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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ALPHACYPERMETHRIN

SUMMARY REPORT (1)

1. Alphacypermethrin consists of 2 *cis*- isomers from the 8 isomers present in cypermethrin. These 2 isomers comprise the most biologically-active enantiomer pair. Alphacypermethrin is used as an insecticide on a range of growing crops. In veterinary medicine it is applied topically, as a spray or pour-on to cattle and sheep (150 mg alphacypermethrin/animal), and as a spray to poultry (8 to 10 mg per bird), for the control of ectoparasites such as ticks, fleas, lice and blowflies.

2. Alphacypermethrin is a type II synthetic pyrethroid and causes a long-lasting prolongation of the normally transient increase in sodium permeability of the nerve membrane during excitation, resulting in long-lasting trains of repetitive firing. The presence of the α-cyano group on the phenoxybenzyl alcohol moiety is considered to be responsible for the long-lasting prolongation of sodium permeability. The type II pyrethroids produce a distinct poisoning syndrome which is characterised by choreoathetosis and salivation.

3. The pharmacokinetics of alphacypermethrin was compared with that of cypermethrin in rats and in human volunteers. After oral administration of doses in the range 0.25 to 0.75 mg alphacypermethrin to humans, 43% of the administered dose was excreted in the urine within 24 hours, mostly as the free and conjugated cyclopropanecarboxylic acid metabolite. A similar pattern of excretion was observed following repeated dosing. After oral administration of 2 mg alphacypermethrin/kg bw to rats, around 50% of the dose was recovered from the urine and 40% from the faeces within 96 hours of dosing. 75% of the material in faeces was unmetabolised alphacypermethrin; the urine consisted of a mixture of metabolites. Residues in all tissues were very low with the exception of fat in which residues of 0.42 and 0.22 µg alphacypermethrin equivalents/kg were found in males and females respectively, 96 hours after treatment. More than 95% of the residue in fat was unmetabolised alphacypermethrin. There was no evidence for any racemisation of the chiral centres of alphacypermethrin from residues in faeces, the intestine or the fat. In these experiments, the distribution and excretion of cypermethrin and alphacypermethrin were very similar.

4. The *in vitro* metabolism of alphacypermethrin was compared with that of cypermethrin using liver microsomal preparations from rats, rabbits and humans. Both substances were metabolised by oxidative and esteratic routes. The respective cyclopropane carboxylic acid derivative was the major metabolite of both substances, together with several metabolites derived from the phenoxybenzyl alcoholic moiety of the compound.

5. The acute toxicity of alphacypermethrin was variable and depended on factors such as the solvent vehicle and the concentration of the substance in the vehicle. The acute oral LD_{50} was 35 mg/kg bw in mice when administered as a 5% solution in corn oil but was 798 mg/kg bw when administered as a 50% aqueous suspension. In Wistar rats, the acute oral LD_{50} was in the range 40 to 80 mg/kg bw when administered as a 10% solution in corn oil but was greater than 5000 mg/kg bw when administered as a 50% aqueous suspension. The signs of toxicity included ataxia, salivation, choreoathetosis, abnormal gait, tremor and clonic convulsions.

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6. Repeated dose toxicity studies were carried out in rats in which alphacypermethrin was administered in the diet at concentrations equivalent to 0, 1.25, 5, 10, 20 or 40 mg/kg bw/day for 5 weeks, 0, 2.5, 10, 40 or 60 mg/kg bw/day for 6 weeks or 0, 1, 3, 9 or 27 mg/kg bw/day for 13 weeks. Doses of 40 mg/kg bw/day and above caused severe toxicity with cachexia, high-stepping and splayed gait, hunched posture, thin appearance, hypersensitivity to stimuli, reduced bodyweight gain and food consumption. Tests for neurotoxicity including assessments of grip strength and motor activity and a detailed functional observational battery were made during weeks 2 and 6 of the 6-week study. Changes in gait were observed at 40 mg/kg bw and above but there were no significant changes in mean fore- or hind-limb gripstrength or mean hindlimb landing footsplay. A NOEL of 10 mg/kg bw/day was established in this study. However, a lower NOEL of 3 mg/kg bw/day was established in the 13-week study, based on reduced bodyweight gain in males given the next dose.

