities at the surface. Excess undissolved material was retained in the test system. At the start (after filtration) a concentration was measured of 0.040 mg decoquinate l\(^{-1}\). After 24 h the concentration was below the LOD (0.008 mg l\(^{-1}\)). After 96 h no mortality was observed.

**Effect on sediment dwelling organisms**

The toxicity of [14C] decoquinate to the sediment dwelling larvae of the midge *Chironomus riparius* was determined in test using spiked sediment. In a range-finding test no effects on developing larvae were observed at up to 1000 mg decoquinate kg\(^{-1}\) dry weight. The exposure concentration for the definitive limit test normally is taken as 1000 mg kg\(^{-1}\). However, this would have resulted in the extensive dilution of the radio label. Therefore the definitive limit test was made at a measured concentration of 16.3 mg decoquinate kg\(^{-1}\) dry weight for 28 days\(^{42}\). At this concentration no effect on survival and development was observed.

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Bioaccumulation

No data on bioaccumulation have been provided. However, since the log $K_{ow}$ is between 5.2-5.5 decoquinate has a potential for bioaccumulation.

7.3. Conclusions

Decoquinate has a log $K_{ow}$ of > 5.6. The DT$_{50}$ of decoquinate in soil is between 96 and 140 days (mean 117 days). No information is provided on the degradation in manure and water and therefore could not be considered in the risk assessment.

The available information covers the three key relevant taxonomic groups for soil (microorganisms, earthworms and plants). For earthworms, plants, and soil microorganisms the NOEC is $>$ 1000, $>$ 100 and $>$ 9.2 mg decoquinate kg$^{-1}$, respectively. Applying a safety factor of 10 to the lowest NOEC of $\geq$ 9.2 mg decoquinate kg$^{-1}$, gives a PNEC for the soil compartment of $\geq$ 0.9 mg decoquinate kg$^{-1}$.

Since in all aquatic toxicity tests the concentrations after 24 h were below the LOQ or LOD, the actual concentration could not be determined. Consequently, the LC$_{50}$ values for the aquatic organisms cannot be estimated. The NOEC for Chironomus riparius is $>$ 16.3 mg decoquinate kg$^{-1}$ dw. In addition, in a range finding test no effects were observed up to a concentration of 1000 mg decoquinate kg$^{-1}$ dw.

7.4. Risk Characterisation

7.4.1. Risk for soil

Comparison of the PNEC ($> 0.9$ based on soil microorganisms) with the maximum PEC soil gives a PEC/PNEC ratio below 1 for both vulnerable areas and non-vulnerable areas. Therefore, a low risk for soil organisms arising from the intended use is to be expected.

7.4.2. Risk for groundwater

The PEC groundwater for both vulnerable and non-vulnerable areas are both below the trigger value of 0.1 µg l$^{-1}$. Therefore the ecological risk for groundwater is considered to be low.

7.4.3. Risk for surface water

Although it was not possible to determine an acute toxicity value for the aquatic organism, the tests do show that after renewal of the test medium with decoquinate concentration at maximum water solubility no (acute) toxicity to fish and daphnids was observed. As the PEC surface water is < 0.1 µg l$^{-1}$, the risk for aquatic organisms via drainage of decoquinate is considered to be low. It is expected that after an initial peak, the water concentration will rapidly decline due to the high sorption coefficient of decoquinate. Consequently, a risk for water organisms is not expected in either vulnerable or non-vulnerable areas.

7.4.4. Risk for sediment

Since no effects on sediment-dwelling organisms were found up to a concentration of 1000 mg decoquinate kg$^{-1}$, no risk is to be expected.
7.4.5. Risk for secondary poisoning

The log $K_{ow}$ of $5.2 - 5.5$ indicates that decoquinate has a potential for bioaccumulation. However, the bioaccumulation of decoquinate in earthworms and fish was not directly tested. Assuming that the long term PEC for surface water via drainage and run-off will be low, the risk for fish-eating birds and mammals will be correspondingly low. The bioaccumulation concentration factor (BCF) for earthworms is calculated (Montforts$^{27}$) as $1.8 \text{ kg}_{\text{soil}} \text{ kg}^{-1}_{\text{water}}$. The BCF gives a maximum concentration in earthworms of $0.85 \text{ mg kg}^{-1}$, a concentration not considered to pose a risk.

7.4.6. Conclusion

The use of Deccox at the recommended dose range does not pose a risk for the terrestrial and aquatic compartments (including sediment dwelling organisms and secondary poisoning).

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

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

PRESENT EFFICACY

FEEDAP Panel accepts that proof of effect and dose effect studies made when decoquinate was first introduced, demonstrated the value of the active substance as a coccidiostat. The purpose of the present review is to establish that decoquinate in the form of Deccox retains its efficacy and that the proposed dose range is still appropriate.

