COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BAQUILOPRIM

SUMMARY REPORT (1)

1. Baquiloprim is a diaminopyrimidine derivative, acting as a dihydrofolate-reductase inhibitor. It acts synergistically with sulphonamides. Four formulations have been developed: one short acting bolus, two long acting boluses for oral administration in non-lactating cattle (dose: about 4 - 16 mg baquiloprim and 36 - 144 mg sulphadimidine/kg bw per treatment) and a formulation for intravenous and intramuscular use in lactating cattle (dosage: 1.67 g baquiloprim and 2.09 g sulphadimidine per kg bw per day during 4-5 days or twice this dosage at 48 h intervals for a maximum of 3 treatments) and intramuscular use in swine (dosage: 1.67 mg baquiloprim and 2.09 mg sulphadimidine per kg bw per day during 6 days or 1.5 times this dose at 48 hr intervals for a maximum of 3 doses). Indications in cattle are respiratory tract and gastro-intestinal tract infections, foul-in-the-foot and mastitis. Indications in swine are respiratory and gastro-intestinal infections and mastitis-metritis-agalactia.

2. Baquiloprim has a high oral bioavailability in laboratory animals.

3. Baquiloprim is widely distributed in the body and slowly eliminated. Both urine and bile are important routes of elimination.

4. Baquiloprim is extensively metabolised in laboratory animals as well as target species. Information on metabolism is not complete, because low concentrations of the individual metabolites hinder their identification. The available information indicates that metabolism in laboratory animals and target animals is qualitatively comparable.

5. The following metabolites have been identified: des-methyl-baquiloprim, bis-des-methylbaquiloprim, baquiloprim-1-N-oxide, baquiloprim-3-N-oxide, 6-hydroxy-baquiloprim. The major compound found in tissues and milk after administration is unchanged baquiloprim.

6. A high percentage (increasing during the first weeks after treatment) of the total residue in liver, kidney and injection site is covalently bound.

7. The oral bioavailability in rats of covalently bound residues as well as total residues in liver of cattle (at a withdrawal time of 28 days) was estimated to be maximally 15%.

8. Baquiloprim has moderate acute toxicity. The acute oral LD$_{50}$ values are about 500-1000 mg/kg bw. Important signs of toxicity are CNS toxicity and liver damage.
9. The following oral repeated dose toxicity studies were provided: 6-10 days in the rat (0, 6.25, 25 and 100 mg baquiloprim/kg bw/day), 21 days in the rat (0, 5, 20 and 80 mg/kg bw/day), two and 4 weeks in the dog (pilot studies, 10-20 mg baquiloprim/kg bw/day), 90 days in the rat (0, 4, 16 and 64 mg baquiloprim/kg bw/day), 6 months in the dog (0, 2, 5, 8-12.5 mg baquiloprim/kg bw). In the different repeated dose rat studies and the 6 months dog study hepatotoxicity was found, consisting of accumulation of haemosiderin in hepatic cells (Kupffer cells), and signs of inflammation and necrosis. In addition, effects on plasma enzymes (e.g. alkaline phosphatase) and other biochemical blood parameters (e.g. cholesterol, protein, albumen) were found. In the 6 month dog study at the lowest tested dose of 2 mg/kg bw there were indications of slight histopathological effect in liver, and small but at this dose level not statistically significant effects on clinical chemistry, therefore this dose was considered to be a marginal NOEL. In a 90-day rat study at the lowest tested dose of 4 mg/kg bw small but statistically significant dose related effects on clinical chemistry parameters were found (e.g.: decreased plasma cholesterol in males and total protein in females), therefore this dose was considered to be a marginal NOEL. The effects were reversible.

10. In a rat and a rabbit teratogenicity test, both with oral doses of 0, 3, 10 and 30 mg baquiloprim/kg bw/day, only foetotoxicity - probably related to maternal toxicity - was found at the maximum tested dose of 30 mg/kg bw. No signs of teratogenicity were found. The dose without foetotoxic effect was 10 mg/kg bw/day, the dose without teratogenic effect was greater or equal to 30 mg/kg bw/day.

