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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

PERMETHRIN

SUMMARY REPORT (3)

1. Permethrin (CAS No 52645-53-1) is a type I synthetic pyrethroid. It is an ester of the dichloro-analogue of chrysanthemic acid, and 3-phenoxybenzyl alcohol. The permethrin used in veterinary medicines is a mixture of four stereoisomers of the configuration [*1R,3S*], [*1R,3R*], [*1S,3R*] and [*1S,3S*]. Permethrin (*cis:trans* 80:20, 40:60 or 25:75) is used in veterinary medicine in cattle; a dose of about 4 mg/kg bw is applied in the form of sprays (including udder sprays), powders, pour-ons or ear-tags for external application for the control of ectoparasites. Permethrin has also been used in sheep, goats, horses, pigs and poultry. In pigs and poultry the doses were reported to be about 6 mg/kg bw.

   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	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	

   Additional data on residues were provided for bovine species only 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 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 4.9 hours) permethrin was eliminated with a $t_{1/2}$ 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. At its year 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 only conducted over a 28-day period.

14. 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 µgequivalents/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.

    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.

15. 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.

    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.

16. 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"-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.

    A new radiometric residue depletion study was provided in which cattle were dermally dosed with 20 ml of a solution containing 800 mg ^{14}C-permethrin. Two cattle (1/sex) were killed on days 7 14 and 21 after dosing. In a cow a C_{max} of 0.181 ng-equivalents/g was attained after a t_{max} of 92 hours. The area-under-curve ($AUC_{0-\infty}$) was 63 µg.h/l and the $t_{½}$ was 202 hours. Seven days after dosing 23% of the administered dose was recovered (5% in urine, 14% in faeces and 4% in metabolism cage washing). Tissue samples were assessed for total residue and *cis* and *trans*-isomer concentrations by the proposed routine analytical method and radio-TLC respectively. The average residue concentrations in liver were 650, 454 and 237 µg equivalents/kg on days 7, 14 and 21 after dosing. At the same time points the average residue concentrations were 263, 159 and 69 µg equivalents/kg in kidney; 22, 13 and 13 µg equivalents/kg in muscle; and 1537, 1127 and 735 µg equivalents/kg in fat. The marker residue concentrations, as a percentage of the total residue, in liver were 3%, undetectable and undetectable on days 7, 14 and 21 after dosing. At the same time points the average marker residue concentrations, as a percentage of the total residue, were 15, 5 and 13% in kidney; 66%, undetectable and undetectable in muscle; and 89, 47 and 55% in fat.

    Non-radiometric 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.

17. Peak ^{14}C-labelled residues in the range 4 to 11 µg equivalents/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.

18. 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.

19. Goats were dosed orally with 0.2 to 0.3 mg/kg bw permethrin, 14C-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 µg/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 50µg/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.

20. For the pesticidal use, maximum residue limits (MRLs) for permethrin in animal tissues have been established (Commission Directive 98/82/EC). These MRLs are 500 µg/kg for fat and 500 µg/kg for meat and offal, 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. Commission Decision 2000/817/EC withdrew the authorisations for all uses of plant-protection products containing permethrin except those for young plants for forestry which are to be withdrawn by 25 July 2003, on the grounds that the information submitted was insufficient to satisfy the requirements of Directive 91/414/EEC, Articles 5(1)(a) and (b) with regard to influence on the environment and Article 5(2)(b) with regard to operator exposure.

21. A routine analytical method based on capillary gas chromatography with electron capture detection was presented, in the ISO 78/2 format, for determining residues of permethrin in edible tissues and milk from cattle. The analytical method detected permethrin in extracts from bovine tissues and milk as a single chromatographic peak consisting of all isomers of permethrin. The proposed routine analytical method had been validated for bovine species in accordance with CVMP Guidelines and Volume VI of the Rules Governing Medicinal Products in the European Community. The limit of quantification for all edible bovine tissues was 25 µg/kg and 5 µg/kg in milk.

Conclusions and recommendation

Having considered that:

- a toxicological ADI of 0.01 mg/kg bw (i.e. 600 µg/person) was established,
- permethrin (sum of isomers) was identified as the marker residue and accounted for 5% and 50% of the total residue in bovine kidney, and fat 14 days after treatment and 3% and 66% of the total residues in bovine liver and muscle 7 days after treatment,
- the marker residue accounted for 80% of the total residue in cow's milk 3 to 5 milkings after treatment,
- MRLs for the pesticidal use of permethrin were previously established in the EU by Commission Directive 98/82/EC for products of animal origin,
- the recommended MRLs of 500 µg/kg for bovine fat and 50 µg/kg for muscle, kidney and liver are compatible with those already set by Commission Directive 98/82/EC for cattle fat and meat (with fat content 10% or less); the MRL for muscle was established at a value equivalent to twice the limit of quantification of the analytical method,
- a validated analytical method based on capillary gas chromatography with electron capture detection was available for determining residues of permethrin in bovine edible tissues and milk,
- it was not possible to recommend final MRLs for pigs, goats and chickens because no information concerning the ratio of marker to total residues and no validation data for the proposed routine analytical methods were provided for these species;

the Committee recommended inclusion of permethrin into Annex I of Council Regulation No 2377/90 as amended, 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	50 µg/kg 500 µg/kg 50 µg/kg 50 µg/kg	Muscle Fat Liver Kidney	
			50 µg/kg	Milk	Further provisions in Commission Directive 98/82/EC are to be observed (OJ L 290, 29.10.1998, p. 25)

Based on these MRLs, the daily intake of total residues was estimated to be 383 µg/day, representing about 64% of the ADI.

MRLs have also been established in food products of plant origin, however, the use of permethrin as pesticide was discontinued (except for young plants for forestry) and therefore residue intake for food of vegetable origin is no longer relevant.