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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

PERMETHRIN

SUMMARY REPORT (2)

1. Permethrin is a type I synthetic pyrethroid. It is an ester of the dichloro-analogue of chrysanthemic acid, and 3-phenoxybenzyl alcohol. Permethrin used in veterinary medicines is a mixture of four stereoisomers of the configuration ([*1R,trans*], [*1R,cis*], [*1S,trans*] and [*1S,cis*]. The optical ratio of *1R:1S* is 1:1 (racemic). Permethrin used in veterinary medicinal products is in the form of sprays (including udder sprays), powders, pour-ons or ear-tags for external application to cattle, pigs, sheep, goats and poultry, for the control of ectoparasites. The dosages used are about 4 mg/kg bw for cattle, about 6 mg/kg bw for sheep and poultry. The isomer ratios in these products are *cis:trans* 80:20, 40:60 or 25:75.

   Currently permethrin is included in Annex III of Council Regulation (EEC) No 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Permethrin	Permethrin (sum of isomers)	Bovine, caprine	100 µg/kg 500 µg/kg 50 µg/kg 50 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.1.2001
			50 µg/kg	Milk	Further provisions in Commission Directive 98/82/EC are to be observed (OJ L 290, 29.10.1998, p. 25.) Provisional MRL expires on 1.1.2001
		Chicken, porcine	100 µg/kg 500 µg/kg 50 µg/kg 50 µg/kg	Muscle Skin + fat Liver Kidney	Provisional MRLs expire on 1.1.2001
		Chicken	50 µg/kg	Eggs	

   Additional data were provided in response to the list of questions, further to the establishment of provisional MRLs for permethrin.

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2. Permethrin binds to sodium channels causing a slowing of their rate of closure resulting in repetitive firing of nerves, depolarisation and nerve block. This property underlies the insecticidal action and the mammalian toxicity of pyrethroids. The action of pyrethroids on sodium channels shows a negative temperature coefficient, favouring effects in cold blooded insects over warm-blooded mammals. The type I pyrethroids produce a distinct poisoning syndrome characterised by progressive fine whole body tremor, exaggerated startle response, uncoordinated muscle twitching and hyperexcitability. The effects are generated largely by effects in the central nervous system. The type I response is associated with kinetically distinct effects on sodium channels as compared with type II compounds. Permethrin also induces hepatic microsomal enzymes.

3. Studies in rats have shown slow and partial absorption of permethrin (*cis:trans* 25:75) administered orally in corn oil to rats ($t_{½}$ absorption 0.9 hours, oral bioavailability 61%). Peak concentrations and area-under-curve values were higher in brain and sciatic nerve than in plasma. Following a distribution phase ($t_{½}$ 4.9 hours) permethrin was eliminated with a $t_{½}$ of 12.3 hours.

   In mammals, almost all of an oral dose is excreted as metabolites in the urine and faeces within a few days. In rat, goat, cow and hen, the major routes of metabolism are similar and involve hydrolysis of the ester bond and oxidation followed by conjugation. Excretion of the *trans*-isomer is more rapid than that of the *cis*-isomer and this is related to the lower susceptibility of the *cis*-isomer to enzymatic hydrolysis of the ester linkage.

4. The acute oral toxicity of permethrin in rats, mice, rabbits and guinea-pigs is relatively low. The rat appeared to be the most sensitive species with an oral LD_{50} of 400 mg/kg bw for *cis:trans* 40:60 permethrin administered in corn oil. Permethrin was approximately 10-fold more toxic to rodents when administered in corn oil as compared to water. The studies in mice indicated that intravenous or oral *cis*-permethrin is more than 10-fold more toxic than the *trans*-isomer and 2 to 5-fold more toxic than the 40:60 *cis:trans* isomer mix used in the majority of toxicity studies. Neonatal rats are more sensitive than adults to the acute toxic effects of permethrin. This is believed to be related to differences in permethrin metabolism.

