



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### CYFLUTHRIN

#### SUMMARY REPORT (1)

1. Cyfluthrin is a synthetic type II pyrethroid insecticide and acaricide. Its effects are due to changes in permeability of sodium channels of nerve membranes leading to prolonged depolarisation and hyperexcitability. Mammals efficiently reduce the effects of pyrethroids by rapid metabolism. Cyfluthrin for veterinary medicinal and pesticide use consists of a mixture of isomers (8 enantiomers):

Enantiomeric pair:	Isomer I ( <i>cis</i> )	Isomer II ( <i>cis</i> )	Isomer III ( <i>trans</i> )	Isomer IV ( <i>trans</i> )
Proportion:	23-27 %	17-21 %	32-36 %	21-25%

Cyfluthrin is authorised in several EU Member States as a 1% pour-on solution in mineral oil for use in cattle of all ages for the control of flies and tabanids (dosage: 10 ml/animal, approximately 100 mg cyfluthrin; 0.2-0.5 mg/kg bw). Cyfluthrin is also used as a pesticide for the protection of crops.

2. Most submitted studies were carried out using cyfluthrin with the isomers in the ratios given above, i.e. *cis:trans* approximately 45:55. The *cis:trans* ratio of parent cyfluthrin residues in animal tissues appear to be unchanged or only slightly changed. No information on the toxicity of individual isomers is supplied. In rats, the acute oral toxicity of *cis:trans* 55:45 cyfluthrin in polyethylene glycol is approximately twice that of *cis:trans* 45:55 cyfluthrin in the same vehicle. The acute inhalation toxicity is also enhanced when the *cis:trans* ratio of cyfluthrin is changed to 55:45.
3. The bioavailability and toxicity of cyfluthrin were highly dependent on the vehicle used. Highest values for both were observed in cremophor EL emulsions. Cremophor EL is a complex mixture of polyoxylated castor oil derivatives with surface active properties and some inherent toxicity. Increased bioavailability and toxicity of type II pyrethroids have also been described from other emulsions, e.g. 20% lecithin in water.
4. The pharmacodynamic effects of cyfluthrin include prolongation of the barbiturate sleeping time in mice at an oral dose of 1 mg/kg bw in 2% cremophor and shortening at 30 mg/kg bw in polyethylene glycol. Latter vehicle and dose induced seizures and caused death in 60% of the mice.

Single oral doses greater than or equal to 0.03 mg cyfluthrin/kg bw in 2% cremophor significantly changed neuromotor function in rats in the inclined plane test. The NOEL is 0.01 mg/kg bw, the lowest observed in all submitted studies.

Oral cyfluthrin doses of 10 and 30 mg/kg bw in polyethylene glycol led to elevated blood glucose levels, decreased urine volume and sodium excretion in rats, and had small effects on haemodynamic parameters (slight increase of heart rate, cardiac output, and contractility) in dogs.

*In vitro* cyfluthrin had no antiallergic or pseudo-allergic effects on rat mast cells. Two metabolites inhibited Na<sup>+</sup>, K<sup>+</sup> or Mg<sup>++</sup> dependent ATPase in brain tissue of rats and chickens more effectively than parent cyfluthrin.

5. Bioavailability of cyfluthrin in rats (10 mg/kg bw, gavage) increased when given in 2 % cremophor as opposed to polyethylene glycol, reaching 5-fold peak plasma concentrations significantly earlier. The isomer composition in blood was slightly changed in favour of the *cis*-components.

Absorption of <sup>14</sup>C-labelled cyfluthrin from the digestive tract reached 80-100% in rats. Single oral or intravenous doses were excreted to 29% (males) and 35% (females) in faeces, and 70% (males) and 60% (females) in urine. Biliary metabolites accounted for about 50% of the faecal cyfluthrin equivalents.

