5.14 FLUFENOXURON (275)

TOXICOLOGY

Flufenoxuron is the ISO-approved common name for *N*-{4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl}-*N*′-(2,6-difluorobenzoyl)urea (IUPAC), which has the CAS number 101463-69-8. Flufenoxuron is a benzoylurea insecticide and acaricide that is used on fruits, vines and ornamentals to control insects and mites. The pesticidal mode of action is the inhibition of chitin synthesis.

Flufenoxuron has not previously been evaluated by JMPR and was reviewed by the present Meeting at the request of CCPR.

All critical studies contained statements of compliance with GLP. The Meeting considered that the database was adequate for the risk assessment.

Biochemical aspects

After oral administration of flufenoxuron to rats, absorption was rapid, with a time to reach C_{max} (T_{max}) of 3–6 hours; there was evidence of saturation at higher dose levels (> 80% absorption at 3.5 mg/kg bw compared with < 15% at 350 mg/kg bw in rats). Absorption was lower in dogs than in rats (< 30% in dogs at 3.5 mg/kg bw). Absorbed flufenoxuron was widely distributed throughout the body, with highest levels in fat and bone marrow. Unchanged flufenoxuron was the major residue in all tissues, faeces and urine. Flufenoxuron is metabolized by cleavage of the bond adjacent to the 2,6-difluorobenzoyl moiety, followed by oxidation or hydroxylation. The major metabolite identified in rats was 2,6-difluorobenzoic acid, together with *N*-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl] urea and 2-amino-5-(2-chloro-4-(trifluoromethyl)phenoxy)-3-fluorophenol; 2,6-difluorobenzamide and 4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorobenzenamine were minor metabolites in rats. After repeated dosing, tissue radioactivity levels decreased slowly in rats (half-life 34 days, range 28–48 days) and in dogs (half-life 33 days). The routes of excretion were faecal and, to a lesser extent, urinary. Flufenoxuron was excreted in the milk of lactating rats. Flufenoxuron was shown to accumulate in body fat.

Toxicological data

The acute toxicity of flufenoxuron is low (oral LD_{50} > 3000 mg/kg bw; dermal LD_{50} > 2000 mg/kg bw; inhalation LC_{50} > 5.1 mg/L). Flufenoxuron was not irritating to the skin or the eyes of rabbits. Flufenoxuron was not a skin sensitizer in a Magnusson and Kligman test in guinea-pigs.

In repeated-dose toxicity studies with flufenoxuron in mice, rats and dogs, multiple adverse effects were observed, in particular body weight changes and toxicity to the haematological system indicative of haemolytic anaemia. Studies in dogs showed that these animals are particularly sensitive to the haematological effects of flufenoxuron. Owing to the saturation of absorption at higher doses, dose–response curves are often flat.

In a 28-day study in mice using dietary flufenoxuron concentrations of 0, 50, 500, 5000, 10 000 and 50 000 ppm (equivalent to 0, 7.1, 71, 710, 1400 and 7100 mg/kg bw per day, respectively), the NOAEL was 10 000 ppm (equivalent to 1400 mg/kg bw per day), based on reduced feed consumption observed at 50 000 ppm (equivalent to 7100 mg/kg bw per day).

In a 13-week study in mice using dietary flufenoxuron concentrations of 0, 50, 500, 5000, 10 000 and 50 000 ppm (equal to 0, 10, 103, 1069, 2139 and 11 071 mg/kg bw per day for males and 0, 12, 124, 1247, 2482 and 12 619 mg/kg bw per day for females, respectively), the NOAEL was 50 ppm (equal to 10 mg/kg bw per day), based on increased serum bilirubin concentrations in males and females at 500 ppm (equal to 103 mg/kg bw per day).

In a 28-day study in rats using dietary flufenoxuron concentrations of 0, 50, 500, 5000, 10 000 and 50 000 ppm (equal to 0, 4.8, 49, 475, 997 and 5147 mg/kg bw per day for males and 0,

5.3, 53, 534, 1067 and 5432 mg/kg bw per day for females, respectively), the NOAEL was 50 000 ppm (equal to 5147 mg/kg bw per day), the highest dose tested.