11. Two range finding reproduction studies and a preliminary report of the first part of a two-generation rat reproduction study with two litters per generation were provided. Because of too high toxicity at the original highest dose level of 30 mg/kg bw, this dose was discontinued and a new lowest dose group of 2 mg/kg /day was started after birth of the F1a generation (final dose levels: 2, 5 and 12 mg/kg bw/day). Results were reported up to the birth of the F2a generation and indicate that maternal toxicity and effects on reproduction occur at doses of 5 mg/kg bw/day and higher. Slight maternal toxicity at 2 mg/kg bw/day cannot be excluded (maternal growth was slightly but not statistically significantly lower than in the negative control, the significance of this possible trend can only be assessed when the complete report of the study has been provided). Some of the effects of 30 mg/kg bw/day on reproduction indices were prevented by folinic acid supplementation, and therefore are likely to be related to the pharmacological mechanism of action of baquiloprim. The preliminary results indicate that 2 mg/kg bw/day probably does not induce reproduction toxicity. The final report of the completed study is expected to be available by May 1996.

12. No evidence was found in the following acceptable set of mutagenicity tests: Ames test (Salmonella typhymurium, TA98, TA100, TA1535, TA1537), fluctuation assays with S. typhymurium (TA 98, TA100, TA1535, TA1537) and E. coli (WP2 pKM101 and WP2 uvrA-pKM101). CHO-HGPRT test, clastogenicity test with human peripheral lymphocytes, in vivo mouse micronucleus test, mouse dominant lethal test. The bacterial tests and CHO test were carried out with and without metabolic activation.

13. No carcinogenicity data were supplied. Since there is no evidence for mutagenicity, neoplastic lesions in the repeated dose toxicity tests, or structural resemblance with known carcinogenic compounds, no carcinogenicity data are required.

14. Baquiloprim elicited signs of hypersensitivity in a skin sensitization test in the guinea pig. In the repeated dose oral toxicity studies no evidence of immunotoxicity was found. Therefore more specific data on immunotoxicity of baquiloprim are not required.

15. Based on a NOEL of 2 mg/kg bw/day in the dog 6 month study and a safety factor of 200 (to correct for the marginal effects found at this dose level and the incompleteness of the reproduction data) a provisional toxicological ADI of 10 µg/kg.bw was established, corresponding to a total intake of 600 µg/person.

16. MIC values of baquiloprim and five of its metabolites in a number of bacterial species isolated from target animals were provided. Des-methyl-baquiloprim and bis-des-methyl-baquiloprim had substantial antimicrobial activity. Based on those MIC50 values of baquiloprim against an adequate set of genera of intestinal bacteria which were less than 100 µg/ml, a geometric MIC50 was calculated.
For the calculation of the microbiological ADI, use was made of the formula that was recommended by the CVMP:

\[
ADI = \frac{\text{geometric mean MIC}_{50} \times CF2}{\text{CF1}} x \frac{\text{daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}}
\]

- **Geometric mean MIC\textsubscript{50} = 1.121 µg/ml**
- **CF1 = 1** (the range of MIC\textsubscript{50} values has already been taken into account in the calculation of the geometric MIC\textsubscript{50})
- **CF2 = 1** (because the MIC values came from tests with high bacterial density)
- The fraction of the microbiologically active residue which is available for the intestinal flora was estimated to be 0.5 (based on the % of the baquiloprim dose excreted in faeces in the oral bioavailability study in rats)
- This ADI is expressed in µg eq of microbiologically active residue. Microbiologically active residue in tissues was considered to consist of the parent compound plus the major metabolites.

17. Taking into account the concentration and the bioavailability of the residues, the identity of the metabolites, the residues of toxicological concern predominate over those with microbiological activity. Hence the most relevant ADI is that derived from toxicological studies, namely 10 µg/kg bw/day.

18. The available MIC data and data on acid production of a large number of cheese and yoghurt starter cultures indicate that acid production of some cultures may be affected by a concentration of 0.1 µg baquiloprim/ml milk. To prevent this effect, the MRL in milk should not exceed 0.1 µg eq of baquiloprim activity/ml. Because an average of 64% of the total baquiloprim derived residue in milk was antimicrobially active, this corresponds with a maximum concentration of 0.150 µg eq total baquiloprim derived residue/ml.