Like other pyrethroids, cyfluthrin is rapidly metabolised by ester cleavage and subsequent oxidation and/or hydroxylation steps and cyano group elimination. The resulting fluorophenoxybenzoic acid and cyclopropanecarboxylic acid derivatives were excreted mainly as glucuronide and sulphate conjugates in urine. In chicken a regular additional metabolite, and, after a large single oral (3000 mg/kg bw) dose of radiolabelled cyfluthrin, a previously unknown metabolite were observed. Other than that metabolism of cyfluthrin in rats, cow and chickens is largely comparable. Many of the metabolites prevail in one isomeric form, e.g. permethramid: 90% *trans*, 10% *cis*.

Tissue levels in rats 48 hours after treatment amounted to less than 2% of the radiolabelled oral dose, fat, spleen, liver, and sciatic nerve containing the highest concentrations. Major residue component in fat was parent cyfluthrin. A lactating cow had highest residues in liver, fat and kidney after 5 oral doses of radiolabelled cyfluthrin in gelatine capsules. Concentrations in other tissues were at least ten-fold lower. Major residue component was parent cyfluthrin (liver: 86%, muscle: 98-100%, fat: 93-100%, heart: 71%, kidney: 56%). Highest concentrations in milk were seen 24 h after the third application, 98% of it parent cyfluthrin. Hens given gelatine capsules containing radiolabelled cyfluthrin on 5 days had highest residues in kidney, liver and gizzard. Other tissues contained at least ten-fold lower levels. Highest concentrations in eggs were seen on the last day of treatment, later time points not being investigated. Percentages of unchanged cyfluthrin in the residues were 9% in kidney, 40% in muscle, 75% in fat, and 56% in eggs. A large single oral dose resulted in unchanged cyfluthrin in the excreta of chickens for 14 days.

No information on pharmacokinetics of dermally applied cyfluthrin was supplied.

6. The acute oral toxicity of cyfluthrin is variable and dependent on factors such as vehicle or sex. Clinical signs of acute oral toxicity were those of the CS-syndrome (choreo-athetosis, salivation, pawing, burrowing, tremor, clonic seizures). Oral LD<sub>50</sub> values in male rats for cyfluthrin in polyethylene glycol, corn oil, or cremophor were 600, 250, or 15-20 mg/kg bw, respectively. The oral LD<sub>50</sub> of cyfluthrin in polyethylene glycol in mice was 291 mg/kg bw for males, 609 mg/kg bw for females, while 100 mg cyfluthrin/kg bw in cremophor killed more than 50% of female mice. The LD<sub>50</sub> values greater than 100 mg/kg bw in dogs are questionable as oral doses greater than 20 mg cyfluthrin/kg bw in polyethylene glycol or cremophor caused vomiting. Chicken have a relatively low susceptibility to the toxic effects of pyrethroids with an oral LD<sub>50</sub> for cyfluthrin greater than or equal to 5000 mg/kg bw independent of the type of vehicle used. In rats using polyethylene glycol as vehicle, the acute oral toxicity of seven cyfluthrin metabolites was lower than that of cyfluthrin.

Polyethylene glycol or oily formulations of cyfluthrin were of low acute dermal toxicity in rats and hens with an LD<sub>50</sub> greater than 5000 mg/kg bw, while 1% cyfluthrin in dipropylene glycol monomethyl ether (for 24 hours under an occlusion) had a LD<sub>50</sub> of 59 mg cyfluthrin/kg bw in male rats.

An LC<sub>50</sub> of 400 mg/m<sup>3</sup> (240 min head/nose exposure, ca. 50-100 mg/kg bw) was seen in acute inhalation toxicity studies in rats.

The intraperitoneal LD<sub>50</sub> for cyfluthrin in polyethylene glycol in rats was 66 mg/kg bw for males and 104 mg/kg bw for females, the subcutaneous LD<sub>50</sub> in mice was greater than 2500 mg/kg bw.

7. Cyfluthrin was mildly irritating to rabbits' eyes but not to their skin. No sensitising potential was observed. The potency of cyfluthrin to induce sensory stimulation of the skin was not investigated.
8. Treatment related effects in two 3-month toxicity studies in rats with dietary cyfluthrin were small changes in haematological parameters at the mid and high dose, and liver enzyme inductions in all dose groups of the first study and decreased serum glucose levels at the mid and high dose in the second. The second was insufficiently documented. No NOELs could be derived.