5. The overall pattern of toxicity in repeated dose studies is similar in mouse, rat, dog and guinea-pig regardless of route of administration or vehicle. Increased liver weight associated with hepatic microsomal enzyme induction and neurotoxic effects appear to be the most sensitive indicators of toxicity. From the repeated oral dose studies, with dosing regimes ranging from 5 to 2000 mg/kg bw/day, a NOEL of 5 mg/kg bw/day for an isomer ratio of *cis:trans* 40:60 would be assigned from the effects on liver weight in 2 year and 26 weeks studies in rats and a 3-month study in dogs. The toxicity of permethrin with a *cis:trans* ratio of 25:75 is lower than that of permethrin with a *cis:trans* ratio of 40:60.

   The comparative hepatotoxicity of *cis*, *trans* and *cis:trans* 40:60 permethrins was investigated in rats in a 28-day GLP-compliant dietary study. The overall NOELs for the 3 substances, based on effects on liver weights and chemistry (microsomal protein and cytochrome P450) were 60 mg/kg bw for the *cis*-isomer, >243 mg/kg bw for the *trans*-isomer, and 118 mg/kg bw for the *cis:trans* 40:60 mixture. It was also noted that pure *cis*-permethrin caused increases in hepatic cytochrome P450 about 2-fold greater than that caused by the 40:60 mixture. Based on the results of this study, it can be concluded that *cis*-permethrin is about twice as potent as *cis:trans* 40:60 permethrin and at least four times more potent than *trans*-permethrin.

6. The reproductive toxicity of permethrin has been tested in 3-generation studies in rats. No effects were found at doses up to 2500 mg/kg feed. Embryotoxicity and teratogenicity has been studied in rats, mice and rabbits using permethrin *cis:trans* 40:60 and 25:75 at doses ranging from 10 to 1800 mg/kg bw in a variety of vehicles, including corn oil. Although the study protocols do not conform with current requirements, they provide adequate assurance that permethrin is not embryotoxic or teratogenic.

7. The mutagenic activity of permethrin has been assessed in tests for mutation in a range of *Salmonella typhimurium* strains in the presence and absence of metabolic activation, in *Echerichia coli* WP2 and *Saccharomyces cerevisiae,* and in *Salmonella typhimurium* in a host-mediated assay in mouse, for chromosome loss in *Drosophila melanogaster mus*-302 and for sex-linked recessive lethal mutations in *Drosophila melanogaster*; for mutations in Chinese hamster V79 and mouse lymphoma L5178Y cells *in vitro,* for chromosomal aberrations in the mouse *in vivo* bone marrow test and in the mouse dominant lethal test and an *in vitro* test for chromosomal damage in human blood cells in cultured in the presence of inhibitors of cytokinesis and DNA excision repair. All the tests gave negative results except the latter, which is not considered relevant for the assessment of the potential human health risk. Permethrin is not considered to be mutagenic.

8. A total of 5 (3 in mice and 2 in rats) long-term chronic toxicity/carcinogenicity studies in rodents for up to 2 years were evaluated by the International Programme on Chemical Safety (IPCS). All dosing was via the diet and the isomer ratio is assumed to be *cis:trans* 40:60. The rat studies gave no indication of carcinogenic potential at up to 250 mg/kg bw/day or 2500 mg/kg feed. The mouse studies did give some indication of an increased incidence of lung tumours in permethrin-treated CD-1 female mice as compared to the concurrent controls. However the incidence of tumours was within the historical control range. Doses in the mouse studies were up to 5000 mg/kg feed. The IPCS classification of permethrin as a possible weak rodent carcinogen is accepted. The carcinogenic potential of permethrin is not a cause for concern.

9. Studies on skin sensitisation were not performed according to currently approved protocols but provide adequate assurance that permethrin does not induce skin sensitisation in the guinea-pig. Although certain synthetic pyrethroids are known to have adverse effects on the immune system, there was no evidence of this type of toxicity in a large number of long term studies in rodents treated with permethrin.

10. Information related to humans is restricted to dermal exposure. Reversible paraesthesia, probably related to local action on sensory nerves in the skin, and mild irritation have been reported to occur at the site of contact 30 minutes to 24 hours after dermal exposure.

