The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TRIMETHOPRIM

SUMMARY REPORT (2)

1. Trimethoprim is a diaminopyrimidine antimicrobial agent which is effective against a wide range of Gram-positive and Gram-negative organisms. In veterinary medicine, trimethoprim is commonly used in combination with a sulphonamide in a concentration ratio of 1:5. It is administered orally, as a bolus, paste or in the drinking water or feed to calves, pigs, horses, poultry and fish (5 mg/kg bw trimethoprim+25 mg/kg bw sulphonamide). Injectable formulations are also available for the treatment of pigs, cattle, goats and horses 15-30 mg/kg bw of combined active ingredients, 1-2 times per day). Treatment is normally repeated for up to 5 days (up to 7 days for fish). There is also an intramammary preparation for cattle containing 40 mg trimethoprim+200 mg sulphadiazine. In some countries trimethoprim was also administered to other species such as sheep and llamas but details of the dosing regimes were not available. Trimethoprim is currently entered into Annex III of Council Regulation (EEC) No 2377/90 as follows:

Phamacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Trimethoprim	Trimethoprim	All food producing species	50 µg/kg	Muscle, liver, kidney, fat, milk	Provisional MRLs expire on 1 January 1998.

2. Trimethoprim is a structural analogue of the pteridine portion of dihydrofolic acid and is a competitive inhibitor of the enzyme dihydrofolate reductase. Trimethoprim inhibits the bacterial enzyme at concentrations many times lower than those required to inhibit the mammalian enzyme. The enzyme in *Escherichia coli* is inhibited by a 5 nM concentration of the enzyme compared with 260000 nM to inhibit the rat liver enzyme. Trimethoprim exerts a bactericidal effect *in vitro* in the presence of methionine, glycine and a purine and this allows inhibition of DNA synthesis without inhibition of protein synthesis. This results in bacterial cell elongation without division and leads eventually to cell death.

3. In humans, trimethoprim was rapidly and almost completely absorbed (more than 95%) after oral administration. Peak plasma concentrations occurred 1-4 hours following a single oral dose and were linearly related to dose; C_{max} after an oral dose of 100 mg was 1 µg/ml. Concentrations in bile were approximately twice those in blood. The mean plasma half-life was 10 hours (range 8.6 to 17 hours). Trimethoprim was widely distributed throughout the body with a volume of distribution of 70-100 litres per person. In humans, trimethoprim readily crosses the placenta and is excreted in breast milk at concentrations which exceed the maternal plasma concentration by 25%. Trimethoprim concentrations measured during pregnancy showed that 75% of the maternal plasma concentration was present in amniotic fluid, 57% in the cord blood and 50% in foetal tissue. Plasma protein binding in humans was 42-46% and was reduced in the presence of sulphonamides. Trimethoprim was excreted chiefly by glomerular filtration and renal tubular secretion; 70-90% of an orally administered dose of ^{14}C-trimethoprim was recovered from the urine within 24 hours and 92-102% within 3 days. Some hepatic biotransformation occurred with a small proportion of the dose excreted in the bile. 80% of the administered dose was excreted unmetabolised.

   Five metabolites were identified in the remaining 20%: trimethoprim-1-oxide, trimethoprim-3-oxide, 4-hydroxytrimethoprim, 3-hydroxytrimethoprim and α-hydroxy-trimethoprim.

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4. Following oral administration of 2 mg ^{14}C-trimethoprim to 9 rats and 2 dogs, around 66% and 83% respectively of the dose was recovered in the urine and around 19% and 6% in the faeces. The metabolic pattern was very similar to that observed in humans. However in rat urine only 21.3% of the excreted radioactivity corresponded to unchanged trimethoprim. In rat plasma, 5.8% of the radioactivity corresponded to trimethoprim.

5. Acute oral LD_{50} values in the range 1500 to 1850 mg/kg bw for rats and 1910-3960 mg/kg bw for mice were reported.

6. A series of repeated dose toxicity studies were carried out in rats during the 1960s and were badly designed and reported. Wistar rats were given daily oral doses of 0 (0.5% aqueous *Celacol*), 100 or 300 mg/kg bw trimethoprim, 0, 400 or 1200 mg/kg bw sulphamethoxazole, or the same doses of trimethoprim + sulphamethoxazole combined. The duration of the studies were one month and 6 months. In the one-month studies, bodyweight gain and food consumption were reduced in the groups given 1200 mg/kg bw sulphamethoxazole and in the groups given 1200 mg/kg bw sulphamethoxazole+300 mg/kg bw trimethoprim. Blood samples were taken for examination only at termination. Combinations of trimethoprim+sulphamethoxazole caused dose-related reductions in leucocyte and neutrophil counts. In the 6-month experiments reductions in erythrocyte counts were also observed - but not in the group given 100 mg/kg bw trimethoprim. Bone marrow hyperplasia was observed after one month of dosing and hypoplasia after 6 months of dosing. A fatty change in the liver was also observed. Histopathological changes were also found in the thyroid and pituitary; these were considered to be typical of those induced by large doses of sulphonamides in rats. In a 13-week study in which Sprague-Dawley rats were given daily oral doses of up to 300 mg/kg bw trimethoprim alone, the effects included changes in the bone marrow and significant effects on some organ weights. A NOEL of 3.3 mg/kg bw per day trimethoprim was established, based on changes in organ weights at the next dose.