In a 13-week study in rats using dietary flufenoxuron concentrations of 0, 50, 500, 5000, 10 000 and 50 000 ppm (equal to 0, 3.5, 35, 351, 689 and 3637 mg/kg bw per day for males and 0, 4.1, 41, 399, 820 and 4151 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 41 mg/kg bw per day), based on slightly higher spleen weights and haematological changes indicative of mild anaemia observed in females at 5000 ppm (equal to 399 mg/kg bw per day).

In a 13-week study in dogs using dietary flufenoxuron concentrations of 0, 500, 5000 and 50 000 ppm (equal to 0, 18, 163 and 1961 mg/kg bw per day for males and 0, 21, 182 and 2039 mg/kg bw per day for females, respectively), the LOAEL was 500 ppm (equal to 18 mg/kg bw per day), based on haemolytic anaemia and associated changes. No NOAEL could be identified.

In a 1-year study in dogs using dietary flufenoxuron concentrations of 0, 10, 100, 500 and 50 000 ppm (equal to 0, 0.36, 3.5, 19 and 1898 mg/kg bw per day for males and 0, 0.36, 3.8, 19 and 1879 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 3.5 mg/kg bw per day), based on evidence of haemolytic anaemia and related changes at 500 ppm (equal to 19 mg/kg bw per day).

The overall NOAEL for the 13-week and 1-year dog studies was 100 ppm (equal to 3.5 mg/kg bw per day). The overall LOAEL was 500 ppm (equal to 18 mg/kg bw per day).

In a 2-year carcinogenicity study in mice using dietary flufenoxuron concentrations of 0, 100, 1000 and 10 000 ppm (equal to 0, 15.3, 152 and 1592 mg/kg bw per day for males and 0, 17.4, 187 and 1890 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 187 mg/kg bw per day), based on decreased body weight gain in females at 10 000 ppm (equal to 1890 mg/kg bw per day). There was no evidence of carcinogenicity.

In a second 2-year carcinogenicity study in mice, using dietary flufenoxuron concentrations of 0, 500, 5000 and 50 000 ppm (equal to 0, 56, 559 and 7356 mg/kg bw per day for males and 0, 73, 739 and 7780 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 56 mg/kg bw per day), based on an increased incidence of lordotic episodes, decreased body weight gain, increased Kupffer cell aggregates in females and increased liver weight relative to brain weight in males at 5000 ppm (equal to 559 mg/kg bw per day). An increased incidence of spleen haemangiosarcomas was observed in females dosed at 50 000 ppm. However, as this finding was not accompanied by an increase in angiomas, it was not considered to be treatment related. An apparent increased incidence of hepatocellular carcinomas in treated male mice in this study was considered not treatment related because the incidence in control males was low.

The overall NOAEL for systemic toxicity in the 2-year studies in mice was 1000 ppm (equal to 187 mg/kg bw per day). The overall LOAEL was 5000 ppm (equal to 559 mg/kg bw per day).

In a 2-year toxicity and carcinogenicity study in rats using dietary flufenoxuron concentrations of 0, 1, 5, 50, 500, 5000 and 50 000 ppm (equal to 0, 0.044, 0.23, 2.2, 22, 230 and 2470 mg/kg bw per day for males and 0, 0.055, 0.28, 2.8, 28, 300 and 3210 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 22 mg/kg bw per day), based on decreased body weight gain, a small increase in non-cyanide-binding haemoglobin, considered to reflect sulfhaemoglobin, and decreased triglyceride levels in both sexes at 5000 ppm (equal to 230 mg/kg bw per day). Flufenoxuron was not carcinogenic under the conditions of the study.

In a second 2-year toxicity and carcinogenicity study in rats, using dietary flufenoxuron concentrations of 0, 500, 5000 and 50 000 ppm (equal to 0, 21.6, 218 and 2290 mg/kg bw per day for males and 0, 25.9, 276 and 2901 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 25.9 mg/kg bw per day), based on reduced body weight gain in females at 5000 ppm (equal to 276 mg/kg bw per day). No carcinogenic potential of flufenoxuron was observed.

The overall NOAEL for systemic toxicity in the 2-year studies in rats was 500 ppm (equal to 25.9 mg/kg bw per day). The overall LOAEL was 5000 ppm (equal to 230 mg/kg bw per day).