11. Neurotoxicity has been studied in rats and hens. Structural damage to nerves is only observed following very high doses (400 mg/kg bw/day for 7 days) of permethrin. The neurotoxic effects diminish with continued exposure and are reversible within a few days.

    A GLP-compliant study was conducted to investigate the acute effects of *cis*- and *trans*-permethrin isomers on acoustic startle response in adult male rats. The study was designed to replicate conditions of an earlier study that established a NOEL for *cis:trans* 40:60 permethrin of 90 mg/kg bw, but indicated effects from *cis*-permethrin at 30 mg/kg bw, the lowest dose tested. In the new study, groups of 10-12 animals received a single oral dose of 3, 10, 30, 60 or 90 mg *cis*-permethrin/kg bw, or 100, 300, 600 or 900 mg *trans*-permethrin/kg bw. The substances were administered in corn oil. In the new study, statistically significant effects on auditory startle response were only observed at 90 mg *cis*-permethrin, although overt effects (hyperaesthesia) were observed at 60 mg/kg bw, giving an overall NOEL for the *cis*-isomer of 30 mg/kg bw. No effects on startle response or overt signs were observed at any dose of the *trans*-isomer. The combined results of the two studies indicate NOELs for acute neurotoxicity of 30 mg/kg bw for the *cis*-isomer, 90 mg/kg bw for the 40:60 *cis:trans* mixture, and >900 mg/kg bw for the *trans* mixture.

12. Permethrin has been assessed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and IPCS. The 1987 meeting of the JMPR confirmed a permanent ADI for *cis:trans* 40:60 permethrin of 0-0.05 mg/kg bw based on a NOEL of 5.0 mg/kg bw/day obtained in a 2-year rat study and applying a standard 100-fold safety factor. This is in accordance with the NOEL of 5 mg/kg bw obtained in a 26-week study in rats used by the IPCS as a safety guideline and that obtained in a 3-month study in the dog. The limiting effects were adaptive liver responses. In the 2-year rat study from which the JMPR NOEL was obtained, liver weight was increased at all dose levels, although the effect was not statistically significant at 5 mg/kg bw/day. The JMPR and IPCS recommendations were restricted to agricultural and horticultural uses of permethrin.

They are not entirely appropriate for use as a basis for establishing an ADI for residues in animal tissues resulting from veterinary medicinal use of products containing isomer ratios up to *cis:trans* 80:20, particularly since the *cis:trans* ratio in tissue residues is expected to be higher than in the medication applied to the target species. At its 2000 meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) confirmed the JMPR ADI of 0-0.05 mg/kg bw for the 25:75 to 40:60 *cis:trans* mixtures, but considered that the database available to JMPR was not adequate to establish an ADI for the 80:20 mixture.

13. An overall NOEL based on chronic studies in the rat and dog on 5 mg/kg bw can be established. These NOELs were all obtained using 40:60 *cis:trans* isomer mixture. The comparative hepatotoxicity and neurotoxicity data obtained for this mixture and the individual isomers indicate that pure *cis*-isomer is about two to three times more potent than the 40:60 mixture, and the potency of the 80:20 mixture would be expected to fall between the two. An ADI of 0.01 mg/kg bw (i.e. 0.6 mg/person) was established based on this NOEL of 5 mg/kg bw/day using a conservative safety factor of 500 to allow for the greater toxicity of the pure *cis*-isomer and the fact that the comparative toxicity studies were conducted were only conducted over a 28-day period.

14. Several studies were carried out in which cattle were dosed orally (1.25 mg) or topically (40 mg) with permethrin ^{14}C-labelled in either the acid or alcohol moiety. Highest residues were found in fat and liver. After topical application, blood permethrin concentrations were undetectable.