7. Repeated dose toxicity studies were carried out in Beagle dogs in which alphacypermethrin was administered in the diet at concentrations equivalent to 0, 0.75, 2.25 or 6.75 mg/kg bw/day for 13 weeks or 0, 1.5, 3 or 6 mg/kg bw/day for 52 weeks. Doses of 6.75 mg/kg bw caused signs of toxicity such as tremors, ataxia, agitation and subdued behaviour. Reddening of the skin and hair loss and associated irritation were observed at 3 and 6 mg/kg bw. In these studies there were no substance-related effects on bodyweight, haematology, clinical chemistry or urinalysis parameters and no gross- or histopathological findings. A NOEL of 1.5 mg/kg bw/day was established in the 52-week study.

8. Repeated dose toxicity studies were carried out in which CD-1 mice were given alphacypermethrin in the feed at concentrations equivalent to 0, 27.4/33.6, 55.9/73.1, 121.4/145.6, 165.9/211.5 or 241.3/294.1 mg/kg bw/day for 4 weeks or 0, 6.3/7.4, 33.2/36.3 or 169.8/184.8 mg/kg bw/day for 13 weeks, for males/females respectively. Signs of toxicity observed in the groups given 33 mg/kg bw and included thin build, ungroomed coat and hair loss. There was evidence of anaemia at 170 mg/kg bw and above with reduced haemoglobin and erythrocyte counts. Increases in aspartate aminotransferase activity and changes in some other clinical chemistry values at 33 mg/kg bw and above did not correlate with any histopathological changes. No NOEL was established in the 13-week study due to 2 males with hair loss in the 6.3 mg/kg bw/day group and the slightly reduced bodyweight gain in this group.

9. No multigeneration study with alphacypermethrin was provided. Groups of Wistar rats were fed diets equivalent to 0, 0.5, 5 or 25 mg/kg bw/day cypermethrin for 5 weeks prior to mating and then throughout gestation and lactation for three successive generations. Parental bodyweight gain and food consumption were reduced at 25 mg/kg bw. Litter size and total litter weight were reduced in the F1a litters of the 25 mg/kg bw group. The NOEL was 5 mg/kg bw/day.

10. Groups of female Sprague-Dawley rats were given daily oral doses of 0 (corn oil), 3, 9 or 18 mg/kg bw/day of alphacypermethrin from days 6 to 15 of gestation. On day 10 of gestation, the top dose was reduced to 15 mg/kg bw due to maternal toxicity and an additional group of rats was administered 15 mg/kg bw/day from days 6-15 of gestation. Overt signs of toxicity together with reduced maternal bodyweight gain and food consumption were observed at 15 mg/kg bw and above and foetal weights were significantly reduced in these groups. There was no evidence of teratogenicity at any dose level. The NOEL for both maternal toxicity and foetotoxicity was 9 mg/kg bw/day.

11. Groups of New Zealand White rabbits were given daily oral doses of 0 (corn oil), 3, 15 or 30 mg/kg bw/day of alphacypermethrin from days 7-19 of gestation. Maternal bodyweight gain and food consumption were reduced in the 30 mg/kg bw group. There was no evidence of teratogenicity or foetotoxicity at any dose level. The NOEL for maternal toxicity was 15 mg/kg bw/day.

12. A battery of mutagenicity assays with alphacypermethrin investigating all the appropriate end-points gave negative results. The studies included *in vitro* assays for gene mutation in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538, *E. coli* WP2 and WP2uvrA and *Saccharomyces cerevisiae* XV 185-14C, an *in vitro* assay for mitotic gene conversion in *Saccharomyces cerevisiae* JD1, an *in vitro* assay for gene mutation at the TK locus of L5178Y mouse lymphoma cells, an *in vitro* cytogenetics assays in rat liver (RL4) cells and in cultured human peripheral lymphocytes, an *in vivo* alkaline elution assay for DNA single strand damage in rat liver (following a single oral dose of 40 mg/kg bw) and an *in vivo* cytogenetics assay in rat bone marrow (following single oral doses of 2, 4 or 8 mg/kg bw in corn oil). Negative results were also obtained in an *in vivo* micronucleus test in which mice were given single oral doses of 1, 5 or 10 mg/kg bw alphacypermethrin in corn oil and the bone marrow harvested 24, 48 or 72 hours later. Although some published studies with cypermethrin had claimed positive results, it was considered that these studies were inadequately conducted and reported and that no reliance could be placed on the results. It was agreed that alphacypermethrin was not genotoxic.