Dogs receiving dietary cyfluthrin for 6 months showed vomiting, diarrhoea, and neuromotor dysfunctions (unsteady gait, uncoordinated hind leg movements) at the high dose (20 mg/kg) and reduced mean bodyweight gain at the two high doses. The low dose, 2 mg/kg bw, was the NOEL.

The NOEL in rats for repeated inhalation exposure to cyfluthrin was 0.09 mg/m<sup>3</sup>, analytical concentration (6 h/day for 63 days, ca. 30 µg/kg bw/day), concentrations greater than 0.5 mg/m<sup>3</sup> led to reduced respiration rates and changes in the acid/base status, and greater than or equal to 1.4 mg/m<sup>3</sup> caused behavioural effects, signs of acute toxicity.
9. Long term toxicity of cyfluthrin in the diet was studied in rats, dogs (both 24 months), and mice (23 months). In rats the two high dose levels resulted in dose dependent weight gain retardation, all dose levels caused deviations of some haematological parameters from the control values. The low dose of 2 mg/kg bw/day, on which the ADI established by the Joint FAO/WHO Meeting on Evaluation of Pesticide Residues in Food (JMPR) is based, therefore, is a questionable NOEL. In mice, as in rats, deviations from haematological and clinical-chemical parameters of the controls were recorded in all dose groups. The high dose caused weight gain retardation. No clear NOEL can be established from the study results. Treatment related effects were observed in dogs receiving the high dose (approximately 20 mg/kg bw/day). They consisted of loose stools, vomiting, reduced mean bodyweight gain, and uncoordinated hind limb movements in 2 out of 12 dogs on one or more occasions. The average dose of 5 mg/kg bw/day was stated as a NOEL in dogs.
10. No teratogenic potential of cyfluthrin was recorded even at maternotoxic doses (rats: greater than 3 mg/kg bw in polyethylene glycol, rabbits: greater than 30 mg/kg bw in 0.5% cremophor, and greater than 20 mg/kg bw in corn oil). An abortifacient effect of cyfluthrin in 0.5% cremophor in rabbits cannot be ruled out, since 2 does in the high dose group (45 mg/kg bw) aborted and another resorbed all foetuses.

Another study in rats with orally administered cyfluthrin in 1% cremophor revealed no maternotoxic or teratogenic effects at doses up to 10 mg/kg bw. The results conflict with acute toxicity studies, where 0.5-10 mg cyfluthrin in 2% cremophor/kg bw had toxic effects in rats. No explanation of the conflicting results is given.

Impaired placental and foetal development in rats was caused by inhaled cyfluthrin concentrations greater than or equal to 1.1 mg/m<sup>3</sup> (approximately 0.4 mg/kg bw, 6 h/day for 10 days, analytical concentrations). Reflex bradypnoea due to sensory irritation, in turn inducing hypothermia and hypoxia may be the reason. The incidence of bone alterations, runts per litter and resorptions was increased at 4.7 and 23.7 mg/m<sup>3</sup> but no clear teratogenic effects were identified. The NOEL was 0.59 mg/m<sup>3</sup>, approximately 0.2 mg/kg bw.
11. A multigeneration study (F0-F3) with dietary cyfluthrin in rats revealed no specific reproduction toxicity. Several nursing pups in the high dose group and one in the medium dose group had seizures. This may be attributable to a high susceptibility of pups to cyfluthrin and/or metabolites or to a high exposure to the substances via milk. The NOEL was 5 mg/kg bw per day.

No studies specifically investigating the pre- and perinatal influence of cyfluthrin were submitted.
12. Most of the mutagenicity studies on cyfluthrin were not GLP compliant. With this reservation no mutagenic or genotoxic effect was observed in the Salmonella/microsome assay (Ames test) and other bacterial test systems with and without metabolic activation (S9 mix), in the unscheduled DNA synthesis test in rat hepatocytes, and *in vivo* micronucleus and dominant lethal tests in mice.