    Residues were highest in fat (up to 528 µg-equivalents/kg) and skin (up to 25035 µg-equivalents/kg) with significant residues remaining at the site of application. Residues in liver, kidney and muscle remote from the application site were very low (up to 7, 5, and less than 3 µg-equivalents/kg respectively). Seven and 14 days after topical treatment, more than 80% of the radio-labelled material in fat and 98% of the radio-labelled material in skin were extractable and consisted of unmetabolised permethrin. Extractability of radio-labelled material from liver depended on the position of the ^{14}C-label with around 30% extracted after labelling in the acid moiety and around 60% after labelling in the alcohol moiety. Approximately 50% of the extractable residues in liver consisted of unmetabolised permethrin. The remainder consisted chiefly of the cleavage products 3-2(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and 3-phenoxybenzyl alcohol. From this study it can be concluded that approximately 80% of the total residues in bovine fat 7 and 14 days after topical treatment was unmetabolised permethrin but that only 15-30% of the total residues in liver was unmetabolised permethrin. No conclusions can be drawn regarding the ratio of marker to total residues for muscle and kidney.

15. Peak ^{14}C-labelled residues in the range 4 to 11 µg/kg were found in cows' milk 3 to 5 milkings after treatment. The residues were concentrated in the fat phase. Of the radio-labelled material in cows' milk 70 to 90% was extractable and more than 80% of the extractable residues consisted of unmetabolised permethrin.

16. Goats were dosed orally with 0.2 to 0.3 mg/kg bw permethrin, ^{14}C-labelled in the acid or alcohol moieties. Residues were generally higher in tissues from goats given the *cis*-isomer than from goats given the *trans*-isomer. Concentrations of radioactivity in the fat of goats given the *cis*-isomer (218 to 252 µg-equivalents/kg) were 10 times higher than those found in goats given the *trans*-isomer (13 to 25 µg-equivalents/kg). All of the radioactivity in fat was extractable. Unmetabolised permethrin accounted for 38 to 59% of the radioactivity in fat from goats given the *cis*-isomer and 75 to 80% from goats given the *trans*-isomer. Concentrations in liver in goats given the *cis*-isomer (121 to 132 µg-equivalents/kg) were also higher than those found in goats given the *trans*-isomer (10 to 40 -equivalentsµg/kg). From liver 36 to 59% of the radioactivity was extractable; at least 5 components were present but were not characterised due to the small amounts present. Total residues in kidney were 30 to 50 µg/kg; there was no information concerning the extractability or identity of these residues.

    Residues were higher in milk from goats given the *cis*-isomer. Of the radioactivity in goats' milk 80 to 100% was extractable. Unmetabolised permethrin accounted for 43 to 68% of the residues in milk from goats given the *cis*-isomer but only 21 to 45% of the residues in milk from goats given the *trans*-isomer.

17. Following oral administration of ^{14}C-labelled permethrin (10 mg/kg bw/day for 3 days) to laying hens, around 50% of the residues in eggs consisted of unmetabolised permethrin. Total residues in yolk and albumin peaked at 3000 µg-equivalents/kg and 600 µg-equivalents/kg, 5 days after the first dose. *Cis*-permethrin resulted in significantly higher residues in egg yolk and fat than the *trans*-isomer. Ten days after the first dose, highest total residues were found in fat (up to 1360 µg equivalents/kg) and skin (up to 470 µg-equivalents/kg) and consisted mostly of unmetabolised permethrin. In liver, total residues of up to 270 µg-equivalents/kg were found and 96% of the radiolabelled material was extractable. No residues of permethrin were found in liver which consisted of a mixture of unidentified metabolites. Total residues of up to 340 µg equivalents/kg were found in kidney and consisted of a mixture of metabolites. From the way the results were presented, it was not possible to deduce the ratios of residues of permethrin to total residues in this study.

    In another study using topical application of 3.77 or 11.94 mg/bird permethrin ^{14}C-labelled only in the alcohol moiety, concentrations of radio-labelled material were up to 80 and 110 µg equivalents/kg, from the low and high dose treatment respectively in fat , up to 414 and 6690 µg equivalents/kg in skin and up to 49 and 121 µg equivalents/kg in egg yolk. Peak total residues in kidney, muscle and liver were 153 and 718, 30 and 46, and 40 and 178 µg equivalents/kg, respectively. The distribution of residues was similar to that observed in the oral study. However, the nature of the residues in tissues was not investigated.

