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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TAU FLUVALINATE

REVISED SUMMARY REPORT

1. Tau fluvalinate is a synthetic pyrethroid used for topical treatment of honey bees against the parasitic mite Varroa jacobsoni. Tau fluvalinate contains two of four isomers of the racemic mixture, fluvalinate. The mode of action is based on changes in the permeability of sodium channels of nerve membranes causing prolonged depolarisation and hyperexcitability. Mammals metabolise pyrethroids very efficiently and possess a relatively high tolerance towards these compounds.

   Tau fluvalinate is also used as a pesticide for the protection of crops, fruits, and other plants.

2. Absorption from the digestive tract of racemic fluvalinate varied between 30 - 80% in rats, Rhesus monkeys and mice. Up to 70% of the administered oral dose of tau fluvalinate is absorbed in rats.

   Dermal absorption of racemic fluvalinate dissolved in acetone appeared to be low in rats.

3. The metabolism of racemic fluvalinate after oral administration in rats, mice and Rhesus monkeys, was similar to other type II pyrethroids. It involved ester bond cleavage and subsequent hydrolysation and oxidization steps to form phenoxybenzoic acid derivatives, mainly excreted as conjugates in urine, and anilinic acid which is also mainly excreted as glucuronide or sulphate conjugate in urine. For the latter the formation of certain amino acid and bile acid conjugates and the formation of the amide of the chloroanilinic acid were also observed.

   Comparable results were observed after gavage administration of single doses of a mixture of ^{14}C-benzyl-labelled and ^{14}C-anilino-labelled tau fluvalinate in corn oil to rats (200 mg/kg bw, 1 mg/kg bw with or without 14 day pretreatment with 1 mg/kg bw/day). The amount of parent compound in the faecal residues depended on the dose and was 55-60% in low dosed animals, the main metabolite being anilinic acid, and 80-90% in the high dose group. Main urinary metabolites were 3-(4-OH-phenoxy)-benzoic acid (about 35-50%) and 3-phenoxybenzoic acid (about 12-20%).

4. Excretion rates of racemic or tau fluvalinate are variable. Single oral doses of racemic trifluoromethyl ^{14}C-fluvalinate in rats were excreted to nearly 80% in faeces (30% of it metabolites) and 10-20% in urine, in mice 60% (80% of it metabolites) in faeces and 30 % in urine, and in Rhesus monkeys more than 50% in faeces and roughly 37% in urine. For ^{14}C-benzyl-labelled tau fluvalinate excretion rates in rats were about 50% in urine and faeces, respectively. Urinary excretion in rats at 96 h after oral administration of a mix of ^{14}C-benzyl- and ^{14}C-anilino-labelled tau fluvalinate (dosage as above) amounted to about 28% in the low dose group without pretreatment, 40% with pretreatment, and 17% in the high dose group. The data are difficult to interpret as a mixture of tau fluvalinate radiolabelled a either of two molecular sites was used.

   In rats, 50% of an oral dose of racemic trifluoromethyl ^{14}C-fluvalinate were excreted within 24 h. Tissue residues 96 h after oral administration of benzyl-U-ring ^{14}C-tau fluvalinate amounted to 2-4% of the applied dose with highest concentrations in blood, fat, ovaries, liver and kidneys. Major residue component in fat was tau fluvalinate.

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5. Acute oral toxicity of tau fluvalinate following application by gavage in corn oil is characterized by LD-50s of 100-300 mg/kg bw for rats and mice. Clinical signs of acute toxicity resembled the CS-syndrome (choreoathetosis, salivation, pawing, burrowing, tremor, clonic seizures) observed for most alpha cyano pyrethroids.

   The acute dermal toxicity of tau fluvalinate in rabbits was low (LD 50 > 20 g/kg bw) with little percutaneous absorption. Similar results were found in rats for racemic fluvalinate in acetone.