13. A carcinogenicity study was carried out in which groups of 52/sex/dose CD-1 mice were fed diets containing 0, 30, 100 or 300 mg alphacypermethrin per kg feed for 78 weeks. Further groups of 20/sex/dose mice were subjected to interim necropsy after 52 weeks. Only a 52-week interim report of the study was available. Over the first 52 weeks of the study, the achieved substance intakes were 0, 3.1/3.8, 11.1/12.4 and 36.8/40.7 mg/kg bw/day in males/females respectively. Signs of toxicity (thin build, hunched posture, ungroomed coat, skin encrustations and hair loss in males and overactivity in females) were observed at 300 mg/kg feed. Bodyweight gain was reduced in males given 100 and 300 mg/kg feed and in females given 300 mg/kg feed. There were no effects on haematology values measured at 50 weeks and no gross or histopathological findings attributable to treatment. The NOEL was 3.1 mg/kg bw/day in males and 3.8 mg/kg bw/day in females. Carcinogenicity studies with cypermethrin in rats and mice had given no indication of carcinogenic potential. It was concluded that alphacypermethrin was not carcinogenic.

14. Fischer 344 rats were given oral doses of 0, 4, 8 or 12 mg alphacypermethrin/kg bw/day for 28 days, in a published study investigating potential immunotoxicity. The test substance was administered in soybean oil vehicle. Six rats from each group were immunised with 10% sheep red blood cells in phosphate buffered saline. A range of immunological, haematology and pathological tests were carried out. There was no effect on the immune system at any dose level.

15. Alphacypermethrin was not classifiable as a skin sensitiser when tested in Dunkin-Hartley guinea pigs using the method of Magnusseon & Kligman. In this test, challenge was carried out with 50% alphacypermethrin in corn oil.

16. The time-course for development and recovery from pyrethroid-induced nerve lesions was investigated by measuring β-glucuronidase and β-galactosidase activities in the sciatic/posterior tibial nerve, trigeminal nerve and trigeminal ganglion of Wistar rats given daily oral doses of 0 or 37.5 mg/kg bw/day of alphacypermethrin, 5 days per week for 4 weeks. The test substance was administered in dimethyl sulfoxide for the first 10 doses and in arachis oil for the remaining doses. 21% of the rats given alphacypermethrin died; the signs of toxicity included ataxia, abnormal gait, lethargy, salivation and hypersensitivity. The β-glucuronidase and β-galactosidase activities in the sciatic/posterior tibial nerve were increased at 5, 6 and 8 weeks in treated rats compared with the controls; maximal increase was at 5 weeks. No significant changes were found in the trigeminal nerve and trigeminal ganglion. The experiment was repeated with groups of rats given daily oral doses of 0, 10, 20 and 40 mg/kg bw/day, 5 days per week for 4 weeks. The NOEL was 10 mg/kg bw/day.

17. In a study to investigate the acute neurotoxic potential of alphacypermethrin, groups of Crl:CD rats were given a single oral dose of 0, 4, 20 or 40 mg alphacypermethrin/kg bw in an oily vehicle. A detailed clinical assessment for neurotoxic effects was carried out prior to treatment and on days 1, 7 and 14; this included a functional observational battery and measurements of fore- and hind limb grip strength, hind limb landing foot splay and motor activity. The rats were killed on day 15 and certain tissues including brain, nerves, spinal cord and ganglia were examined histopathologically. In the functional observational battery, clinical signs were observed 5 hours after dosing at 20 and 40 mg/kg bw but there were no effects on grip strength, hind limb landing foot splay or motor activity. The NOEL was 4 mg/kg bw. Another study was carried out using the same dosing regimen to confirm histological effects on the sciatic nerve since this was the only histopathological finding which showed any relation to treatment. Evidence of slight sporadic sciatic nerve degeneration was observed at 20 mg/kg bw. The NOEL was 4 mg/kg bw.

18. No studies regarding antimicrobial activity were provided. It was agreed that such studies were not necessary for the pyrethroids.

19. Alphacypermethrin has been used in many countries as a crop spray since 1983. In common with the other pyrethroids, the most frequently reported symptom of human occupational exposure was reported to be skin paraesthesia. This has been attributed to the repetitive firing of sensory nerve endings and is generally reversible within a few hours.