18. In pigs, 1% of a topically-applied dose of 18 mg ^{14}C-labelled permethrin/pig remained at the site of application for at least 14 days after treatment and more than 95% of this was permethrin. Seven days after treatment, residues in fat were 50 µg-equivalents/kg and consisted almost entirely of permethrin. Residues in fat samples taken 14 days after treatment were undetectable (less than 12 µg-equivalents/kg). In a second study, a residue of 10 µg-equivalents/kg permethrin was found in muscle beneath the site of application, 7 days after treatment. Residues in distant muscle, liver and kidney were below the limit of quantification (1 µg/kg) 7 and 14 days after treatment. No other details were provided and no conclusions could be drawn regarding the ratios of residues of permethrin to total residues.

19. Residues depletion studies were carried out in cattle using a number of proprietary products at the recommended dose rates. Residues in tissues were very low. In many studies residues in all tissues were below the limit of detection of the analytical method. Residues in tissues resulting from the use of ear-tag formulations were detectable only in occasional samples of fat taken 1 to 91 days after treatment and were in the range 10 to 20 µg/kg. In a study using a pour-on formulation, residues in muscle and in peri-renal fat were less than 5 µg/kg in all samples; residues in liver declined from 70 to 280 µg/kg 24 hours after treatment, to less than 5 to 25 µg/kg 72 hours after treatment; over the same time period residues in kidney declined from 30 to 110 µg/kg to 5 to 15 µg/kg.

20. Residues in whole cows' milk following application of a number of permethrin-based products at the recommended dose rates were always below the limit of detection of the analytical method employed. For these assays, the limits of detection ranged from 1 to 10 µg/kg and milk samples were taken from 7 hours up to 72 hours post-treatment. Following the use of a spray formulation, residues of 79 µg/kg were found in rendered butterfat made from milk taken 7 hours after treatment; the residues declined to around 25 µg/kg in butterfat made from milk taken 46 hours after treatment.

21. Pigs were slaughtered one day after the 6th application of a mist treatment. Residues of 20 µg/kg were found in both subcutaneous and intestinal fat but residues in all other tissues were below 10 µg/kg.

22. Following treatment of hens with a spray formulation at an intended dose of 30 mg of active ingredient per bird, residues in skin showed only a small decline from 169 to 224 µg/kg 6 hours after treatment to 50 to 102 µg/kg, 21 days after treatment. The mean residues in eggs reached a maximum of 10.4 µg/kg 5 days after treatment and declined to 3.2 µg/kg 21 days after treatment. Residues in both tissues and eggs were less persistent in another study in which a spray formulation was directed to the vent area at an intended dose of 20 mg of active ingredient per bird.

23. For the pesticidal use of permethrin maximum residue limits (MRLs) have been established in the EU (Directive 93/57/EEC). These MRLs are 500 µg/kg for fat and 500 µg/kg for meat and offals, expressed in terms of the fat content, equating to 50 µg/kg for tissues with a fat content of less than 10%. MRLs of 50 µg/kg were also established for eggs and milk. In order to harmonise the MRLs for veterinary and pesticidal use, the provisional MRL for muscle previously established for veterinary use should be reduced from 100 to 50 µg/kg.

24. It was agreed that the HPLC method in which the residues of the *cis*- and *trans*-isomers were eluted separately from the GLC column and quantified using ECD was suitable as an analytical method. However, the method was not validated in accordance with Volume VI of the Rules Governing Medicinal Products in the Community. The limit of quantification for tissues appeared to be 50µg/kg (sum of isomers) in an experiment in which the isomers were not separated. The limit of quantification for egg yolk and white appeared to be 5 µg/kg. The limit of quantification for milk appeared to be 20 µg/kg.

25. It was agreed that the GC/MS method was suitable as a confirmatory method. The limit of quantification was 5 µg/kg (sum of isomers) for bovine tissues and milk. However, the method was not fully validated in accordance with Volume VI.