7. A series of repeated-dose toxicity studies were carried out in rhesus monkeys and patas monkeys. Dosing was by oral gavage from 14 days for up to 6 months. In some studies the monkeys were given trimethoprim alone (doses of 100 and 300 mg/kg bw per day), in others trimethoprim was administered in a 1:5 combination with a sulphonamide (doses of trimethoprim were 33 and 100 mg/kg bw). The effects on haematology values and the effects on bone marrow were similar to those observed in rats. Again, fatty changes were found in the liver. In an experiment with groups of 4 patas monkeys given daily oral doses of 300 mg/kg bw trimethoprim for 14 days, marked changes in the bone marrow were found in the monkeys killed at the end of treatment. In a group of 4 monkeys killed 4 weeks after the end of dosing, there was a significant recovery of the bone marrow effects in all animals. There were no histopathological changes in the thyroid or pituitary. Leucopenia was observed in only one (out of 4) patas monkeys given 300 mg/kg bw per day trimethoprim + 2 mg/kg bw per day leucoverin indicating that the effects were due to folate inhibition. The studies in monkeys were poorly designed and reported. It was not possible to draw any conclusions regarding a NOEL.

8. Repeated-dose toxicity studies were carried out during the 1960s in which Beagle dogs were administered either trimethoprim alone or mixtures of trimethoprim and sulphadiazine for up to 90 days. Following oral (gavage) dosing with 135 mg/kg bw trimethoprim alone, the effects included reduced white blood cell counts, increased serum cholesterol concentrations and changes in thyroid weights. Minor changes in haematology parameters were observed following oral dosing with 45 mg/kg bw trimethoprim for 3 months. The NOEL was 2.5 mg/kg bw per day of trimethoprim.

9. Trimethoprim is generally well tolerated by the target species when administered by the oral route.

10. Groups of 20 female Sprague-Dawley rats were given daily oral doses of 0 (carboxymethylcellulose gel), 20, 180 and 420 mg/kg bw of a trimethoprim:sulfamoxole 1:5 combination, for 2 weeks prior to mating with untreated males (2 females: 1 male). On day 13 of gestation, 10 females/dose were subjected to laparoscopic examination. The remaining dams were allowed to litter down and suckle the offspring. Groups of 20 male Sprague-Dawley rats were given the same daily oral doses of the same trimethoprim:sulfamoxole combination for 10 weeks prior to mating with untreated females. In this experiment mating was 1 male: 1 female. The uterine contents of the dams were examined on day 19 of gestation. In these 2 experiments there were no substance-related effects on male or female fertility, mating activity, the numbers of *corpora lutea* or implantation rate. Treatment of the male rats had no dominant lethal effect. Following treatment of the dams with 420 mg/kg bw, pup weights at birth and at 4 weeks of age were significantly depressed in comparison with the other groups. The NOEL was 180 mg/kg bw of combined active ingredients, equivalent to 30 mg/kg bw per day of trimethoprim.

11. In a peri- and post-natal study, groups of 20 mated female Sprague-Dawley rats were given daily oral doses of 0 (carboxymethylcellulose gel), 10, 30, 100, 300 or 600 mg/kg bw per day of a trimethoprim:sulfamoxole 1:5 combination, from day 16 of gestation up to the end of lactation. The top dose level of 600 mg/kg bw caused signs of toxicity in the dams including sedation and reduced bodyweight gain. In the 600 mg/kg bw group, pup weights at birth and pup weight gain during lactation were significantly reduced. The NOEL was 300 mg/kg bw of combined active ingredients, equivalent to 50 mg/kg bw per day of trimethoprim.