Equivocal results were obtained with cyfluthrin in a reverse mutation reduction assay with *Saccharomyces cerevisiae*, a CHO/HGPRT mutation assay, an *in vitro* cytogenetic study in human lymphocytes for the detection of clastogenic effects, and an sister chromatid exchange (SCE) assay in Chinese hamster ovary (CHO) cells.

Seven cyfluthrin metabolites gave negative results in the Ames test (with and without S9 mix).

13. No tumorigenic effects of cyfluthrin were seen in lifetime carcinogenicity studies in rats and mice.
14. Neurotoxicity of cyfluthrin was studied in rats and chickens. Repeated oral administration of sublethal doses to rats for several days to weeks caused motor function disturbances with slight histopathological correlates in nerve tissue, which were largely reversed during a 1-3 month recovery period. Near lethal single or repeated oral doses of cyfluthrin (1500-5000 mg/kg) in hens resulted in motor function losses, severely impaired health state and, frequently, death 3-6 weeks following treatment. Possible explanations were either an unknown type of delayed neurotoxicity or late effects of general toxicity. Five repeated dermal applications of 5000 mg cyfluthrin/kg bw caused death in two of ten treated chickens, and slight brain and sciatic nerve fibre degeneration in two other. The latter symptoms were also observed in hens used as controls in other studies.
15. Tolerance studies in cattle with dermal application of multiples of the recommended cyfluthrin dose in not unambiguously specified formulations showed irritation of the application site in some animals. No treatment related effects were seen after dermal treatment of single sheep, dogs, and pigs with unspecified formulations containing cyfluthrin.
16. Human occupational exposure (dermal contact) resulted in itching and burning sensations of exposed areas of the body, which lasted for several hours.
17. The CVMP noted that cyfluthrin had been reviewed by the Joint FAO/WHO Meeting on Evaluation of Pesticide Residues in Food (JMPR) in 1987. An ADI for cyfluthrin of 1.2 mg/person was established, based on a NOEL of 2 mg/kg bw/day derived from a 2 year oral toxicity study in rats using a safety factor of 100. For the following reasons the CVMP was unable to adopt the JMPR ADI. Cyfluthrin orally administered in cremophor had effects on motor functions in rats in the inclined plane test at 0.03 mg/kg bw (NOEL: 0.01 mg/kg bw). This result was presented to the JMPR for evaluation. The JMPR ADI of 1.2 mg/person, i.e. 0.02 mg/kg bw, lies in the same range. In comparison to other vehicles, cyfluthrin has a high acute oral toxicity in rats (LD<sub>50</sub>: 15-20 mg/kg bw) when administered in cremophor and a corresponding high dermal toxicity following application in dipropylene glycol monomethyl ether (LD<sub>50</sub>: 60 mg/kg bw). The results and the underlying mechanisms, particularly of the increased oral toxicity, were not discussed. They may include enhanced bioavailability of cyfluthrin from cremophor. In many cases consumers can be expected to ingest cyfluthrin residues in emulsions (e.g. milk, sauces). The relevance of the cremophor vehicle to dietary emulsion is unclear. No human data on the relevance of the vehicle to pharmacological/toxicological effects of cyfluthrin are available. Therefore the evidence of neurotoxic properties of cyfluthrin (inclined plane test) and the dependency of the biological effects of cyfluthrin on the vehicle must be considered in respect to their relevance for consumer safety.

The CVMP agreed to set a provisional ADI of 0.001 mg cyfluthrin/kg bw, i.e. 0.06 mg cyfluthrin per person, based on the NOEL of 0.01 mg cyfluthrin/kg bw of the inclined plane test in rats using a safety factor of 10. The safety factor of 10 is considered appropriate, as the NOEL is based on the most sensitive pharmacological endpoint available in a vehicle which may enhance the toxicity of cyfluthrin above that expected to result from residues in normal human diet.