Conclusions and recommendation

Having considered that:

- a toxicological ADI has been set at 0.01 mg/kg bw (i.e. 0.6 mg/person),
- it was estimated that consumer intake of residues from meat, milk, eggs, fruit and vegetables could amount to 102% of the ADI. It was considered that there would be no undue risks to human health from veterinary use for the following reasons:
  - the ADI was exceeded by a relatively small amount, and consumers would be unlikely to consume all the different food commodities containing residues at the maximum residue limits at the same time,
  - only 21% of this theoretical intake would result from veterinary use, compared to the 81% from pesticidal uses.
  - the intake from the pesticidal uses represented an extreme (97.5th percentile) consumer of fruit and vegetables.
  - the ADI established by CVMP was more conservative than the JMPR ADI, and intakes from veterinary use took less than half of the 45% of the ADI normally allocated for such purposes,
- the ratios of marker to total residues in tissues of the target species require clarification,
- residues depletion data for sheep and goats, and pharmacokinetic data in goats are required,
- a physico-chemical analytical method is available but not fully validated,
- the applicant has committed to address the outstanding issues;

the Committee recommends according to Article 4 of Council Regulation No 2377/90 as amended, a 2-year extension of the provisional MRL for permethrin in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Permethrin	Permethrin (sum of isomers)	Bovine, caprine	50 µg/kg 500 µg/kg 50 µg/kg 50 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.1.2003
			50 µg/kg	Milk	Further provisions in Commission Directive 98/82/EC are to be observed (OJ L 290, 29.10.1998, p. 25.) Provisional MRL expires on 1.1.2003
		Chicken, porcine	50 µg/kg 500 µg/kg 50 µg/kg 50 µg/kg	Muscle Skin + fat Liver Kidney	Provisional MRLs expire on 1.1.2003
		Chicken	50 µg/kg	Eggs	

LIST OF OUTSTANDING ISSUES FOLLOWING THE ASSESSMENT OF THE RESPONSE TO THE LIST OF QUESTIONS

1. The Applicant should clarify the relationship between the ratios of permethrin (sum of isomers) to total residues and should clarify the percentage of extractable residues for all edible tissues of the target species.

2. There were some data on total ^{14}C-residues in goat tissues but no pharmacokinetic or residues depletion data. Data for goats should be provided taking into account the CVMP Note for Guidance on the Establishment of Maximum Residue Limits for Minor Species (Doc. EMEA/CVMP/153a/97-FINAL).

3. Information concerning residues depletion in goat and sheep milk would be required to support Annex I entries for these commodities taking into account the CVMP Note for Guidance on the Establishment of Maximum Residue Limits for Minor Species (EMEA/CVMP/153a/97-FINAL).

5. The routine analytical method based on HPLC and involving separation of the *cis*- and *trans*-isomers should be fully validated in accordance with Volume VI of the Rules Governing Medicinal Products in the Community and submitted in an internationally recognised format (e.g. ISO 78/2). In particular, the following should be provided:

   - limits of quantification for the *cis*-isomers and the *trans*-isomers (separately) should be determined in accordance with Volume VI for all edible tissues, milk and eggs and taking into account the CVMP Position Paper on Requirements for LOQ/MRL ratio (EMEA/CVMP/274/96-FINAL);
   - the limit of detection should be properly determined for all edible tissues, milk and eggs;
   - information should be provided concerning possible interference from residues of other pyrethroids;
   - the stability of permethrin in extracts, standard solutions and as residues in stored edible tissues, milk and eggs should be documented.

6. The confirmatory method based on GC/MS should also be fully validated in accordance with Volume VI for all edible tissues, milk and eggs and should be re-presented in an internationally recognised format (e.g. ISO 78/2). In particular the following should be provided:

   - justification for using only one molecular ion;
   - the method should be improved to get better recovery of the internal standard from bovine liver samples and better recovery of permethrin from bovine fat samples
   - the limit of detection should be determined;
   - information concerning possible interference from residues of other pyrethroids should be provided;
   - the stability of permethrin in extracts, standard solutions and as residues in stored edible tissues, milk and eggs should be documented.