12. Groups of 20 mated female Sprague-Dawley rats were given daily oral doses of 0 (tragacanth), 180, 420 or 600 mg/kg bw per day of a trimethoprim:sulfamoxole 1:5 combination, from days 8-15 of gestation. The dams were euthanased on day 19 of gestation and the uterine contents examined. Signs of maternal toxicity (sedation, reduced bodyweight gain and food consumption) were observed at 420 and 600 mg/kg bw. Teratogenicity was observed at 420 mg/kg bw and 600 mg/kg bw; the malformations included micrognathia, cleft palate and phocomelia. Foetotoxicity (increased post-implantation losses, reduced foetal weight, increased incidence of skeletal variations and retarded ossification) was also observed at 420 and 600 mg/kg bw. 180 mg/kg bw (combined active ingredients) was a NOEL for maternal toxicity, teratogenicity and foetotoxicity; corresponding to 30 mg/kg bw trimethoprim.

13. In another study in Sprague-Dawley rats, groups of 15-18 dams were given daily oral doses of 0, 50, 200 or 350 mg/kg bw per day of a trimethoprim:sulfamethoxypyrazine combination from days 6-15 of gestation. The dams were euthanased of day 20 of gestation and the uterine contents examined. There was a low incidence of foetal malformations in the 350 mg/kg bw group including 2 foetuses with microphthalmia/anophthalmia. Foetal weights were significantly reduced in the 200 and 350 mg/kg bw groups. Over 90% of the foetuses in a positive control group which received 800 mg/kg bw of carbutamide were malformed. In this study, the NOELs for teratogenicity and foetotoxicity were 200 and 50 mg/kg bw (combined active ingredients), corresponding to 33.3 and 8.3 mg/kg bw of trimethoprim respectively.

14. Groups of 15 mated female rabbits were given daily oral doses of 0 (aqueous carboxymethylcellulose), 60, 180 or 600 mg/kg bw of a trimethoprim:sulfamoxole 1:5 combination from days 8-14 of gestation. Five dams per group were euthanased on day 16 or 17 of gestation and the uterine contents examined. The remaining dams were allowed to deliver naturally. 600 mg/kg bw caused reduced maternal bodyweight gain and food consumption. There was no evidence of teratogenicity at any dose level. Foetotoxicity (increased incidence of resorptions) was observed at 600 mg/kg bw. The NOEL was 180 mg/kg bw (combined active ingredients) for both maternal toxicity and foetotoxicity, corresponding to a dose of 30 mg/kg bw trimethoprim.

15. No evidence of teratogenicity was observed in another study in which groups of 5-8 pregnant female New Zealand White rabbits were given daily oral doses of 0, 150, 300 or 600 mg/kg bw of a trimethoprim:sulfamethoxypyrazine combination from days 6-18 of gestation. 600 mg/kg bw caused an increased incidence of dead foetuses. No malformations were observed in the groups given. However 25% of the foetuses were malformed in a positive control group given 100 mg/kg bw thalidomide. The NOEL for foetotoxicity was 300 mg/kg bw (combined active ingredients), equivalent to 50 mg/kg bw trimethoprim.

16. No evidence of teratogenicity was observed in a study in hamsters in which oral doses of 0, 10, 16 or 32 mg/kg bw of a trimethoprim:sulfamethoxazole combination were administered to groups of 10 dams at various stages of organogenesis. However, foetal weight and size were significantly reduced in all treated groups in comparison with the controls.

17. Human epidemiology studies, including a large collaborative perinatal study, revealed no association between the use of trimethoprim during pregnancy, at the therapeutic doses indicated in paragraph 25, and subsequent birth defects in the offspring.

18. Negative results were obtained in an *in vitro* assay for gene mutation using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538. The negative result was confirmed in an assay with the strains TA98, TA100, TA1535 and TA1537 carried out as part of the US National Toxicology Programme and in a published study using one strain (TA100). In contrast, positive results were reported in a published study in strains TA98 and TA1538, in both the presence and absence of S9 metabolic activation; however there was no information concerning the purity of the material tested and the assay used concentrations which were several times those shown to cause toxicity in the other studies; the Committee agreed that no reliance could be placed on the result of this assay in the absence of independent confirmation. Negative results were obtained in *in vitro* assays for gene mutation at the HGPRT locus in Chinese Hamster V79 cells and at the TK locus in mouse lymphoma cells; both these assays were carried out in accordance with GLP and to modern protocols. A GLP-compliant *in vitro* assay for unscheduled DNA synthesis in cultured A549 cells also gave negative results.

19. Negative results at concentrations which were moderately toxic were obtained in an *in vitro* assay for chromosomal damage using human lymphocytes; the study was GLP-compliant but there was no independent repeat of the assay. Negative results were also obtained in 2 published *in vitro* chromosomal aberration assays in human lymphocytes though full details of the experiments were not available. In contrast, positive results were obtained in a GLP-compliant chromosomal aberration assay using Chinese hamster V79 cells at doses which caused significant toxicity; in the presence of S9, the positive result was not confirmed on repeating the assay. Positive results were claimed in a published study investigating chromosomal aberrations and sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells; however there was no clear dose-response and conflicting results were reported by the 2 laboratories participating in the study.