In the single floor pen study presented, decoquinate at $40 \text{ mg kg}^{-1}$ feed (measured value $32 \text{ mg}$) gave better control of coccidial challenge than the lower dose ($20 \text{ mg kg}^{-1}$, although no birds died in either dose group. Both concentrations of decoquinate demonstrated protection when compared to the infected controls. From this study, decoquinate would appear to be at least as efficacious in protecting chickens against a virulent coccidial challenge as the two other commercial coccidiostats used. Under field conditions, coccidiosis also appeared as well controlled by Deccox ($24 \text{ mg decoquinate kg}^{-1}$ feed) as with another synthetic coccidiostat.

However, these results derive from only two trials and provide insufficient evidence of the present value of Deccox in the control of *Eimeria* infections. That resistance may develop is suggested by some laboratory studies in which *Eimeria* spp. repeatedly exposed to sub-

lethal concentration develop a tolerance and by the Notifiers recognition that the minimum dose of 20 mg decoquinate kg\(^{-1}\) feed previously claimed may no longer be sufficient.

**Resistance of Bacteria**

Most of the strains of bacteria investigated occurring in the digestive tract of chickens appear resistant to the effects of decoquinate at concentrations \(>64\, \text{mg l}^{-1}\) a concentration substantially higher than that expected in the digestive tract of poultry. Consequently, effects on the bacterial flora of chickens are likely to be minimal. In the absence of demonstrable effects on the major species of gut bacteria it can be reasonably concluded that the use of decoquinate would not select for strains resistant to clinically important antibiotics or lead to the development of any form of cross-resistance.

**Safety for the Target Species**

There were no adverse effect on the health and growth detected in birds administered Deccox in their diet for 42 consecutive days at the recommended incorporation rate of 40 mg kg\(^{-1}\) complete feedingstuffs or at ten times the recommended incorporation rate (400 mg kg\(^{-1}\) complete feedingstuff).

Since its introduction decoquinate has been extensively used in combination with a variety of other medicinal compounds and ingredients in animal feed with only one incompatibility (with bentonite), reported.

**Safety for the Consumer**

Toxicity studies generally were done to standards appropriate to the time but many were not in accordance with GLP and would not be considered satisfactory by present standards. A NOEL of 15 mg kg\(^{-1}\) bodyweight day\(^{-1}\) was identified from a 12 week repeat dose dog studies based on reduced activity. No treatment-related adverse effects were seen in a three-generation rat study or in a developmental study in rabbits. Retarded skeletal development was observed at 300 mg kg\(^{-1}\) bodyweight day\(^{-1}\) in a developmental study in rats. The NOEL for this study was 100 mg kg\(^{-1}\) bodyweight day\(^{-1}\).

The results of the in vitro genotoxicity tests indicated that decoquinate was not genotoxic. The clearly negative results from bacterial tests and from cytogenetic studies gave sufficient reassurance of safety to overcome any doubts generated by the equivocal result from a mouse lymphoma assay. In the view of FEEDAP Panel the adverse response seen with 2500 \(\mu\text{g ml}^{-1}\) in the mouse lymphoma assay was a consequence of the general cytotoxicity of decoquinate.

No reports of carcinogenicity bioassays were available, but the results of a two-year rat study gave limited evidence of an absence of carcinogenicity. Furthermore, the results of repeated dose toxicity studies gave no suggestion of any effects of decoquinate that might be associated with a non-genotoxic mechanism of carcinogenicity. FEEDAP Panel therefore concluded that decoquinate is unlikely to be a carcinogen either by a genotoxic or non-genotoxic mechanism.

The NOEL of 15 mg kg\(^{-1}\) bodyweight day\(^{-1}\) may be used as the basis for setting an acceptable daily intake (ADI) for decoquinate. Applying a 200-fold uncertainty factor to this
NOEL gives an ADI of 0.075 mg kg\(^{-1}\) bodyweight. A higher than usual uncertainty factor was used to derive the ADI to compensate for concern about the precision of the results from the older studies not performed to modern standards. The ADI of 0.075 mg kg\(^{-1}\) bodyweight allows an adequate margin of safety for the other toxicological effects seen in the studies.

**SAFETY FOR THE USER**

Two studies showing essentially an absence of effects on the respiratory system were both made with the active agent rather than the product Deccox and would of relevance mainly for those exposed during manufacturing process. However, Deccox has been shown to have a very low of dusting potential. Consequently, in the view of FEEDAP, exposure will therefore occur primarily by skin contact. The extremely low acute toxicity combined with lack of irritant or sensitisation potential of the product suggests that only minimal precautions would need to be taken during handling. The proposed material safety data sheet suggest to avoid direct contact with skin and to wash hands after use. This advice would be compatible with the safety data provided.

**SAFETY FOR THE ENVIRONMENT**

The use of Deccox at the recommended dose range does not pose a risk for terrestrial or aquatic environment.

**MONITORING**

An adequate and fully validated method was described allowing monitoring of the active substance in premixes and complete feedingstuffs. Modifications to this method also allow the detection of the active compound decoquinate in tissues. However, it should be noted that the method was introduced considerable later than most of the metabolism and residues studies described which made use of an older and less accurate method for determining the parent compound.

