The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CHRYSANTHEMI CINERARIIFOLII FLOS and PYRETHRUM EXTRACT

SUMMARY REPORT

1. *Chrysanthemi cinerariifolii flos* is the dried flower heads of *Chrysanthemum cinerariifolium* (Trev.) Vis. This crude drug is generally called pyrethrum and is often used as an extract (concentration of pyrethrins 25% (w/w)) in an isoparaffinic hydrocarbon solvent. It contains 0.4 to 2% of pyrethroids, mainly pyrethrin I and II, cinerarin I and II and jasmolin I and II. Other constituents are stachhydrin (L and D,L), taraxasterin, chrysanthin, chrysanthen, sesamin, β-cyclopyrethrosin, volatile oil, fatty acids, resin, azelainic acid, tannin, wax, sugar and cerylalcohol. The pyrethroids are the main pharmacologically active compounds.

2. Commercial veterinary products based on *Chrysanthemi cinerariifolii flos* generally contain around 3.4% of pyrethrum extract. They are indicated for topical use in all food producing species, against lice, ticks, fleas and other ectoparasites. The animals are washed 1 to 2 times a week with a 0.5% solution of the preparation.

   Products based on *Chrysanthemi cinerariifolii flos* are also used in human medicine.

3. The pyrethroids are insecticides which kill the insects on direct contact. Pyrethroids affect the Na^+-, K^+- and Cl^-- channels of the nerve cell membranes causing disturbances of the axonal transmission of excitations and partly blockage of ganglia. The effect is about 100 times higher in arthropods than in mammals.

4. Pharmacokinetic data were available in humans.

   A commercial formulation containing 0.3% ^{14}C-pyrethrins was applied to the skin of 6 human volunteers at a rate of 5.5 mg pyrethrins/cm^2. The unabsorbed material was washed off 30 minutes later. It was calculated that 2% of the dose was absorbed (range 0.06 to 4.1%). Following oral dosing of rats with 50 or 100 mg/kg bw ^{14}C-pyrethrin I, peak blood concentrations were found 5 to 6 hours after dosing in male and 6 to 8 hours after dosing in females. Most radioactivity was excreted during the first 72 hours. In males, 32 to 47% of the dose was recovered from urine and 53 to 71% from faeces. The corresponding values for females were 50 to 57% and 50 to 52%. Several metabolites were identified in urine indicating metabolism by 2 major pathways: oxidation of the double bond on the cyclopentene or cyclopropane moieties to form a diol, and hydrolysis of the ester bond to form the corresponding acid and alcohol. The major metabolite in urine samples was chrysanthemum dicarboxylic acid.

5. The acute toxicity of pyrethrum extract was variable and depended on factors such as grade of material used and solvent vehicle. For pure pyrethrins I and II, the acute oral LD_{50} in rats were reported to be 260 to 420 mg/kg bw and more than 600 mg/kg bw respectively. In rats, the acute oral LD_{50} values for different grades of pyrethrum extract ranged from 200 to 4910 mg/kg bw. Signs of toxicity in these studies included diarrhoea, increased respiratory rate, tremors and convulsions. Females were more sensitive to the toxicity of the substance than males. Acute toxicity was lower following dermal application indicating low percutaneous absorption.

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6. Repeated-dose toxicity studies with pyrethrum extract were carried out in rats and dogs using oral and inhalational dosing, in rabbits using dermal dosing and in mice using oral dosing. Hepatotoxicity was observed in rats and mice (increased liver weight, hepatocellular hypertrophy), and renal toxicity in rats (renal tubular degeneration). A NOEL of 600 mg/kg feed (approximately 15 mg/kg bw) was established in an 8-week dietary study in dogs with pyrethrum extract, based on increased liver weights and decreased testes weights at the next dose of 1000 mg/kg feed (around 25 mg/kg bw).

7. No target species tolerance data were provided. Pharmacovigilance data indicated that adverse reactions to products containing pyrethrum extract were uncommon and mostly involved signs consistent with contact sensitisation.