20. In an *in vivo* micronucleus test, NMRI mice were given a single oral dose of 2000 mg/kg bw trimethoprim and killed 12, 24 or 48 hours later; there was no increase in micronuclei in any of the trimethoprim-treated groups. A second GLP-compliant micronucleus test employed CD-1 mice and 3 daily oral doses of 100, 250, 500, 700 and 1000 mg/kg bw trimethoprim; negative results were again obtained and the ratio polychomatic erythrocytes (PCE) : normochromatic erythrocytes (NCE) was increased in several of the groups confirming that the test substance had reached the bone marrow. Negative results were also reported in a published dominant lethal assay in rats though few details of the experiment were available.

21. Four published studies on the effects of trimethoprim therapy in both adults and children on chromosomal aberrations in human peripheral blood lymphocytes reported negative results. Another study reported an increased incidence of micronuclei in 2 out of 12 patients undergoing trimethoprim therapy but no increase in chromosomal damage on metaphase analysis. The smoking status of the patients was not mentioned.

22. Although positive results were reported for trimethoprim in some *in vitro* assays for chromosome damage, the *in vivo* studies gave negative results. The related substance, baquiloprim, had given

negative results in an *in vitro* clastogenicity study in human peripheral lymphocytes, an *in vivo* mouse micronucleus test and an *in vivo* mouse dominant lethal assay. The Committee concluded that trimethoprim was not genotoxic.

23. No carcinogenicity studies were carried out with trimethoprim. The absence of such data was considered justified by results of the mutagenicity studies and the lack of evidence of pre-neoplastic lesions in the repeated-dose toxicity studies.

24. A sensitisation study was carried out in guinea pigs using a 25% suspension of trimethoprim+sulphadiazine (1:5) in 0.5% Celacol. A negative result was claimed but full details of the test were not provided. The negative result is surprising in view of the known potential of the sulphonamides for inducing sensitization.

25. Trimethoprim has been used in human medicine as an antimalarial and in the treatment of respiratory and urinary tract infections for over 40 years. It is available as both oral and parenteral formulations. It is commonly used in combination with a sulphonamide but, because of adverse reactions, trimethoprim is increasingly used on its own. The usual dose is 100 or 200 mg, twice daily, orally or 200 mg every 12 hours by intravenous injection or infusion. When given as a mono-preparation, trimethoprim is generally well tolerated; the majority of adverse reactions are mild and include gastrointestinal disturbances such as nausea, vomiting and sore mouth. Skin rashes have been reported but are generally mild and a rare occurrence unless larger doses (greater than 400 mg/day) are used for extended periods (more than 10 days). Haematological adverse effects with trimethoprim are rare but the possibility of megaloblastic changes due to folic acid inhibition exists; patients deficient in folate should therefore be given folic acid supplements.

26. A toxicological ADI of 12.5 µg/kg bw per day was established by applying a safety factor of 200 to the NOEL of 2.5 mg/kg bw per day which was established in the 90-day study in Beagle dogs. The safety factor of 200 was considered justified by the poor quality of the 90-day study.

27. In a series of tests on 3 commercial dairy starter cultures, concentrations of up to 200 µg/kg of trimethoprim were usually without effect. However, in one test, a trimethoprim concentration equivalent to 50 µg/kg caused a 20% inhibition of L(+)-lactate formation in one starter culture but did not affect overall acid production. A trimethoprim concentration greater than 5000 µg/kg was necessary to produce a 50% inhibition in L(+)-lactate formation.

28. *In vitro* MIC data were obtained at 3 different inoculum densities for trimethoprim against 100 isolates representing 10 genera of human gut flora. *Lactobacillus* was the most sensitive species with an *in vitro* MIC_{50} value of 0.25 µg/ml at an inoculum density of 10^9 cfu/ml.

For the assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP:

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

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

$$\text{ADI} = \frac{\frac{0.25 \times 1}{3} \times 150}{0.05 \times 60} = 4.2 \text{ µg/kg bw i.e. } 252 \text{ µg/person}$$

The following assumptions were made:

- 0.25 µg/ml was the *in vitro* MIC_{50} value for the most sensitive species (*Lactobacillus* spp) at an inoculum density of 10^9 cfu/ml.
- CF1 = 3, based on a factor of
  - 1 because the most sensitive, predominant, organism was used
  - 3 because trimethoprim may induce both chromosomal and plasmid-mediated resistance
- CF2 = 1, because there were no reliable data to correct for differences in growth conditions between the *in vitro* and *in vivo* situation. No correction was considered necessary to correct for bacterial density. There was no information to correct for possible effects of pH.
- 0.05 = fraction of oral dose available to micro-organisms at the distal part of the gastro-intestinal tract, calculated from human data indicating that more than 95% of an oral dose is bioavailable.