6. In 13-week toxicity studies in rats tau fluvalinate was given in the diet or per gavage in corn oil. Pharmacotoxic effects and skin lesions were observed at a dose of 1 mg/kg bw/day by gavage (NOEL proposed by expert). Dietary exposure to the same dose also produced skin lesions. Clipping of the toenails, a variation from the standard protocol, reduced the severity of the lesions without resolving them. Similar findings were recorded in mice receiving tau fluvalinate in the diet for 13 weeks (lowest dose tested: 1 mg/kg bw). The NOEL in the rat study was 0.3 mg/kg bw.

   6-month toxicity studies on tau fluvalinate were not submitted. Administration of racemic fluvalinate in gelatine capsules for 6 months to dogs resulted in skin lesions at the lowest dose tested and emesis, depression, diarrhoea, and dehydration at higher doses.

7. Teratogenicity studies on tau fluvalinate in rabbits showed increased incidences of delayed ossification, and visceral and skeletal malformations at an overt maternotoxic dose (125 mg/kg bw). This dose also caused increased numbers of resorptions and poorer viability of fetuses. No teratogenic effects occurred at lower doses. No reproduction studies with tau fluvalinate in other species were submitted and the compound has to be classified as possibly teratogenic.

   No teratogenic effects of the less toxic racemic fluvalinate were seen in rats. Doses of 10 and 50 mg/kg bw/day were materno- and fetotoxic.

8. In a 2-generation rat study with racemic fluvalinate reproduction was subnormal in all groups, including controls. At dietary doses of 100 ppm and 500 ppm pup body weights were reduced. At 500 ppm, the offspring showed reduced viability, survival and growth rates and parent animals showed skin lesions and reduced body weight gains. Beginning with the low dose (20 ppm), a dose dependent impairment of spermatogenesis was observed in males of the F0 generation.

   A 2-generation reproduction toxicity study in rats with tau fluvalinate showed parental and developmental effects in the mid and high dose group. 2 of 4 males of the F1-generation from the median dose group failing to mate had atrophic seminiferous tubules in both testes and spermatozoa were partly absent from the epididymides. Litter and mean pup weights of the F2 generation of the median dose group were lower between days 8 and 21. Incidence of fur loss and scabbing was a marginally increased among adults. F1 and F2 pups of the highest dose group had tremors, mainly around lactation day 14, indicating toxic effects of tau fluvalinate excreted in rat milk. As toenails of all animals were clipped prior to treatment and later at weekly intervals a final NOEL can not be derived. No adverse substance related effects were observed at 0.5 mg/kg bw/day.

9. No mutagenic or genotoxic effect of tau fluvalinate was observed in the Salmonella/microsome assay (Ames test), and the L5178Y TK +/- mouse lymphoma assay both without and with metabolic activation (S9 mix), an in vitro transformation of BALB/3T3 cell assay without and with activation (primary rat hepatocytes), an in vivo rat bone marrow test, and in an unscheduled DNA synthesis test in rat hepatocytes with the reservation that the GLP status of most of the studies is unclear and/or test and control substances have not been sufficiently characterised.

10. A 2-year study in mice showed no tumorigenic effects of dietary tau fluvalinate. An increase of mammary fibroadenomas in female rats in a 2-year gavage study is judged to be a chance effect.

11. Neurotoxic effects of tau fluvalinate were seen in rats after administration by gavage of 60 mg/kg bw/day in corn oil for 7 days. Body weight decrease, symptoms such as fear, ruffled fur, ataxia, startle response hyperreactivity, spasms, and signs of neurotoxicity (reduced grip strength, irritability, reduced motion, spasms and abnormal gait) were observed, accompanied by nerve fibre degeneration correlating to incidence and severity of the symptoms. Severity and number of the lesions were reduced in animals allowed to recover for 7 days. 10 mg/kg bw/day resulted in a marginal increase in vocalisation when handled and hyperalgesia.

