European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

CYPERMETHRIN
(Extrapolation to all ruminants)

SUMMARY REPORT (4)

1. Cypermethrin is a synthetic pyrethroid insecticide which which is applied topically for the control of ectoparasites such as ticks, fleas, lice and blowflies. Cypermethrin is currently included 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
Cypermethrin	Cypermethrin (sum of isomers)	Bovine	20 µg/kg 200 µg/kg 20 µg/kg 20 µg/kg	Muscle Fat Liver Kidney	
			20 µg/kg	Milk	Further provisions in Council Directive 93/57/EC are to be observed.
		Ovine	20 µg/kg 200 µg/kg 20 µg/kg 20 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk are produced for human consumption
		Salmonidae	50 µg/kg	Muscle and skin in natural proportions	

2. In reviewing the availability of endo- and ectoparasiticides for sheep and goats, cypermethrin was considered for extrapolation from bovine and ovine species to all ruminants. The considerations and criteria leading to the identification of cypermethrin are described in the Position Paper Regarding Availability of Veterinary Medicines – Extrapolation of MRLs (EMEA/CVMP/457/03-FINAL).

3. The scientific justification for this extrapolation was assessed in accordance with the Notes for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL) and on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL).

4. In setting the ADI in the original assessment of cypermethrin, the data summarised in the paragraphs below were considered.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 47
E-mail: mail@emea.eu.int http://www.emea.eu.int

5. In humans, laboratory animals and the target species, cypermethrin was rapidly absorbed after oral administration and was rapidly excreted in both the urine and faeces with the major portion excreted in the urine. Cypermethrin is lipophilic and highest residues were found in the fat, the liver and kidney. Metabolism was very similar in all species and involved cleavage of the ester bond to form phenoxybenzoic acid and a cyclopropanecarboxylic acid derivative which were excreted as conjugates. Absorption was much slower after dermal administration and the extent of metabolism was also reduced.

6. Many of the toxicity studies were carried out approximately 20 years ago and do not meet modern standards of design or reporting. Most of the studies were carried out using cypermethrin of *cis:trans* isomer ratio 50:50 to 40:60.

7. The acute toxicity of cypermethrin was very variable and depended on factors such as age, sex and strain of the animal, nature of the solvent vehicle and the environmental conditions. The *cis*-isomers were more acutely toxic than the *trans*- isomers. The acute oral LD_{50} of cypermethrin of cis:trans isomer ratio 90:10, in corn oil, was 367 mg/kg bw in female rats. The acute oral LD_{50} of cypermethrin of *cis:trans* isomer ratio 40:60 to the same species, under the same conditions, was 891 mg/kg bw. The toxic signs were characterised by salivation, increased startle response, ataxia, splayed gait, tremors and convulsions. Myelin and axon degeneration were observed at lethal dose levels.

8. Signs of central nervous system toxicity were observed in 3-month repeated dose studies in rats and dogs. The NOELs were 100 and 50 mg/kg feed, respectively, corresponding to 5 and 12.5 mg/kg bw/day.

9. Reduced litter size and weights were observed in a 3-generation study in rats at dose levels which also caused reduced bodyweight gain in the parents. The NOEL was 5 mg/kg bw/day. Cypermethrin was not teratogenic or foetotoxic in rats or rabbits at dose levels which caused maternal toxicity. In these studies, oral doses of 0, 20, 50 or 120 mg/kg bw/day of cypermethrin in corn oil were administered to rabbits from days 6 to 18 of gestation and oral doses of 0, 17.5, 35 or 70 mg/kg bw/day of cypermethrin in corn oil were administered to rats from days 6 to 15 of gestation.

10. Cypermethrin was not mutagenic in *in vitro* assays for gene mutation in bacteria and in V79 Chinese hamster cells, in a cytogenetics assay and in a sister chromatid exchange (SCE) assay in human lymphocytes. However *in vivo* assays for mutagenicity gave conflicting results. Positive results were obtained in a mouse bone marrow micronucleus test following oral and dermal administration but not following intraperitoneal administration. A dose-related increase in sister chromatid exchange frequency was observed in mouse bone marrow after subcutaneous administration. Cypermethrin did not induce chromosomal aberrations in Chinese hamster bone marrow and a dominant lethal assay was also negative. Positive results were reported in several published studies including a chromosomal aberration assay in mouse bone marrow, a micronucleus test, a sperm abnormality test and an *in vitro* cytogenetics assay in mouse spleen cells. However the published studies were often poorly reported and did not comply with Organization for Economic Cooperation and Development (OECD) guidelines for the numbers of animals, numbers of polychromatic erythrocytes scored, presence of a positive control group, etc and it was agreed that no reliance should be placed on these reports. The overwhelming evidence suggested that cypermethrin was not mutagenic.