19. Several residue distribution and depletion studies in calves and lactating cows were provided, using oral and intramuscular formulations of labelled as well as unlabelled baquiloprim. In some studies residue composition of residues in liver, kidney and/or injection site tissue was investigated. Withdrawal periods from 5 up to 42 days were studied. These studies showed that the highest concentrations of residue occur in liver, with lower levels in kidney and injection site. For example, in one study, 14, 28 and 42 days after intramuscular treatment of calves with three doses of 3.3 mg of radiolabelled baquiloprim + 16.2 mg sulphadimidine/kg bw with intervals of 48 hrs the average concentrations or ranges of total baquiloprim derived residues vs. the range of parent compound concentrations in liver were (in mg/kg) 4.629 vs 0.0206-0.0432 (14 days), 1.983 vs less than 0.005 to 0.0189 (28 days), 2.511 vs less than to 0.005-0.0146 (42 days). Similar findings were made in another study in calves. Here, the mean percentage of parent compound decreased between withdrawal day 5 (liver: 8.5%, kidney: 17%, intramuscular injection site: no exact % known but most of the residue was parent compound) and 14 (liver: 1.8%, kidney: 2.0%, injection site 3%). In the same period the percentage of bound residue increased (from about 64 % on day 5 to 73 % on 14 days in liver and from about 60% on day 5 to about 71 % on day 14 in kidney). The composition of residues in normal muscle and fat tissue was not investigated on time points earlier than 14 days. Between 14 and 42 days changes in residue composition were relatively small. On 42 days the percentage of marker residue in liver was 1.3%, in kidney 3.1%, in muscle and fat it was too low to be determined, in injection site tissue it was 1.7%.

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\text{ADI} = \frac{1.121 \times 1/1}{0.50} \times \frac{150}{60} = 5.6 \mu g eq/kg bw = 336 \mu g eq/person
\]
Intramuscular residue distribution and depletion studies in pigs were provided, with withdrawal periods of 7 up to 28 days and with labelled as well as unlabelled baquiloprim. In two studies residue composition of residues in liver, kidney and/or injection site tissue was investigated. These studies showed that the highest concentrations of residue occur in liver, with lower levels in kidney, injection site and skin. For example, in a radiolabel study, 14 and 28 days after intramuscular treatment of pigs with three doses of 5.0 mg of radiolabelled baquiloprim+25 mg sulphadimidine/kg bw with intervals of 48 hrs the average concentrations or ranges of total baquiloprim derived residues vs. the range or average of parent compound concentrations in liver were (in mg/kg): 2.514 vs less than 0.005 to 0.007 (14 days), 1.304 vs less than 0.005 (28 days).

In another radiolabel study, pigs were slaughtered 7-28 days after the same dosing schedule. At 7 days after administration the following residue concentrations were found (mg/kg): liver: 2.82 (parent compound: 0.052), kidney: 0.82 (parent compound: 0.018), muscle: 0.03, fat: 0.05, skin: 0.32 and injection site: 2.577. The percentage of parent compound in liver and kidney decreased between withdrawal day 7 (liver: 1.8 %, kidney: 2.2%) and 14 (liver: 1.2 %, kidney: 0.7 %). Percentages of parent compound in muscle and fat were not measured on day 7, on 14 days the percentage in fat was about 7%, the concentrations of marker compound in fat on 28 days and in muscle on 14 and 28 days were too low to calculate meaningful percentages. The percentage in skin was not determined on day 7, 14% on day 14 and 7% on day 28. The percentage of marker in injection site tissue was not determined on day 7, 0.3% on day 14 and too low to be determined on day 28. The percentage of bound residue increased between 7 and 14 days: using a protease digestion and ultrafiltration method the percentage in liver was about 68% on 7 days and 74% on 14 days, for kidney 75% was found on day 7, and on day 14 the residue concentration was too low for analysis with this method).

Residues in milk from cattle treated with baquiloprim were initially high but rapidly depleted. For example, after treatment with the commercial formulation, residues at the first and second milking were in a range of 300-800 µg/kg but these had depleted to 20-40 µg/kg by the eighth milking.