30. The metabolism of trimethoprim in the target species followed the same metabolic pathways as in humans and laboratory rats and dogs. A significant proportion of the residues in tissues consisted of unmetabolised trimethoprim. Residues of the 1-N-oxide and 3-N-oxide metabolites, which do not retain the pteridine structure and would be expected to have little or no microbiological activity were detected together with residues of 4-hydroxy- , 3-hydroxy- (also referred to as the 4-desmethyl and 3-desmethyl derivatives) and the α-hydroxy metabolite. The studies in the target species using ^{14}C-trimethoprim indicated each metabolite comprised less than 5% of the total residues present in tissues. The desmethyl metabolites and the α-hydroxy metabolite retain the pteridine structure and would therefore have some microbiological activity, though this had not been experimentally determined. No data were available on the residue composition of milk. The nature of the residues in fish tissues had not been studied.

31. Metabolism was investigated in neonatal and young pigs following intravenous injection. Unchanged trimethoprim, the α-hydroxy, 1-N-oxide and the conjugated 3'-demethyl and 4'-demethyl metabolites were detected in plasma, urine bile, liver and kidney. In another study, a female pig was given a single intramuscular injection of 5 mg/kg bw ^{14}C-trimethoprim and kept in a metabolism crate. The peak blood concentration (3300 µg/kg) was found in the first sample taken one hour after dosing. The half-life for elimination was 2.7 hours. 49% of the administered dose was excreted within 8 hours of dosing, 43% in the urine and 3% in the faeces. 22% of the material in urine was unchanged trimethoprim. The pig was killed 8 hours after dosing. Total residues in liver, kidney, fat and muscle were 2030, 3560, 270 and 340 µg/kg respectively. Total residues at the injection site were 1000 µg/kg. Radioprofiling showed that 39% and 48% of the residues in liver and kidney were unchanged trimethoprim.

32. In a new GLP-compliant residues depletion study, pigs were fed twice a day a medicated diet containing the equivalent of 5 mg trimethoprim+25 mg sulphadiazine per kg bw per day. The treated feed was fed for 10 consecutive days and on the morning of the 11th day. The pigs were killed (2/sex/time point) at 3, 5, 10, 15 and 20 days after the last dose. Residues of trimethoprim and sulphadiazine were determined in edible tissues using HPLC (trimethoprim: limit of quantification 25 µg/kg, limit of detection 15 µg/kg). Residues of trimethoprim were detectable only in one muscle sample taken 3 days after the end of treatment (34 µg/kg) and two samples of skin/fat taken 5 days (31 µg/kg) and 10 days (27 µg/kg) after the end of treatment.

33. A calf was given a single intramuscular injection of 5 mg/kg bw ^{14}C-trimethoprim and kept in a metabolism cage. Plasma concentrations had peaked by the time the first blood sample was taken 3 hours after treatment (630 µg/kg). The half-life for plasma elimination was approximately 4.5 hours. Most of the administered dose was excreted within 2 days, 67% in the faeces and 33% in the urine. The calf was killed 7 days after treatment. Total residues in liver, muscle, kidney and fat were 100, 10, 30 and 20 µg/kg respectively. Residues at the injection site were 220 µg/kg. In another study, 5 calves were given a single oral dose of 250 mg ^{14}C-trimethoprim + 1250 sulphadiazine, equivalent to 30 mg/kg bw of combined active ingredients. The calves were killed 6 hours, 24 hours, 3 days (2

calves) and 7 days after dosing. Total residues in liver, kidney, muscle and fat, 3 days after dosing, were 140, 180, below 100 and 420 µg/kg bw respectively. Seven days after dosing, total residues in all tissues were below 100 µg/kg. The livers from the animals killed 6 and 24 hours after dosing (total residues 4300 and 1440 µg/kg respectively) were analysed for trimethoprim. The livers contained 54% and 50% trimethoprim respectively. Trimethoprim was the major component of the residues in kidney, muscle and fat at the 6 and 24 hour time points (values were not given in the report).