20. An ADI of 15 µg/kg bw (900 µg/person) was calculated by applying a safety factor of 100 to the NOEL of 1.5 mg/kg bw/day which was established in the one-year study in dogs. It was noted that the Joint WHO/FAO Expert Committee on Food Additives (JECFA) had established an ADI on the same basis, but had rounded the ADI to 20 µg/kg bw (1200 µg/person).

21. Total alphacypermethrin-derived residues were determined in milk and meat from 4 cows treated with 10 ml of a 1.5% pour-on formulation of ^{14}C-alphacypermethrin and killed 7, 14, 28 or 35 days after treatment. Mean total residues of radioactivity in milk rose to 7 ($\pm$5) µg equivalents/kg, 2 days after treatment and declined to the limit of quantification (1 µg/kg), 7 days after treatment. Residues of alphacypermethrin in milk, determined by GLC, were 5 µg/kg at 2 days after treatment. The total residues of radioactivity in liver, kidney, muscle, and fat were mostly below the limits of quantification (10 to 30 µg/kg). Only in the subcutaneous fat of one cow slaughtered 35 days after treatment was a residue measured at the limit of quantification of 30 µg equivalents/kg. Perirenal and fat samples from the cows killed 7 and 14 days after treatment were also analysed for alphacypermethrin using GLC and that the concentrations found were not significantly different from those resulting from scintillation counting.

22. In another study, a lactating cow was given oral doses of 125 mg ^{14}C-alphacypermethrin, twice daily, for 4 days. Another cow was given unlabelled alphacypermethrin according to the same dosage regimen. The cows were killed 6 hours after the last dose. Over this time period, 34% of the administered dose was excreted *via* the faeces and 23% *via* the urine. Less than 1% of the dose was secreted in milk. Highest residues were found in liver (561 µg equivalents/kg) followed by renal fat (476 µg equivalents/kg), omental fat (426 µg equivalents/kg) and subcutaneous fat (390 µg equivalents/kg). Kidney contained 217 µg equivalents/kg and residues were below 30 µg equivalents/kg in muscle. At least 8 radioactive components were found in liver and 9 in kidney. The major component in both tissues (16% and 20% the radioactivity in liver and kidney, respectively) appeared to be unmetabolised alphacypermethrin. Four radioactive components were found in muscle and 2 in fat and in milk. However, a single component which chromatographed with unmetabolised alphacypermethrin predominated: 85% in muscle 85%: 91% in fat; and 97% in milk.

23. There was no information concerning the ratio of marker to total residues in sheep or poultry.

24. Residues of alphacypermethrin were measured in calves after topical application of a 1.5% w/v pour-on formulation at the rate of 10 ml/animal (equivalent to approximately 1.2 mg/kg bw), using GLC with confirmation by MS. Residues in all samples of muscle and liver taken 3, 7 and 14 days after treatment were below the limit of quantification (10 µg/kg). Residues in kidney were less than 10 to 20 µg/kg on day 3, less than 10 to 30 µg/kg on day 7 and less than or equal to 10 µg/kg on day 14. In subcutaneous fat, residues were 30 to 150 µg/kg on day 3; 20 to 140 µg/kg on day 7 and declined to 10 to 20 µg/kg at 14 days after treatment. The corresponding values for perirenal fat were 140 to 300 µg/kg, 190 to 270 µg/kg and 60 to 150 µg/kg, respectively. The persistence of residues in fat was confirmed in a second study; (also using a dose of 10 ml/animal i.e. 150 mg/animal); residues in subcutaneous and perirenal fat declined to less than 10 to 30 µg/kg and less than 10 to 40 µg/kg respectively, 28 days after treatment.

25. Residues of alphacypermethrin in bovine milk after topical application of various pour-on formulations at rates of 0.1 to 0.2 g active ingredient per cow reached peak values of around 4-5 µg/l, 2-7 days after treatment. The doses were in the range 0.1 to 0.3 mg/kg bw, depending on the weight of the cow.

26. Sheep plunge-dipped in 80 mg/litre alphacypermethrin for one minute had higher residues in the fat, wool and skin than those treated with the same formulation as a pour-on at a rate of 100 mg/sheep. Only one sheep was slaughtered at each time point with each treatment. Residues in subcutaneous fat were not detectable (less than 10 µg/kg) in the sheep slaughtered 7 days after treatment with the pour-on but residues of 40 µg/kg alphacypermethrin were found in the fat of the sheep slaughtered 7 and 14 days after the dip treatment. High residues were found in skin (up to 1400 µg/kg) and wool (greater than 1000 µg/kg) indicating that the majority of the dose remained at the site of application.

