



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BAQUILOPRIM

SUMMARY REPORT(2)

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 boli 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 mg baquiloprim and 2.09 mg sulphadimidine per kg bw per day for 4-5 days or twice this dosage at 48 hours 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 for 6 days or 1.5 times this dose at 48 hours 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 syndrome.
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 in the 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 and 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 LD₅₀ values are about 500-1000 mg/kg bw. Important signs of toxicity are central nervous system 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), 2 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) and 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-month 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 a slight histopathological effect in liver (e.g. hepatocyte degeneration, fibrous scarring), and small, but at

this dose level, not statistically significant effects on clinical chemistry. Since the effects were only of little significance 2 mg/kg bw/day was concluded to be the NOEL for this study. 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). Since these effects were only of little significance, 4 mg/kg bw/day was considered to be the NOEL for this study. The effects were reversible.

10. Two dose-finding reproduction studies (doses: 0-60 mg/kg bw/day; 0-30 mg/kg bw/day) showed that 45 and 60 mg/kg bw/day were highly maternally toxic. In a two-generation study doses of 0, 2, 5 and 12 mg/kg bw/day were tested for two generations with two litters per generation. A higher dose of 30 mg/kg bw/day was discontinued after weaning of the first litter of the first generation because of too high a maternal toxicity. The incidence of cleft palate was increased by 30 mg/kg bw/day in the formal two-generation study and by 45 mg/kg bw/day in one range-finding study, but absent in pups from dams receiving 8 mg/kg bw/day folinic acid in addition to 30 mg baquiloprim. Other treatment-related effects in the two-generation study were parental hepatotoxicity, decreased litter size and pup birth weight at 12 and 30 mg/kg bw/day and decreased bodyweight, growth and food intake at 5-30 mg/kg bw/day. Only in the F2 generation slightly decreased bodyweight, growth and food intake were found at 2 mg/kg bw/day. Since the effects at 2 mg/kg bw/day were only of little significance, this dose was considered to be the NOEL for general (post weaning, parental) toxicity. 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/day. No signs of teratogenicity were found. Based on these reproduction and teratogenicity studies the NOEL for teratogenicity was 12 mg/kg bw/day and the NOEL for reproduction toxicity was 5 mg/kg bw/day.
11. No evidence was found in the following acceptable set of mutagenicity tests: Ames test (*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537), fluctuation assays with *S. typhimurium* (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 and mouse dominant lethal test. The bacterial tests and Chinese hamster ovary (CHO) cells test were carried out with and without metabolic activation.
12. No carcinogenicity data were supplied. Since there is no evidence for mutagenicity, no neoplastic lesions in the repeated dose toxicity tests and no structural resemblance with known carcinogenic compounds, no carcinogenicity data are required.
13. 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.
14. 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) a toxicological ADI of 10 µg/kg bw was established, corresponding to a total intake of 600 µg/person.
15. 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 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 assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP:

$$\text{ADI} = \frac{\frac{\text{geometric mean MIC}_{50} \times \text{CF2}}{\text{CF1}} \quad (\mu\text{g/ml}) \times \text{daily faecal bolus (150 ml)}}{\frac{\text{fraction of an oral dose available for microorganisms} \quad \times \text{weight of human (60 kg)}}{}} \quad (\mu\text{g/kg bw})$$

Based on the above formula, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{1.121 \times 1/1}{0.50} \times \frac{150}{60} = 5.6 \text{ } \mu\text{g equivalent/kg bw (336 } \mu\text{g/person)}$$

The following assumptions were made:

- Σ geometric mean MIC₅₀ = 1.121 mg/ml;
- Σ CF1 = 1 because the range of MIC₅₀ values has already been taken into account in the calculation of the geometric MIC₅₀;
- Σ CF2 = 1 because the MIC values came from tests with high bacterial density;
- Σ 150 g was the weight of the daily faecal bolus;
- Σ the fraction of the microbiologically active residue which is available for the intestinal flora was estimated to be 0.5 (based on the percentage of the baquiloprim dose excreted in faeces in the oral bioavailability study in rats).

The ADI is expressed in µg equivalent of microbiologically active residue. The 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 and 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.
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 equivalent of baquiloprim activity/ml. Because an average of 64% of the total baquiloprim derived residue in milk was antimicrobially active, this corresponds to a maximum concentration of 0.150 µg equivalent 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 the 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 hours, the average concentrations or ranges of total baquiloprim derived residues versus the range of parent compound concentrations in liver were 4629 µg/kg versus 20.6-43.2 µg/kg (14 days), 1983 µg/kg versus less than 5 to 18.9 µg/kg (28 days), 2511 µg/kg versus less than 5 to 14.6 µg/kg (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 percentage 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 day 14. 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 and in injection site tissue it was 1.7%.
20. Intramuscular residue distribution and depletion studies in pigs were provided, with withdrawal periods of 7 to 28 days and with labelled as well as unlabelled baquiloprim. In two studies the composition of residues in liver, kidney and/or injection site tissue was investigated. These studies showed that the highest concentrations of residue occur in the 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 hours, the average concentrations or ranges of total baquiloprim derived residues versus the range or average of parent compound concentrations in liver were: 2514 µg/kg versus less than 5 to 7 µg/kg (14 days) and 1304 µg/kg versus less than 5 µg/kg (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: liver: 2820 µg/kg (parent compound: 52 µg/kg), kidney: 820 µg/kg (parent compound: 18 µg/kg), muscle: 3 µg/kg, fat: 5 µg/kg, skin: 320 µg/kg and injection site: 2577 µg/kg. The percentage of parent compound in liver and kidney decreased between withdrawal days 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 day 14 the percentage in fat was about 7%, the concentrations of marker compound in fat on day 28 and in muscle at 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 approximately 68% at 7 days and 74% at 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.

21. 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.
22. 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.
23. 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.
24. Target tissues are liver, kidney, fat, 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 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 in natural proportions.
25. Based on the toxicological ADI, the bioavailability of residues, the residues depletion profiles and the standard food package, the following MRLs were proposed :

Bovine	liver	300mg
	kidney	150mg
	fat	10mg
	milk	30mg
Porcine	liver	50mg
	kidney	50mg
	fat +skin	40mg

For cattle fat, 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 a limit of quantification of 20 µg of parent compound/kg and have a limit of detection of 10 µg/kg. HPLC methods with MS detection were validated for residues in fat and pig skin (limit of quantification = 5 µg/kg and limit of detection = 2.5 µg/kg). For milk an HPLC-UV method with a limit of quantification of 10 µg/kg and a 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:

Having considered that:

- Σ the provisional toxicological ADI of 10 µg/kg bw (i.e. 600 µg/person) should be accepted as a final ADI,
- Σ no adjustments were required to the provisional MRLs;

the Committee recommends the inclusion of baquiloprim in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Baquiloprim	Baquiloprim	Bovine	10 µg/kg 300 µg/kg 150 µg/kg 30 µg/kg	Fat Liver Kidney Milk	
		Porcine	40 µg/kg 50 µg/kg	Skin+fat Liver, kidney	