34. In an old, pre-GLP study, calves were given a daily oral dose of a trimethoprim/sulphadiazine (1:5) bolus formulation for 5 days. The dose level was at least 30 mg/kg bw per day of combined active ingredients and was significantly more than this for a number of calves. The calves were slaughtered (3/time-point) 1, 6, 7, 8 and 9 days after the last dose. Residues of trimethoprim in tissues were determined using GLC; the limits of quantification and detection for trimethoprim seemed to be 100 and 50 µg/kg respectively. One day after the last dose, mean residues of trimethoprim were 117, 183 and 47 µg/kg in liver, kidney and muscle respectively. Residues of trimethoprim were below the limit of quantification at later time points. Residues of sulphadiazine were still detectable in kidney and muscle samples taken 9 days after the end of treatment. A second study used 4 lactating Friesian cows, mean body weight 504 kg. An intramammary suspension containing 40 mg trimethoprim + 200 mg sulphadiazine was administered to each quarter, at 3 consecutive milkings. All 4 cows were killed 7 days after the last treatment. Residues of trimethoprim in liver, kidney, muscle and fat were determined using a non-specific microbiological assay employing *Bacillus pumilus* CN 607 as the test organism. The limit of detection was 20 µg/kg. Residues in all tissue samples were below the limit of detection.

35. A milk residues depletion study used 8 lactating Friesian cows of mean bodyweight 546 kg and milk yield range at initiation 14.6-29.4 litres. An intramammary suspension containing 40 mg trimethoprim+200 mg sulphadiazine was administered to each quarter, at 3 consecutive milkings. Residues of trimethoprim in quarter milk samples were determined using a non-specific microbiological assay employing *Bacillus pumilus* CN 607 as the test organism. The limit of detection was 30 µg/kg. Mean concentrations of trimethoprim were 4749 µg/kg 6 hours after the first infusion and declined to 215 µg/kg, 12 hours after the first infusion. Twenty-four hours after the first infusion (12 hours after the second infusion, mean residues were 70 µg/kg. Thirty hours after the first infusion (6 hours after the last infusion) mean residues were 2805 µg/kg. Twenty-four hours after the last infusion, mean residues were 32 µg/kg and were below the limit of detection at subsequent time points.

36. Six broilers were given a daily oral dose of 7.5 mg/kg bw ^{14}C-trimethoprim + 22.5 mg/kg bw sulphaquinoxaline for 5 days. The birds were killed (2/time point), 1 day, 3 days and 7 days after the last dose. Mean total residues of trimethoprim in kidney and liver declined from 1000 µg/kg and 1340 µg/kg, one day after the end of dosing, to 60 µg/kg and 30 µg/kg, 7 days after dosing. Mean total residues in muscle and fat one day after dosing were 110 and 90 µg/kg and had declined to below 10 µg/kg 7 days after dosing. Mean total residues in skin declined from 210 µg/kg one day after dosing to 30 µg/kg 7 days after dosing. Radioprofiling of the kidney and liver samples taken one day after the end of treatment showed that 29-51% of the residues were present as trimethoprim.

37. In a non-GLP study, broiler chickens were housed individually in cages and fed a medicated diet containing trimethoprim/sulphadiazine (1:5) designed to achieve a dose level of 33 mg/kg bw per day of combined active ingredients for 5 days.The birds were killed (6/time point) 1, 2 and 3 days after the end of treatment. Tissues were analysed using an HPLC assay with limits of quantification and detection of 25 µg/kg and 7.5 µg/kg respectively for trimethoprim. Residues of trimethoprim were below the limit of quantification in all samples of muscle and skin/fat. 2 days after the end of treatment, residues of 29 and 46 µg/kg trimethoprim were found in 2 samples of kidney and residues of 34 and 24 µg/kg trimethoprim were found in 2 samples of liver; residues of trimethoprim in all other samples were below the limit of quantification. In a second, GLP study, male and female Ross chickens were group housed and fed a medicated diet containing trimethoprim/sulphadiazine (1:5) designed to achieve a dose level of 30 mg/kg bw per day of combined active ingredients for 10 days. The birds were killed (6/time point) 0, 1, 2, 3 and 5 days after the end of treatment. Tissues were analysed using an HPLC assay with limits of quantification and detection of 50 µg/kg and 25 µg/kg

respectively for trimethoprim. One day after the end of treatment, residues of trimethoprim were below the limit of detection in all samples of muscle and liver. One day after the end of treatment, residues of trimethoprim in kidney ranged from below the limit of detection to 80 µg/kg. Two days after the end of treatment, residues of trimethoprim in all kidney samples were below the limit of detection. Detectable residues were found in one kidney sample taken 3 days after the end of treatment (61 µg/kg) and one kidney sample taken 5 days after the end of treatment (26 µg/kg). Residues of trimethoprim in samples of skin + fat were in the range from below the limit of detection to 87 µg/kg, one day after the end of treatment, and from below the limit of detection to 62 µg/kg, 2 days after the end of treatment. Residues in all subsequent samples of skin + fat were below the limit of detection.

