The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CYPERMETHRIN

SUMMARY REPORT (2)

1. Cypermethrin is a synthetic pyrethroid insecticide which is applied topically for the control of ectoparasites such as ticks, fleas, lice and blowflies. It consists of a mixture of 4 *cis*- and 4 *trans*-isomers. The ratio of *cis*- : *trans*- isomers in commercial products depends on the manufacturing source. In Member States cypermethrin is authorised for administration to cattle, sheep, goats, pigs and chickens, including laying birds and lactating cattle, sheep and goats.

   Currently, cypermethrin is included in Annex III 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, ovine, caprine	20 µg/kg 200 µg/kg 20 µg/kg 20 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 01.01.2002
		Bovine, ovine, caprine	20 µg/kg	Milk	Provisional MRLs expire on 01.01.2002 Further provisions in Council Directive 93/57/EC are to be observed
		Porcine	20 µg/kg 200 µg/kg 20 µg/kg 20 µg/kg	Muscle Skin+fat Liver Kidney	Provisional MRLs expire on 01.01.2002
		Chicken	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Skin+fat Liver Kidney Eggs	
		Salmonidae	50 µg/kg	Muscle and skin in natural proportions	

   Additional data were provided in response to the list of questions, further to the recommendation of provisional MRLs for cypermethrin, regarding ovine species only.

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2. 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.

3. 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.

4. 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.

5. 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.

6. 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.

7. 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 dosing. 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.

8. 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/sex/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.

9. 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.

10. *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.

11. 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.

12. 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 dosing 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.

13. 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.

14. In a new 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 dosing 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 dosing. 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.

15. In another new study, 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 dosing. The identities and concentrations of residues in tissues were determined using LSC, HPLC-Radio/UV, LC-MS, GC-MS and EC-ECD. 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 dosing.

16. Sheep were plunge-dipped in a commercial dip preparation diluted to a nominal concentration of 150 mg/litre of cypermethrin. 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. 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 were 427 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 dosing, 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 dosing and declined to 20 µg/kg at 28 days after dosing. Residues of cypermethrin were measured in ewes' milk after plunge dipping in a preparation diluted to a nominal concentration of 150 mg/litre cypermethrin. Mean values of 13, 10, 9, 7 and 7 µg/kg cypermethrin were found 1, 3, 7, 10 and 15 days after treatment respectively.

17. 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 dosing. Residues of cypermethrin were determined using GLC. 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 dosing to 40 µg/kg at 14 days after dosing. 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 dosing; mean residues in these tissues were 140 and 330 µg/kg 14 days after dosing. 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 dosing, 48 µg/kg at 48 hours after dosing 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.

The residue data available regarding bovine species (edible tissues and milk) were insufficient to establish the ratio of marker residue to total residues.

14. 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 dosing. 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 dosing, 25 µg/kg at 32 hours after dosing and below 10 µg/kg at 96 hours after dosing.

15. Pigs were treated topically with 100 mg cypermethrin/kg bw. The treatment was repeated 3 times at 72-hour intervals. The pigs were slaughtered (2 per time-point) 1, 3, 7 or 14 days after the last treatment and the residue in tissues determined using GLC. Mean residues of cypermethrin in fat samples were 16 µg/kg and 33 µg/kg at 3 and 7 days after dosing. Residues in most other tissues were below the limit of quantification of the assay (10 µg/kg).

    The residue data available regarding porcine species were insufficient to establish the ratio of marker residue to total residues.

16. Groups of laying hens were sprayed with 0.05% or 0.1% solutions of cypermerthrin (corresponding to 10 or 20 mg cypermethrin per bird). Residues of cypermethrin in tissues and in eggs were determined using GLC. In the group treated at the lower rate, residues of cypermethrin in muscle depleted from 20 µg/kg at 1 to 2 days after dosing to 10 µg/kg at 4 days after dosing. Residues in all samples of liver and kidney were below 10 µg/kg (the limit of quantification of the assay). Residues in abdominal fat were 80 µg/kg at 1 day after dosing and depleted to 40 µg/kg at 8 days after dosing. Residues of cypermethrin in samples of skin+fat were 400 µg/kg at 1 day after dosing and depleted to 100 µg/kg at 8 days after dosing. In the group treated at the higher rate, residues in liver, kidney and muscle were again very low. Residues in abdominal fat were 250 µg/kg at 1 day after dosing and depleted to 50 µg/kg at 8 days after dosing. Residues of cypermethrin in samples of skin+fat were 1300 µg/kg at 1 day after dosing and depleted to 160 µg/kg at 8 days after dosing. Residues of cypermethrin in all samples of whole eggs were below 10 µg/kg.

    The residue data available regarding chicken (edible tissues and eggs) were insufficient to establish the ratio of marker residue to total residues.

    An analytical method based on GC-ECD to determine cypermethrin in ovine tissues has been presented in the ISO 78/2 format. The limits of quantification of the method were 10 µg/kg in ovine edible tissues. This method had not been validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community as the limits of detection were not determined for ovine liver and muscle and specificity (interference from other synthetic pyrethroids was not tested) were inadequate.

    The analytical methods available for bovine (tissues and milk), porcine, caprine (tissues and milk), chicken (tissues and eggs) and ovine milk were not validated.

Conclusions and recommendation

Having considered that:

- an ADI of 15 µg/kg bw (900 µg/person) was established for cypermethrin,
- MRLs were set in Council Directive 93/57/EEC for cypermethrin for products of animal origin;
- cypermethrin (sum of isomers) was identified as the marker residue and represents 4, 1, 22, and 86% of the total residue in ovine liver, kidney, muscle and fat respectively;
- an analytical method for determination of residues of cypermethrin in ovine tissues was available but specificity was not adequately demonstrated and the limit of detection was not determined for ovine liver and muscle;
- the Applicant has committed to address the outstanding issues;

the Committee recommends, in accordance with Article 4 of Council Regulation (EEC) No 2377/90 as amended, a 18 months extension of the provisional MRLs for cypermethrin in ovine species, in accordance with the following table:.

Pharmacologically active substance(s)	Marker residue	Animal Species	MRLs	Target tissues	Other provisions
Cypermethrin	Cypermethrin (sum of isomers)	Ovine	20 µg/kg 200 µg/kg 20 µg/kg 20 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 01.07.2003 Not for use in animals from which milk are produced for human consumption
		Bovine	20 µg/kg 200 µg/kg 20 µg/kg 20 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 01.07.2003
		Bovine	20 µg/kg	Milk	Provisional MRLs expire on 01.07.2003 Further provisions in Council Directive 93/57/EC are to be observed

Based on these MRLs, the daily intake of total residues was estimated to be 226 µg/day, representing about 25% of the ADI.

Based on a global diet and Codex MRLs, the World Health Organisation had calculated the Theoretical Maximum Daily Intake (TMDI) arising from the use of cypermethrin as a pesticide was 280 µg/day. It was concluded that the ADI would accommodate both the veterinary and the pesticide uses of cypermethrin.

Before the Committee for Veterinary Medicinal Products can consider the inclusion of cypermethrin in ovine in Annex I of Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The Applicant should provide a routine analytical method, validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Union and described in an internationally recognised format (ISO 78/2). In particular, the limits of detection should be determined for ovine liver and muscle and specificity (potential for interference from other synthetic pyrethroids) should be adequately demonstrated. Full validation data should be provided for all edible tissue and milk of bovines.
2. The Applicant should provide data to clarify the ratio of marker residue to total residues for all edible bovine tissues and bovine milk