8. In a two-generation, reproduction study, with two litters per generation, male and females rats were fed diets containing 0, 100, 1000 or 3000 mg/kg feed pyrethrum extract, with treatment commencing at least 11 weeks prior to the first mating. Signs of parental and neonatal toxicity (reduced food consumption and body weight gain) were observed at 3000 mg/kg feed throughout the study. Similar though less severe effects were occasionally observed in the 1000 mg/kg feed group. There were no effects on any reproductive parameters. The NOEL was 100 mg/kg feed (equivalent to approximately 5 mg/kg bw).

   No evidence of teratogenicity or foetotoxicity was observed in a teratogenicity study in which rats were given oral doses of 0, 5, 25 or 75 mg/kg bw per day from days 6 to 15 of gestation. No evidence of teratogenicity or foetotoxicity was observed in a teratogenicity study in which rabbits were given oral doses of 0, 25,100 or 250 mg/kg bw per day of pyrethrum extract from days 7 to 19 of gestation; however maternal toxicity (weight loss, salivation) was observed at 100 and 250 mg/kg bw.

9. Pyrethrum extract was not mutagenic in an *in vitro* bacterial assay for gene mutation using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, in an *in vitro* unscheduled DNA-synthesis (UDS) assay in primary rats hepatocytes using concentrations in the ranges 0.003 to 3.0 and 0.03 to 1.0 µl/ml, nor in an *in vitro* chromosomal aberration assay in Chinese hamster ovary cells using dose levels in the range 0.005 to 0.08 µg/ml. An *in vivo* micronucleus assay in which mice were given two oral doses of 0.25, 0.5 or 1.0 ml/kg bw of the test substance, 24 hours apart, also gave negative results.

10. There was no evidence of carcinogenicity in a study in which mice were fed diets containing 0, 100, 2500 or 5000 mg/kg feed pyrethrum extract for 18 months. In a combined chronic toxicity and carcinogenicity study, rats were fed diets containing 0, 100, 1000 or 3000 mg/kg feed (4.6, 49 or 151 mg/kg bw per day) pyrethrum extract for 2 years. Body weight gain was reduced in both sexes given the top dose. The incidence of follicular cell adenomas of the thyroid was significantly increased in males given 1000 and 3000 mg/kg feed and in females given 3000 mg/kg feed and the incidence of thyroid hyperplasia was increased in males given 1000 mg/kg feed. The incidence of benign keratoacanthomas was increased in males given 3000 mg/kg feed. Although the incidence of thyroid adenomas exceeded the historical control range for the laboratory concerned, the incidence was within the range encountered for the same strain of rat at other laboratories. There was no increase in the incidence of malignant tumours in this study. Taking into account the negative results obtained in the mutagenicity studies, it was agreed that pyrethrum extract was not carcinogenic. The NOEL was 100 mg/kg feed (4.6 mg/kg bw per day) based on hepatotoxicity (changes in liver enzymes and increased lobulation) and effects on the thyroid at the next dose.

11. In human medicine pyrethrum extract is used externally as insecticide and antiscabies remedy and used internally in traditional medicine against worms. No doses are reported.

    A number of allergic reactions in humans to crude extracts of pyrethrum have been reported.

12. In a neurotoxicity study, groups of 15 female rats were given an oral dose of 0 (corn oil solvent vehicle) 20, 63 or 200 mg/kg bw of pyrethrum extract and groups of 15 males were given 0 (corn oil), 40, 125 or 400 mg/kg bw. Five males given 400 mg/kg bw and 2 females given 200 mg/kg bw died. The signs of toxicity observed in these groups were reduced activity, salivation, tremors, pyrexia, exaggerated startle response, decreased grip strength, splayed hind legs and changes in certain fine movements. Alterations in motor activity were also seen in the males given 125 mg/kg bw and 3 females in the 63 mg/kg bw group had tremors. The surviving rats were killed 15 days after dosing. No gross pathological changes were found and brain weight was unaffected by treatment. Substance-related microscopic changes were found in the sciatic nerves of females given 200 mg/kg bw. The NOELs were 20 and 40 mg/kg bw for males and females respectively.