38. In a GLP-compliant residues depletion study, turkeys were given medicated feed at a nominal concentration of 50 mg/kg trimethoprim + 250 mg/kg sulphadiazine, in the feed, for 10 days. Groups of 3 birds/sex/time-point were killed 2, 3, 5 and 10 days after the end of treatment. Edible tissues were analysed by HPLC for residues of trimethoprim (limit of quantification 25 µg/kg, limit of detection 10 µg/kg). Residues of trimethoprim were found in all 6 kidney samples taken 2 days after the end of treatment (mean 20.4 µg/kg) and in 4 out of 6 samples taken 3 days after the end of treatment (mean 15.1 µg/kg). Trimethoprim was not detectable in any of the liver samples. Trimethoprim was detectable in only one muscle sample (44.6 µg/kg) which was taken 2 days after the end of treatment. Mean residues of trimethoprim in skin+fat samples declined from a mean of 32.4 µg/kg 2 days after the end of treatment, to 13.7 µg/kg 5 days after the end of treatment. Ten days after the end of treatment, residues of trimethoprim were detectable in only one (15.8 µg/kg) out of six skin+fat samples.

39. Several pharmacokinetic studies were carried out in goats using various dosage regimes. Following intravenous dosing with 10 mg/kg bw trimethoprim, 44% of the administered dose was excreted in the urine within 24 hours. Three male and 2 female goats were given a single intravenous injection of 13 mg/kg bw trimethoprim + 67 mg/kg bw sulphadoxine and killed 3 hours later. Residues of trimethoprim in tissues were determined by HPLC with fluorescence detection. Mean (SEM) residues in muscle, liver and kidney were 1100 (+200) µg/kg, 800 (+100) µg/kg and 2100 (+400) µg/kg respectively. In another experiment, male goats were given an initial intravenous dose of 10 mg/kg bw per hour of ^{14}C-trimethoprim followed by continuous intravenous infusion of 4 mg/kg bw per hour of ^{14}C-trimethoprim. The infusion was stopped after 2.5 - 3 hours, the goats were killed immediately and the residues in tissues of trimethoprim and total trimethoprim metabolites were determined. Mean (SEM) residues of trimethoprim and metabolites were in muscle: 4700 (+300) µg/kg and 600 (+500) µg/kg, in kidney: 22000 (+3000) µg/kg and 65000 (+27000) µg/kg and in liver: 700 (+200) µg/kg and 3900 (+700) µg/kg.

40. A male sheep was given a single intramuscular injection of 5 mg/kg bw ^{14}C-trimethoprim and kept in a metabolism crate. Plasma concentrations had peaked by the time the first blood sample was taken 3 hours after dosing (980 µg/ml); the half-life for elimination was 4.5 hours. Within 3 days of dosing, approximately 75% of the administered dose recovered from the urine and 25% from the faeces. The sheep was killed 7 days after treatment. Total residues in liver, muscle and fat were 400, 30 and 40 µg/kg respectively; residues were undetectable in fat. Radioprofiling showed that 30% of the residues in liver was unchanged trimethoprim.

41. Several pharmacokinetic studies were carried out in horses using various dose regimes. Trimethoprim was well absorbed after oral administration and rapidly eliminated. Following oral dosing of 3 horses with 5 mg/kg bw trimethoprim, the half-life for elimination was 1.05 hours. In 3 horses given an intravenous dose of 8 mg/kg bw trimethoprim, 46% of the dose was excreted in the urine and 51% in the faeces. In a residue study, 2 adult horses (body weights 393 and 457 kg) and 2 foals (body weights 152 and 177 kg) were given daily oral doses of a trimethoprim:sulphadiazine (1:5) paste formulation at a rate of 30 mg/kg bw per day of combined active ingredients for 5 days. The animals were killed 6 days after the last dose and residues of trimethoprim in tissues were determined using a GLC analytical method with a limit of quantification of 100 µg/kg and limit of detection of 10 µg/kg. Residues in all samples of liver, kidney, muscle and fat were below 10 µg/kg.

42. Six neutered male llamas, body weight 101-124 kg, were given intravenous doses of 3 mg/kg bw trimethoprim, every 12 hours (exact number of doses not stated). Plasma C_{max} (peak) and C_{min} (trough) values of 15.0 ± 3.1 µg/ml and 0.66 ± 0.38 µg/ml were attained. The elimination half-life was 3.31 ± 0.56 hours and the mean residence time (MRT) was 4.83 ± 1.74 hours. The volume of distribution at steady state (Vd_{ss}) was 0.404 ± 0.151 l/kg and the $AUC_{0-\infty}$ was 39.94 ± 16.63 µg/h/ml. No residue data were provided for llamas.