    In a second study in which groups of 5 sheep were killed 7 days after treatment with 2 different 1.25% alphacypermethrin pour-on formulations at rates of 1 ml or 2 ml/5 kg bw, equivalent to 2.3 or 4.6 mg/kg bw, residues of up to 18 µg/kg and 19 µg/kg were found in perirenal and omental fat respectively. None of the sheep studies investigated residues in edible tissues other than fat.

    However, as it was shown in *in vitro* studies that alpha-cypermethrin and cypermethrin have the same metabolic pathway the depletion data provided for cypermethrin can be considered.

    Residues in all edible tissues were determined in studies in which sheep were treated with cypermethrin. Following topical treatment with a pour-on formulation at approximately 10 mg/kg bw, residues of cypermethrin were detectable only in fat; mean residues in renal and omental fat were 40 µg/kg at 7 days after dosing and 20 µg/kg at 28 days after dosing. Residues in all tissues were detectable following plunge-dipping of sheep in a commercial dip preparation diluted to a nominal concentration of 150 mg/litre of cypermethrin. Mean residues in muscle were 176 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 3 out of 4 samples of liver were undetectable at 1 day after treatment but mean residues in liver of 260 µg/kg were found 3 days after treatment. Mean residues of 280 µg/kg were found in 2 out of 4 kidney samples taken 1 days after treatment; residues in the other 2 samples were undetectable. At 3 days after treatment, mean residues in liver were 427 µg/kg.

27. Laying hens were treated with a spray formulation at a dose corresponding to 8.6 mg alphacypermethrin per bird and eggs were collected 2 to 14 days after treatment. Residues in all samples of albumin were less than 5 µg/kg. Residues in yolk peaked at 26 $\pm$ 11 µg/kg 5 days after treatment and declined to 16 $\pm$ 12 µg/kg 14 days after treatment. There was no information concerning residues in poultry meat. However, tissue residues depletion data were available for hens sprayed with 0.05% or 0.1% solutions of cypermethrin (corresponding to 10 or 20 mg cypermethrin per bird). 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 skin+fat were 400 µg/kg at one 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.

28. The proposed routine analytical methods were based on GLC with electron capture detection followed by confirmation by GC-MS. Under standard GC operating conditions, the 2 isomers of alphacypermethrin were not resolved. Resolution was possibly 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. The methods were robust and used commercially-available materials. Limits of quantification, expressed as the sum of the isomers, of 10 µg/kg and 1 µg/kg were claimed for meat tissues and for milk respectively. However, there was no information concerning accuracy and precision at the claimed limits of quantification and no information concerning limits of detection, possible interference from residues of other pyrethroids and stability of the residues in extracts, standard solutions and in frozen tissue samples.

Conclusions and recommendation

Having considered that:

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

the Committee recommends the inclusion of alphacypermethrin 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
Alphacypermethrin	Cypermethrin (sum of isomers)	Bovine, ovine	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	20 µg/kg	Milk	Further provisions in Council Directive 94/29/EC are to be observed. 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	Provisional MRLs expire on 01.01.2002

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 Theoretical Maximum Daily Intake (TMDI) arising from the use of cypermethrin as a pesticide was 280 µg/day. Because alphacypermethrin was more biologically active than cypermethrin and therefore used at lower rates, the TMDI was considered to exaggerate the case for alphacypermethrin. It was concluded that the ADI calculated above would accommodate both the veterinary and the pesticide uses of alphacypermethrin.

LIST OF QUESTIONS

1. The metabolic profile of alphacypermethrin should be investigated in the target species following topical administration. It should be clarified whether any inter-conversion of isomers occurs during metabolism in the target species.
2. The applicant should establish the ratio of total to marker for sheep and poultry tissues and eggs.
3. The applicant should provide tissue residues depletion data for sheep and poultry in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community
4. The Applicant should provide routine analytical methods for the determination of residues of alphacypermethrin in milk, eggs and tissues of the target species validated in accordance with Volume VI. The analytical method should be described in an internationally recognized format (e.g. ISO 78/2).