12. Toxicity in the target species was examined in an acute toxicity study on the knock down effect of tau fluvalinate in bees. Tau fluvalinate is less toxic to bees than other alpha cyano pyrethroids. The intended formulation renders environmental aspects a matter of limited concern.

13. A NOEL of 1 mg/kg bw for tau fluvalinate, derived from a 13-week gavage study in rats is not acceptable as this dose led to pharmacodynamic effects and skin problems. Only limited information about biological effects of tau fluvalinate in mammals, particularly in regard to acute neurotoxicity is given. Subchronic studies in dogs and teratogenicity studies in rats were only conducted with racemic fluvalinate.

    A tentative NOEL of 0.5 mg tau fluvalinate/kg bw derived from the 2-generation rat study is retained. A safety factor of 1000 is used to calculate a preliminary ADI to compensate the inadequacies of the pharmacological/toxicological data:

NOEL:	0.5 mg tau fluvalinate/kg bw/day
Safety Factor:	1000
ADI:	0 - 0.5 µg tau fluvalinate/kg bw 0 - 30 µg tau fluvalinate/person (60 kg bw)

    Data from France indicate that intake of tau fluvalinate from treated agricultural products amounts to approximately 13 µg/person and day. An acceptable maximum intake in honey of 17 µg tau fluvalinate/person and day, 57% of the ADI, remains.

14. Two polymer matrix strips, each 8 g in weight containing 800 mg tau fluvalinate were suspended midway for a period of 6-8 weeks between the brood frames (recommended dose) so that the bees can walk on both sides of the strip. Residues in honey were investigated in a total of 25 hives.

    Tau fluvalinate was only found in 2 samples of the hive super of one colony with residue concentrations of 12 and 42 µg/kg. In all other samples residue concentrations were below the limit of detection of the analytical method (10 µg/kg). Contamination during handling is a possible explanation for the residues found in one hive. Furthermore, increased dose and duration of treatment (4 or 8 strips/hive for 11 weeks) did not cause increased residues (all samples < limit of detection).

15. Investigations on the degradation of tau fluvalinate showed stability in honey and wax. Less than 1.5 % of tau fluvalinate was degraded to chloroanilinoacid at room temperature within 4-6 months.

16. Considerable accumulation of tau fluvalinate was observed in wax. Depending on the location of the samples tau fluvalinate concentrations varied from 0.2 mg/kg - 5.5 mg/kg, with a maximum of 26.9 mg/kg in one wax sample collected from a frame positioned next to a strip.

    Accumulation in wax is the result of the stability of tau fluvalinate in this matrix, its lipophilic character and the fact that wax is normally reused over several seasons. Monitoring of tau fluvalinate residues in honey and wax in Belgium in 1989-1992 revealed that residues in wax increased exponentially when the wax was reused over the years. Transfer of tau fluvalinate residues from wax to honey was shown to be negligible. However, the high tau fluvalinate residues in wax should be taken into consideration in the evaluation of tau fluvalinate since

contamination of honey with wax particles has to be expected (0.5 % content of water insoluble particles in honey is allowed).

17. A capillar GC-ECD method was developed for routine measurements of tau fluvalinate in honey, wax and propolis. The detection limit of the method was given as 10 µg/kg in honey, 0.1 mg/kg in wax and 1 mg/kg in propolis. A complete documentation and validation of the analytical method was not provided.

Conclusion and recommendation

Considering that following treatment of bees with the intended pharmaceutical formulation and dose as well as 4 times the intended dose at a prolonged treatment period residues of tau fluvalinate in honey were always below the detection limit of the analytical method (10 µg/kg), it is concluded that it is not necessary to establish a MRL for tau fluvalinate. Inclusion of tau fluvalinate into Annex II of Council Regulation (EEC) 2377/90 as indicated in the following table is recommended:

Pharmacologically active substance	Animal species	Other provisions
Tau fluvalinate	Honey bees	