11. No increase in tumour incidence was observed in a 2-year study in rats fed dietary concentrations of cypermethrin equivalent to approximately 0, 0.05, 0.5, 5 or 50 mg/kg bw/day, though the group sizes were too small for a satisfactory assessment of carcinogenic potential. The NOEL was 5 mg/kg bw/day, based on reduced bodyweight gain at the higher dose. According to the summary of a combined chronic toxicity and carcinogenicity study in rats which had been provided to the US Environmental Protection Agency (EPA), groups of 52 per sex and dose rats were fed diets which corresponded to intakes of 0, 1.0, 7.5 or 75 mg/kg bw/day of cypermethrin for 2 years.

There was no evidence of carcinogenicity and the US Environmental Protection Agency concluded that the NOEL was 7.5 mg/kg bw/day, based on bodyweight loss, minor haematological and clinical chemistry changes and adaptive changes in the liver at the higher dose. An increased incidence of benign alveolar lung tumours was observed in mice fed 1600 mg/kg feed. The study was evaluated by a WHO Task Group which considered that the increase in benign lung tumours was insufficient to warrant concern when compared with the historical control incidence. A published report suggested that cypermethrin might act as a tumour promoter; however this was contradicted by the results of another study in which 800 mg/kg feed cypermethrin did not enhance GST-P positive foci in rodent liver. It was concluded that cypermethrin did not possess carcinogenic potential.

12. Cypermethrin was shown to be a potential skin sensitiser in 2 separate studies using cypermethrin from the same source. Two further studies using cypermethrin from a different manufacturing source indicated that cypermethrin had only mild sensitisation potential.

13. *In vitro* studies suggested that cypermethrin might possess an immunomodulatory effect and it was shown to affect antibody production in rats and rabbits *in vivo*. However, in another study using alphacypermethrin, no effects on a range of immunological, haematology and pathological tests were observed following oral doses of 0, 4, 8 or 12 mg alphacypermethrin/kg bw/day to rats for 28 days.

14. Human occupational exposure to cypermethrin has been reported to cause transient paraesthesia on the face and other exposed areas of the body. It was considered that the paraesthesia was due to a spontaneous repetitive firing of the local sensory nerve endings, with thresholds temporarily lowered by the substance.

15. Groups of Wistar rats were given daily oral doses of 0 (dimethyl-sulphoxide), 25, 50, 100, 150 or 250 mg/kg bw/day of cypermethrin for 7 days. The rats were killed 3 to 4 weeks after the start of treatment and the right and left sciatic/posterior tibial nerves were analysed. Neuromuscular function was assessed by means of the inclined plane test and peripheral nerve damage by reference to β-glucuronidase and β-galactosidase activity increases in nerve tissue homogenates. Rats given 100 mg/kg bw and above showed signs of toxicity and increased enzyme activity was observed at 150 mg/kg bw. The overall NOEL for neurotoxicity was 50 mg/kg bw/day.

16. An ADI of 50 µg/kg bw (3000 µg/person) was established for cypermethrin by applying a safety factor of 100 to the NOEL of 5 mg/kg bw/day which was established in the 3-month and 2-year studies in rats, and the 3-generation study in rats. It was noted that this was identical to the ADI which had been adopted by the Joint WHO/FAO Expert Committee on Food Additives (JECFA). However, a lower ADI of 15 µg/kg bw (900 µg/person) had been established for alphacypermethrin, which comprised the 2 most toxic of the cis- isomers. The isomeric composition of cypermethrin varied considerably with the manufacturing source. Cypermethrin from one source consisted of more than 90% of the 4 cis-isomers (known as high-cis cypermethrin). For these reasons, it was agreed that the ADI which had been established for alphacypermethrin should be used in elaborating MRLs.

17. For the extension to include all ruminant species in Annex I the information summarised in the paragraphs below was taken into account.