18. To evaluate the pharmacokinetics in the target species (cattle) only distribution and metabolism were investigated (after oral application of <sup>14</sup>C-cyfluthrin). Data on absorption, excretion and the metabolic fate of cyfluthrin after dermal application were not provided. However, the results of the radiolabel study (see above) justify the choice of parent cyfluthrin as the marker residue.
19. Fat was the tissue with highest concentrations of residues after cattle were treated with the recommended therapeutic doses. For example, residue levels ranged from 53 µg/kg at 7-14 days

after treatment to 27-40 µg/kg at days 21-28. In other edible tissues, residues were below the limit of detection of the analytical methods (10 µg/kg) at various times after treatment using the recommended therapeutic doses. In several studies in lactating animals, residues in milk were below the limit of detection of the analytical method (10 µg/kg) at 9-96 hours after administration of the therapeutic dose.

20. Following the Guidelines for Predicting Dietary Intake of Pesticide Residues (WHO 1989), a theoretical maximum daily intake of 0.051 mg cyfluthrin from crop protection usage was calculated.
21. A preliminary ADI of 0.06 mg cyfluthrin (parent compound) per person is proposed. However, this amount of 0.06 mg is nearly used up by residues originating from crop protection usage, leaving only 9 µg for residues originating from veterinary use.
22. For milk and fat, the lowest possible MRLs which can be set correspond to the limits of quantification of the proposed routine analytical method. These MRLs are 10 µg/kg for fat and 5 µg/kg for milk. This results in a dietary intake of 0.0125 mg (taking into account the limit of determination for liver, kidney and muscle as well). However, these MRLs do not allow the setting of a withdrawal period for fat (especially when repeated treatments are considered). Moreover, an MRL of 10 µg/kg for fat does not enable the establishment of a meaningful MRL for a target tissue for animal offal (liver or kidney) when the relative distribution between the different tissues is considered.
23. Apart from MRLs for other food commodities derived from crop protection use, MRLs for milk, fat and meat were set by the EU (Council Directive 94/29/EC of 23 June 1994) : 20 µg/kg for cow milk, 50 µg/kg for animal fat, and, taking into account a fat content 10% or less, 10 µg/kg for meat, edible offal.
24. A routine analytical method for the determination of parent cyfluthrin was developed based on GC-ECD. The limit of quantification was 0.01 mg/kg for fat, liver, kidney and muscle, and 0.005 mg/kg for milk. The mean recoveries for the different tissues were in the range of 80% to 89 %, the coefficients of variation were below 15%. The method was shown to be specific relative to cypermethrin, permethrin and flumethrin. However, in view of the methodological problems discussed in one of the 28-day feeding studies, there are some doubts concerning the analysis of incurred liver and kidney samples.

## Conclusions and recommendation

Having considered that :

- a provisional toxicological ADI has been set at 0.001 mg/kg bw,
- no validated physico-chemical analytical method is available;

the Committee recommends the inclusion of cyfluthrin 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
Cyfluthrin	Cyfluthrin	Bovine	10 µg/kg	Muscle	Provisional MRLs expire on 01.01.2001
			50 µg/kg	Fat	
			10 µg/kg	Liver	
			10 µg/kg	Kidney	
			20 µg/kg	Milk	Further provisions in Council Directive 94/29/EEC are to be observed
					Provisional MRL expire 01.01.2001

This recommendation is given, irrespective of the following problems : i) these MRLs result in a dietary intake of 0.037 mg cyfluthrin, an amount which exceeds the remaining portion of the ADI by 0.028 mg, and ii) an MRL of 50 µg/kg for fat requires a long withdrawal period.

The CVMP noted that theoretical calculations suggested that consumer intake from the current veterinary and pesticide uses, taken together, would result in the ADI being exceeded. However the CVMP considered that there should be no undue risk to consumers because :

- the intake calculations were based on consumption data which were at the upper limit of the range for individual intake of animal products,
- concentrations of residues in food of animal origin at short withdrawal times are extremely low and are restricted to milk and fat.

It was agreed that realistic estimations of consumer intake from all sources would be required when elaborating final MRLs.