13. An Acceptable Daily Intake (ADI) of 0.046 mg/kg bw per day, i.e. 2.76 mg/person, was calculated by applying a safety factor of 100 to the NOEL of 4.6 mg/kg bw which was established in the chronic toxicity and carcinogenicity study in rats. This was very similar to the ADI of 0.04 mg/kg bw per day which was established by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR).

14. Three cows were treated topically with a spray containing 2% by weight of pyrethrins (equivalent to 1.124 g pyrethrins per day) for 21 days. Residues of pyrethrin I in milk were in the range 2 to 6 µg/kg; residues of pyrethrin II and cinerins I and II were not detected. Another 3 cows were treated with the spray containing 0.031% pyrethrins (equivalent to 17.6 mg pyrethrins per day). No residues were detected in the milk.

15. One calf was sprayed daily for 40 days with a spray containing 2% (0.567 g pyrethrins/day) and killed on the 41st day. Total residues of pyrethrin I, pyrethrin II and cinerin II of 7 µg/kg were found in muscle. In the liver, residues of 5.5 µg/kg pyrethrin I and only trace amounts of pyrethrin II and cinerin II (less than 5 µg/kg) were found. Residues of 3 µg/kg total residues were found in fat. Another calf was sprayed daily for 42 days with a spray containing 0.031% pyrethrins (8.8 mg pyrethrin/day) and killed on the 43rd day. No residues of pyrethrins were detected in tissues. The doses used in these studies were much higher than normally employed.

16. A study was carried out in lactating goats using ^{14}C-pyrethrin I. Three goats were treated orally. Two further goats were treated topically, one with a water-based formulation and the other with an oil-based formulation. For the topical study, the dose was adjusted to represent a dose of approximately 5 mg/kg feed in the diet. The dose was applied for 5 days and the goats were euthanased 5 hours after the last dose. Total residues in muscle, liver, kidney and fat for the goat treated with the oil-based formulation were 5, 22, 22 and 76 µg/kg. For the goat treated with the water-based formulation, total residues in muscle, liver, kidney and fat were 2, 6, 6 and 37 µg/kg respectively. Total residues in milk did not exceed 14 µg/kg following the use of the oil-based formulation and 7 µg/kg following the use of the water-based formulation.

17. Laying hens were treated dermally for 35 to 37 days with a commercial spray formulation intended for the disinfestation of buildings. The dose rate was equivalent to 498 mg total pyrethrins/1500 cubic feet. Using gas-chromatography with electron capture detection, total residues of cinerin I + jasmolin I + pyrethrin I (expressed as pyrethrin I) of up to 14 µg/kg were found in eggs.

18. The calculated consumer intake from residues in milk and meat and eggs represented less than 2% of the ADI.

19. It was noted that EU MRLs had been elaborated for the use of pyrethrins (sum of pyrethrins I and II, cinerins I and II and jasmolins I and II) on cereals. It was estimated that the Theoretical Maximum Daily Intake (TMDI) of residues arising from the use of pyrethrins as a pesticide would amount to around 22% of the ADI.

Conclusions and recommendation

Having considered that:

- an ADI of 0.046 mg/kg bw per day (2.76 mg/person per day) was established for pyrethrum extract,
- the calculated maximum daily intake of total pyrethroid-derived residues from veterinary uses (considering meat, milk and eggs) represents less than 2% of the ADI;

the Committee concludes that there is no need to establish an MRL for *Chrysanthemi cinerariifolii flos* and pyrethrum extract and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Chrysanthemi cinerariifolii flos	All food producing species	For topical use only
Pyrethrum extract	All food producing species	For topical use only

Based on the calculated maximum intakes of residues from both veterinary and pesticide use, the total pyrethroid-derived residues will amount to around 24% of the ADI.