18. In a pharmacokinetic study, 2 sheep were orally dosed with 1 mg ^{14}C-cypermethrin/kg bw and samples of blood faeces and urine were collected at regular intervals. The sheep were killed 7 days after treatment and the identities and concentrations of residues in tissues, blood, faeces and urine were determined using radio-TLC and LSC. The C_{max} were 140 and 144 ng equivalents/g, t_{max} were 8 and 12 hours, $t_{½}$ were 37 and 42 hours and the AUC were 3907 and 4714 ng equivalents.h/g in a male and female sheep respectively. Unmetabolised cypermethrin represented about 4, 1, 22 and 86% of the total residue in liver, kidney, muscle and fat respectively one day after treatment. Faecal elimination accounted for 30 and 35% of the administered dose, urinary elimination accounted for 44 and 35% of the administered dose and the total residue recovered from excrement and the carcass accounted for 75 and 73% of the dose administered to a male and female sheep respectively.

In the same study, sheep were orally dosed with 1 mg ^{14}C-cypermethrin/kg bw then 5 animals were killed 1, 2 and 3 days after. Mean residues in liver were 334, 135, and 66 µg equivalents/kg, 1, 2 and 3 days after treatment. In fat, mean residues were 50, 73, and 52 µg equivalents/kg, 1, 2, and 3 days after treatment. In kidney, residues were 408, 60 and 17 µg equivalents/kg, 1, 2 and 3 days after treatment. In muscle, residues were 13,8 and less than 5 µg equivalents/kg, 1, 2 and 3 days after treatment.

19. Sheep were topically dosed along the back line with 40 ml of 12.5 mg ^{14}C+^{13}C+^{12}C-alphacypermethrin/ml (i.e. about 20 mg/kg bw). Three sheep were killed on days 2, 4, 7 and 14 after treatment. The identities and concentrations of residues in tissues were determined using LSC, HPLC-Radio/UV, LC-MS, GC-MS and EC-ECD methods. Unmetabolised alphacypermethrin represented about 10, 10, 90 and 90% of the total residue in liver, kidney, muscle and fat respectively for up to 4 days after treatment.

20. Sheep were plunge-dipped in a commercial dip preparation diluted to a nominal concentration of 150 mg cypermethrin /litre. The sheep were killed (4 per time-point), 1, 3 or 7 days after treatment and residues of cypermethrin in tissues were determined using GLC method. Mean residues in muscle were 176 µg/kg and 235 µg/kg, 1 and 3 days after treatment. In fat, mean residues were 196 and 775 µg/kg, 1 and 3 days after treatment. Residues in liver were undetectable in 3 samples taken 1 day after treatment whereas mean residues 3 days after treatment were 260 µg/kg. Mean residues of 280 µg/kg were found in 2 out of 4 kidney samples taken 1 day after treatment; residues in the 2 other samples were undetectable. At 3 days, mean residues in kidney were427 µg/kg. Residues in all tissues were below the limit of quantification 7 days after treatment. In another study in which groups of sheep (4 per time-point) were treated topically with a pour-on formulation (at approximately 10 mg/kg bw), residues were detectable only in fat. Twenty-four hours after treatment, mean residues in renal and omental fat were 30 and 20 µg/kg, respectively. Mean residues in both renal and omental fat were 40 µg/kg at 7 days after treatment and declined to 20 µg/kg at 28 days after treatment. Residues of cypermethrin were measured in ewes' milk after plunge dipping in a preparation diluted to a nominal concentration of 150 mg cypermethrin/litre. Mean values of 13, 10, 9, 7 and 7 µg cypermethrin/kg were found 1, 3, 7, 10 and 15 days after treatment, respectively.

21. Calves were treated topically with a pour-on formulation at approximately 41 mg/kg bw and killed in groups of 5, at 3, 7 and 14 days after treatment. Residues of cypermethrin were determined using GLC method. Residues in all liver samples were below the limit of quantification (10 µg/kg). Residues in muscle were detectable only in the day 3 samples (mean residues 24 µg/kg). Mean residues in kidney depleted from 66 µg/kg at 7 days after treatment to 40 µg/kg at 14 days after treatment. Residues were highest in fat: mean residues of 260 µg/kg and 670 µg/kg were found in subcutaneous and peritoneal fat at 7 days after treatment; mean residues in these tissues were 140 and 330 µg/kg 14 days after treatment. In a study in which lactating cows were treated topically with a pour-on formulation at 1.25 mg/kg bw, residues of cypermethrin in milk were determined using GLC; the limit of quantification was 2 µg/kg. Mean residues were 25 µg/kg in milk taken 24 hours after treatment, 48 µg/kg at 48 hours after treatment and 7 µg/kg at 7 days. When cows were treated at 2.5 mg/kg bw, the mean residues in milk at these 3 time-points were 63 µg/kg 99 µg/kg and 13 µg/kg, respectively.