## LIST OF QUESTIONS

### Safety File

1. Information on photochemical and thermal stability of cyfluthrin should be submitted by the applicant.
2. For each impurity (e.g. cis-trans permethric acid) the limit of detection should be given.
3. The content of sulphated ashes and heavy metals in the active ingredient should be specified.
4. Information on the route of synthesis should be submitted.
5. The chemical identity of the test substance has not been stated unambiguously in a number of pharmacological and toxicological studies. Complete information on the respective compounds (e.g. diastereomers, batch number, purity, type of impurities, formulation) should be made available. The applicant should consider the relevance of pharmacological and toxicological studies with the isomeric mixtures used to the assessment of the human health risk posed by residues present in food derived from cyfluthrin-treated animals.
6. The applicant should consider the relevance of pharmacological and toxicological studies with the vehicles used to the assessment of the human health risk posed by residues present in food derived from cyfluthrin-treated animals. In particular, the suitability of cremophor should be assessed. If necessary, key studies, such as the establishment of a NOEL for acute neurotoxic effects should be repeated, using an appropriate vehicle.
7. Evaluation of the pharmacodynamic profile of cyfluthrin is impaired by the fact that due to the insufficient data on bioavailability the results of several relevant studies remain questionable. It is not evident, whether the formulations and time points chosen in the experiments ensure maximal concentrations of the parent compound in blood. The applicant is asked to re-evaluate the submitted data on the pharmacodynamics of cyfluthrin taking into account the bioavailability.
8. The pharmacokinetics and the metabolism of cyfluthrin following dermal application should be discussed by the applicant.
9. The individual animal data and statistics and/or a precise description of studies no. R 2896, 3554, 13 923, report Kazda, S.(dated 30.08.1979, p. 277, Safety File), 215, 221, 11 872, 1632, 10 130, 11 983, 10 562, 264 should be submitted as well as a revised and corrected version of report no. PF 2059.
10. The formulations used to study dermal toxicity and target animal toxicity should be precisely specified, also regarding their content of excipients, to clarify the causes of the differing irritation scores.
11. The applicant is asked to discuss the difference of a factor of 100 in the acute dermal toxicity of cyfluthrin in dependence on the vehicle, which was observed in rats.
12. In view of the potential for perinatal pyrethroid exposure to cause developmental neurotoxicity in mice (Eriksson and co-workers 1990-1994), the applicant should consider the potential risk of developmental neurotoxicity arising from consumption of food derived from cyfluthrin-treated animals.
13. In consideration of the reported dermal sensations following human occupational exposure, the potential of cyfluthrin to induce skin sensations should be tested in an animal model.
14. Information on possible immunological effects of cyfluthrin should be submitted.
15. The applicant should provide a justified proposal for an ADI based on the information derived from above.

## Residue File

1. The precise formulation of the cyfluthrin products used in the different residue studies should be specified.
2. In contrast to the oral studies, liver residues after topical treatment were always found to be lower than the respective analytical limit. The absence of any measurable cyfluthrin concentrations in liver tissues after dermal application should be explained.
3. The additional information about the routine analytical method which has been presented to the German authorities in annex form (national application, RA 200/1991 and RA 241/1991) should be integrated into the documentation. These points are :
  - the specificity of the method with respect to flumethrin and two cyfluthrin metabolites;
  - the purity of the reagents used;
  - the reference substances (the reference substance certificate and the correct naming of the diastereomers in the GC elution order).
4. Information should be provided with regard to :
  - the sample storage conditions to be used;
  - the stability of cyfluthrin in tissue and milk samples during storage;
  - the stability of the analyte (cyfluthrin) in the extract and in the standard solution.
5. The final version of the routine analytical method should be presented in an internationally recognised format (e.g. ISO 78/2).
6. In view of the methodological problems (extraction problems as discussed in the addendum to the Mobay report 86045) concerning incurred liver and kidney samples, it should be demonstrated that these problems will not arise when the routine analytical method is used for residue monitoring. (It should be considered whether additional data on validation for incurred liver and kidney samples should be provided or not).