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 (1)

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.

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.

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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. The data on the nature of the residues in tissues of the target species were very sparce. However, it appeared from the information provided that residues in fat consisted chiefly of unmetabolised cypermethrin. In contrast, unmetabolised cypermethrin comprised only a small percentage of the residues in kidney and liver. The percentage of cypermethrin in the liver and kidney residues was greater following dermal than oral administration, reflecting the reduced metabolism via this route. There was no information concerning the composition of the residues in muscle or eggs. Residues in milk consisted almost entirely of cypermethrin.

15. 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.

16. 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.

17. 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.

18. 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).

19. 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.

20. The analytical methods for the determination of residues of cypermethrin in meat, milk and eggs are available and based on GLC with electron-capture detection or on GC-MS. Under standard GLC operating conditions, the 8 isomers of cypermethrin were not resolved, a single fused peak was obtained. Resolution was possible employing capillary gas chromatography and a run time of over one hour - but this was not appropriate for routine purposes due to the low sample through-put. There is no information on the possible interference from residues of substances such as other pyrethroids. The limits of quantification appeared to be 10 µg/kg for tissues and for eggs and 5 µg/kg for milk but the method was not properly validated in accordance with Volume VI of the Rules Governing Medicinal products in the European Community.

Conclusions and recommendation

Having considered that:

- an ADI of 15 µg/kg bw (900 µg/person) was adopted for cypermethrin,
- MRLs had already been set in Council Directive 93/57/EEC for cypermethrin for products of animal origin; the MRLs for liver, kidney and muscle in this Directive were based on the fat content of these tissues,
- there was a need to establish the same MRLs for cypermethrin and for alphacypermethrin because residues arising from the use of these substances could not be distinguished in the residues surveillance programmes of Member States,.
- the metabolism and radiometric studies in the target species were limited and therefore a very conservative estimate of the marker as a percentage of total residues was proposed: muscle 30%, liver 10%, kidney 5%, fat 60%, milk 80% and eggs 30%,
- the analytical methods for determination of residues in meat, milk and eggs were not fully validated;

the Committee recommends the inclusion of cypermethrin 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 1.1.2002
		Bovine, ovine, caprine	20 µg/kg	Milk	Further provisions in Council Directive 94/29/EC are to be observed. Provisional MRLs expire on 1.1.2002
		Porcine	20 µg/kg 200 µg/kg 20 µg/kg 20 µg/kg	Muscle Skin+fat Liver Kidney	Provisional MRLs expire on 1.1.2002
		Chickens	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Skin+fat Liver Kidney Eggs	

Based on these MRLs, the theoretical maximum daily intake of total residues was calculated to be 172 µg/day.

Based on a global diet and Codex MRLs, the World Health Organisation had calculated the Theto be was 280 µg/day. It was concluded that the ADI calculated above would accommodate both the veterinary and pesticide uses of cypermethrin.

LIST OF QUESTIONS

1. The Applicant should provide data to clarify the ratio of the marker residue to total residues for all edible tissues, milk and eggs of the target species. It should be clarified whether any inter-conversion of isomers occurs during metabolism in the target species.

2. The Applicant should provide routine analytical methods for the determination of residues of cypermethrin in milk, eggs and tissues of the target species validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community. The method should be described in an internationally recognized format (e.g. ISO 78/2).