22. Eight steers and eight lactating cows were treated topically with a ^{14}C-alphacypermethrin pour-on at 3 mg/kg bw. Two steers and two cows were killed at 3, 7, 14 and 21 days after treatment. After 7 days for tissues and 5 days for milk, concentrations of alphacypermethrin on average were 6, 35, 237, 407 and 32 µg/kg of the kidney, muscle, omental fat, back fat and milk of the total residues respectively. This accounted for 12, 90, 92, 76 and 91% of the kidney, muscle, omental fat, back fat and milk of the total radioactive residues respectively. The identities and concentrations of residues in tissues were determined using LSC, HPLC-Radio/UV, LC-MS, GC-MS and GC-ECD methods. The *in vitro* metabolism of alphacypermethrin has been compared with that of cypermethrin and both substances were metabolised by oxidative and esteratic routes. The respective cyclopropane carboxcyclic acid derivative was the major metabolite of both substances together with several metabolites derived from the phenoxybenzyl alcoholic moiety of the compound. More than 95% of the residue in fat was unmetabolised alphacypermethrin. Experiments have shown that the distribution and excretion of cypermethrin and alpahcypermethrin were very similar.

23. When goats were treated topically with a commercial pour-on formulation at a dose rate of 4 mg/kg bw cypermethrin, residues in all samples of muscle, kidney and liver were below the limit of quantification (10 µg/kg) of the GLC analytical method. Mean residues in kidney fat were 70 µg/kg at 7 days, 140 µg/kg at 14 days and 10 µg/kg at 42 days after treatment. In another study, lactating goats were treated with the same pour-on formulation at a dose rate of 4 mg/kg bw. Mean residues of cypermethrin in milk, determined by GLC, were 20 µg/kg at 24 hours after treatment, 25 µg/kg at 32 hours after treatment and below 10 µg/kg at 96 hours after treatment.

24. Ruminant species such as bovine, ovine and caprine share a similar gastro-intestinal physiology. The available pharmacokinetic and residues depletion data do not indicate any significant variability between cattle, sheep and goats, therefore, it was considered that other ruminants were unlikely to show any significant differences in these parameters. The existing tissue MRLs for bovine and ovine species are identical and so it was considered appropriate to recommend the extension of the MRLs so that that the same tissue MRL values would apply to all ruminants, the MRL for bovine milk was also recommended to be extended to all ruminants.

25. An analytical method based on GC-ECD to determine cypermethrin in bovine and ovine tissues has been presented in the ISO 78/2 format. The limits of quantification of the method were 10 µg/kg in bovine and ovine muscle, liver and kidney and 100 µg/kg in bovine and ovine fat. The method was validated in accordance with Volume 8 of the Rules Governing Medicinal Products in the European Union. Information on the specificity in the presence of other pyrethroids was presented and considered adequate. This method should be applicable to other ruminant species and therefore from this aspect extrapolation to the tissues and milk of other ruminants is possible.

Conclusions and recommendation

Having considered that:

- an ADI of 15 µg/kg bw (i.e. 900 µg/person) was previously established for cypermethrin,
- MRLs were established in Council Directive 98/82/EC for cypermethrin for products of animal origin,
- MRLs were previously established in bovine and ovine tissues; these MRLs are identical,
- an MRL in bovine milk was established,
- an analytical method for the monitoring of residues in tissues and milk of all ruminant species was available;

the Committee for Medicinal Products for Veterinary Use recommends the modification of the current entry for cypermethrin for bovine and ovine species 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
Cypermethrin	Cypermethrin (sum of isomers)	All ruminants	20 µg/kg 200 µg/kg 20 µg/kg 20 µg/kg	Muscle Fat Liver Kidney	
			20 µg/kg	Milk	Further provisions in Council Directive 98/82/EC are to be observed.

Based on these MRLs, the daily intake of total residues was estimated to be 263 µg/day, representing about 30% of the ADI.