The limited data available indicates that there are differences in the number and nature of the metabolites produced in the chickens compared to rats. As none of the metabolites have been identified or quantified (in either species) the risk for the consumer exposed to decoquinate residues in chicken tissues cannot be adequately assessed. As a consequence, the FEEDAP Panel is unable to set an MRL for decoquinate.

**RECOMMENDATIONS**

The minimum dose now proposed by the Notifier should be reflected in the Annex description and, under “other provisions”, the incompatibility with bentonite should be noted.

**DOCUMENTATION PROVIDED TO EFSA**

Original dossier of Deccox® (decoquinate) submitted by Alpharma in respect of its application for the re-evaluation and brand specific approval.
Supplementary dossier including additional data generated since 2000 in response to the questions from Member States.

REFERENCES


ACKNOWLEDGEMENTS
The Scientific Panel on Additives and Products and Substances used in Animal Feed wishes to thank Derek Renshaw and Atte von Wright for their contributions to the draft opinion.
ANNEX I

Method to determine the predicted environmental concentrations (PEC)

PEC soil

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

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

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

\[
P\text{EC}_{\text{soil}} = \frac{P\text{EC}_{\text{manure}} \cdot Q}{R\text{HOS}_{\text{soil}} \cdot C\text{ONV}_{\text{area field}} \cdot D\text{EPTH}_{\text{field}}}
\]

<table>
<thead>
<tr>
<th>Input</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHO_{soil}</td>
<td>Bulk density of soil</td>
<td>1500 kg m⁻³</td>
</tr>
<tr>
<td>DEPTH_{field}</td>
<td>Mixing depth with soil</td>
<td>0.2 m (arable land)</td>
</tr>
<tr>
<td>CONV_{area field}</td>
<td>Conversion factor for the area of the agricultural field</td>
<td>10000 m² ha⁻¹</td>
</tr>
<tr>
<td>Q</td>
<td>Nitrogen immission standard</td>
<td>[kg ha⁻¹ yr⁻¹]</td>
</tr>
<tr>
<td>PEC_{manure}</td>
<td>Concentration in manure expressed per amount nitrogen</td>
<td>[mg kg⁻¹]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Output</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEC_{soil}</td>
<td>Highest concentration in the soil</td>
<td>[mg kg⁻¹]</td>
</tr>
</tbody>
</table>
PEC groundwater

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

\[
PECGw = PECporewater
\]

\[
PECPorewater = \frac{PECsoil \cdot RHOSoil}{K_{soil-water} \cdot 1000}
\]

\[
K_{soil-water} = FAir_{soil} \cdot K_{air-water} + F_{water_{soil}} + F_{solid_{soil}} \cdot \frac{K_{p_{soil}}}{1000} \cdot RHOSolid
\]

\[
K_{p_{soil}} = F_{oc_{soil}} \cdot K_{oc}
\]

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

Settings of the module for ground water.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density of fresh soil</td>
<td>RHOSoil</td>
<td>1700 kg m⁻³</td>
</tr>
<tr>
<td>Density of soil solids</td>
<td>RHOSolidsoil</td>
<td>2500 kg m⁻³</td>
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<tr>
<td>Fraction air in soil</td>
<td>FAirsoil</td>
<td>0.2 m³ m⁻³</td>
</tr>
<tr>
<td>Fraction water in soil</td>
<td>F_{water_{soil}}</td>
<td>0.2 m³ m⁻³</td>
</tr>
<tr>
<td>Fraction solids in soil</td>
<td>F_{solid_{soil}}</td>
<td>0.6 m³ m⁻³</td>
</tr>
<tr>
<td>Weight fraction organic carbon in soil</td>
<td>F_{oc_{soil}}</td>
<td>0.02 kg kg⁻¹</td>
</tr>
<tr>
<td>Temperature at air-water interface</td>
<td>TEMP</td>
<td>285 K</td>
</tr>
<tr>
<td>Gas constant</td>
<td>R</td>
<td>8.314 Pa m³ m⁻³ mol⁻¹ K⁻¹</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>VP</td>
<td>[Pa]</td>
</tr>
<tr>
<td>Molar mass</td>
<td>MOLW</td>
<td>[g mol⁻¹]</td>
</tr>
<tr>
<td>Water solubility</td>
<td>SOL</td>
<td>[m g⁻¹]</td>
</tr>
<tr>
<td>Partition coefficient solids and water in soil (v/v)</td>
<td>K_{soil-water}</td>
<td>[m³ m⁻³]</td>
</tr>
<tr>
<td>Partition coefficient solids and water in soil (v/w)</td>
<td>K_{p_{soil}}</td>
<td>[dm³ kg⁻¹]</td>
</tr>
<tr>
<td>Partition coefficient air and water in soil</td>
<td>K_{air-water}</td>
<td>[m³ m⁻³]</td>
</tr>
</tbody>
</table>

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

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

REFERENCES