43. The rates of absorption and elimination of trimethoprim in fish were dependent on water temperature. For 12 hours after oral administration of 220 mg/kg bw ^{14}C-trimethoprim to rainbow trout at 7°C, residues were undetectable in plasma. Residues were detectable in plasma within 6 hours of oral dosing when the experiment was repeated at 15°C. Autoradiography showed that absorption took place in the stomach. Highest concentrations of trimethoprim were present in the uveal tract of the eye. High concentrations were also found in the kidney and concentrations in skin were higher than those in blood. Biliary excretion was shown to be a major excretory pathway in rainbow trout; excretion through the gill was of minor importance. The pharmacokinetics was studied in carp dosed intraperitoneally with 10 mg/kg bw trimethoprim; clearance was 4.17 ml/min/kg and $t_{1/2}$ was 5 hours. In an old, non-GLP study, Atlantic salmon were given feed containing trimethoprim + sulphadiazine (1:5) at a dose rate equivalent to 30 mg/kg bw per day (of combined active ingredients) for 10 days. The fish were killed (6/time point) during treatment for the determination of residues in plasma, and 0, 50, 100, 150, 200, 250, 300 and 400°days after treatment for the determination of residues in muscle. Residues were determined using HPLC with UV detection. The limit of detection for all tissues was 10 µg/kg. Trimethoprim concentrations in plasma peaked during days 7-10 of dosing at around 12 µg/ml. Mean residues of trimethoprim in muscle (taken from the middle) were 10740 µg/kg immediately after the end of treatment and declined to 100 µg/kg after 300°days and less than 10 µg/kg after 400°days. Mean residues in skin were 530 µg/kg after 300°days and 100 µg/kg after 400°days.

44. The routine analytical method for the determination of residues of trimethoprim in tissues was based on HPLC with UV detection. Validation data, in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community, were provided for edible tissues of pigs, turkeys, chickens; the limit of quantification was 25 µg/kg. A similar HPLC assay had been validated for muscle and skin of Atlantic salmon; the limit of quantification was 50 µg/kg. Validation data were also provided for horse tissues indicating a limit of quantification of 100 µg/kg and a limit of detection of 50 µg/kg. In a published HPLC assay which is used as the method for the monitoring of trimethoprim in Germany, the limit of quantification for the edible tissues and milk of cattle was 50 µg/kg.

    The method was not validated for edible tissues of goats, sheep or llamas.

Conclusions and recommendation:

Considering that:

- a microbiological ADI of 4.2 µg/kg bw per day (252 µg/person/day) was established,
- trimethoprim is the marker residue,
- residues of trimethoprim could be found at low concentrations in all tissues at early time points; it was therefore necessary to propose MRLs for all edible tissues and for milk,
- the desmethyl and α-hydroxy metabolites of trimethoprim retained the pteridine structure and would therefore contribute towards the microbiological activity; radiolabelled studies in the target species indicated that each of the desmethyl metabolites accounted for less than 5% to the total residues in tissues,
- in chickens, 29-51% of the total residues in liver and kidney one day after the end of treatment were present as trimethoprim,
- in a pig killed 8 hours after treatment, 39% and 48% of the total residues in liver and kidney were unchanged trimethoprim,

- in calves killed 6 and 24 hours after dosing, 54% and 50% of the total residues in liver were present as trimethoprim,
- a validated analytical method is available for the determination of residues of trimethoprim in edible tissues of cattle, pigs, chickens and turkeys, fin fish, and the milk of cattle,
- an analytical method was available for the determination of residues in horse tissues but data on accuracy and precision were available only at a concentration of 100 µg/kg; this was therefore the limit of quantification; the limit of detection was 50 µg/kg
- the analytical method was not validated for goats, sheep or llamas and therefore no MRLs could be proposed for these species;

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

Phamcologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Trimethoprim	Trimethoprim	Bovine	50 µg/kg	Muscle, fat, liver, kidney, milk	
		Porcine	50 µg/kg	Muscle, skin + fat, liver, kidney	
		Poultry	50 µg/kg	Muscle, skin + fat, liver, kidney	Not for use in animals from which eggs are produced for human consumption
		Equidae	100 µg/kg	Muscle, fat, liver, kidney,	
		Fin Fish	50 µg/kg	Muscle and skin in natural proportions	

Based on these MRLs and assuming that the only metabolites with microbiological activity were the desmethyl- and α-hydroxy metabolites and that these together accounted for no more than 15% of the total residues, it was estimated that the daily consumer intake of microbiologically-active residues in 300 g bovine muscle, 100 g liver, 50 g kidney, 50 g fat and 1.5 litres bovine milk would represent about 46% of the microbiological ADI of 252 µg/person calculated in paragraph 29.

The limitations of the analytical method meant that a higher MRL was proposed for equidae. This was considered acceptable because equidae were regarded as a minor species and it was estimated that consumer intake of microbiologically-active residues from edible tissues would not exceed the ADI.