In cattle and pig slaughtered at 14 days or later after administration, the residue composition in tissues was sufficiently comparable to extrapolate bioavailability data acquired with bovine liver residues to porcine liver. Based on residue composition data these were also used as a basis to estimate bioavailability in kidney at day 14 and later.

Only a small proportion of the total baquiloprim derived residue consisted of the parent compound. However, proportions of identified metabolites were lower or not significantly higher. Therefore the parent compound was considered to be the most suitable marker residue.

Target tissues are liver, kidney, fat tissue, pig skin and bovine milk. Liver, kidney, pig skin and milk were selected as target tissues because they contain relatively high concentrations of residue. Residues in muscle and fat tissue deplete relatively quickly to undetectable levels. However, for monitoring purposes, at least one of these two tissues is indispensable. Fat tissue contained in some studies slightly higher residue concentrations than muscle tissue and was therefore selected as target tissue for cattle. For pigs one MRL was established for fat and skin tissue in natural proportions.

Based on the toxicological ADI, the bioavailability of residues, the residues depletion profiles and the standard food package, the following MRLs were proposed:

<table>
<thead>
<tr>
<th>Bovine</th>
<th>liver</th>
<th>300 µg</th>
<th>kidney</th>
<th>150 µg</th>
<th>fat</th>
<th>10 µg</th>
<th>milk</th>
<th>30 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine</td>
<td>liver</td>
<td>50 µg</td>
<td>kidney</td>
<td>50 µg</td>
<td>fat +skin</td>
<td>40 µg</td>
<td></td>
<td></td>
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</tbody>
</table>
For cattle fat tissues, taking into account the very low residues found, the value of twice the limit of quantification of the analytical method was chosen for the MRL. Similarly, the MRL for pig kidney was based on those for fat and skin. The differences in MRL values between cattle and pigs represent the differences noted in the residue studies between the two species, possibly reflecting differences in pharmacokinetics.

Taking into account the bioavailability of residues and the standard food package, these MRLs will not result in the ADI being exceeded.

26. For monitoring, HPLC methods with UV detection were proposed for bovine and porcine liver and kidney. They are validated for an limit of quantification of 20 µg of parent compound/kg and have an limit of detection (LOD)of 10 µg/kg. HPLC methods with MS detection were validated for residues in fat and pig skin tissue (LOQ = 5 µg/kg and LOD = 2.5 µg/kg). For milk an HPLC-UV method with an limit of quantification of 10 µg/kg and an limit of detection of 2 µg/kg was proposed and validated. The applicant prepared descriptions of these methods in the ISO 78/2 format.

Conclusions and recommendation:

As the complete report of the two generation rat reproduction study was not yet available, a provisional ADI was established.

Consequently, the Committee for Veterinary Medicinal Products recommends to include baquiloprim in Annex III of Council Regulation (EEC) No 2377/90 and that provisional MRLs be set for baquiloprim as indicated in the following table:

<table>
<thead>
<tr>
<th>Pharmacologically active substance(s)</th>
<th>Marker residue</th>
<th>Animal species</th>
<th>MRLs</th>
<th>Target tissues</th>
<th>Other provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baquiloprim</td>
<td>Baquiloprim</td>
<td>Bovine</td>
<td>300 µg/kg</td>
<td>Liver, Kidney, Fat, Milk</td>
<td>Provisional MRLs expire on 01.07.1998</td>
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<td></td>
<td></td>
<td></td>
<td>150 µg/kg</td>
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<td>10 µg/kg</td>
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<td></td>
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<td>30 µg/kg</td>
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<tr>
<td>Porcine</td>
<td></td>
<td>Porcine</td>
<td>50 µg/kg</td>
<td>Liver, Kidney, Skin+fat</td>
<td>Provisional MRLs expire on 01.07.1998</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>50 µg/kg</td>
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<td></td>
<td></td>
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<td>40 µg/kg</td>
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LIST OF QUESTIONS:

1. The applicant should provide the complete report of the rat two-generation reproduction study.