The Meeting concluded that flufenoxuron is not carcinogenic in mice or rats.

Flufenoxuron was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. There was no evidence of genotoxicity in vitro, with the exception of two assays of chromosomal aberrations in Chinese hamster ovary cells, and there was no evidence of genotoxicity in vivo. The Meeting concluded that flufenoxuron is unlikely to be genotoxic in vivo.

In view of the lack of genotoxicity in vivo and the absence of carcinogenicity in mice and rats at exposure levels that are relevant for human dietary risk assessment, the Meeting concluded that flufenoxuron is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation reproductive toxicity study in rats using dietary flufenoxuron concentrations of 0, 50, 190, 710 and 10 000 ppm (premating dietary intakes were equal to 0, 3.8, 14.3, 53.6 and 771 mg/kg bw per day for males and 0, 4.3, 16.0, 61.0 and 907 mg/kg bw per day for females of the F_0 generation and 0, 4.2, 16.1, 62.5 and 865 mg/kg bw per day for males and 0, 4.8, 18.7, 69.2 and 956 mg/kg bw per day for females of the F_1 generation, respectively), the NOAEL for parental toxicity was 50 ppm (equal to 4.2 mg/kg bw per day), based on decreased body weight gain in males of the F_{1B} generation at 190 ppm (equal to 16.1 mg/kg bw per day). The NOAEL for offspring toxicity was 50 ppm (equal to 3.8 mg/kg bw per day), based on a reduction in average body weight gain during lactation of male and female pups of all generations at 190 ppm (equal to 14.3 mg/kg bw per day). The NOAEL for reproductive toxicity was 10 000 ppm (equal to 771 mg/kg bw per day), the highest dose tested. In this study, adverse effects on pup survival and growth were observed at 710 ppm (equal to 53.6 mg/kg bw per day). Subsequent studies, including a cross-fostering study in rats, failed to further elucidate the mechanism for the adverse effects on pup survival.

In a developmental toxicity study in rats using gavage flufenoxuron doses of 0, 7.9, 81 and 967 mg/kg bw per day, the NOAEL for maternal toxicity and for embryo and fetal toxicity was 967 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits using gavage flufenoxuron doses of 0, 7.7, 100 and 1000 mg/kg bw per day, the NOAEL for maternal toxicity and for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

The Meeting concluded that flufenoxuron is not teratogenic.

In a 28-day neurotoxicity study in rats using dietary flufenoxuron concentrations of 0, 1000, 5000 and 20 000 ppm (equal to 0, 88, 435 and 1745 mg/kg bw per day for males and 0, 95, 475 and 1934 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 88 mg/kg bw per day), based on reductions in body weight gain in males at 5000 ppm (equal to 435 mg/kg bw per day). No evidence of neurotoxicity was observed in this or other studies.

The Meeting concluded that flufenoxuron is not neurotoxic.

Toxicological data on metabolites and/or degradates

Acute toxicity and genotoxicity studies were performed with Reg. No. 241208 (4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorobenzenamine), a minor faecal (rats and dogs) and urinary (rats) metabolite and a minor residue in hens; and with Reg. No. 4064702 (*N*-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl] urea), which is a soil metabolite, a minor metabolite found in the urine of rats (at most 1% of the administered flufenoxuron dose), a water–sediment system product and a minor residue in hens.

In studies of acute oral toxicity in mice with Reg. No. 241208, the oral LD_{50} was 1937 mg/kg bw. There was no evidence of genotoxicity in a number of in vitro and in vivo studies, except for one Ames test that indicated a weak positive response.

For Reg. No. 4064702, the oral LD_{50} was 302 mg/kg bw. An Ames test was negative.

The Meeting concluded that these metabolites are not toxicologically relevant for a dietary risk assessment.

Human data

Medical surveillance of personnel at flufenoxuron manufacturing plants revealed no unusual or abnormal health effects, except for one case of skin allergy in a worker potentially exposed to flufenoxuron.

The Meeting concluded that the existing database on flufenoxuron was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.04 mg/kg bw for flufenoxuron, on the basis of the overall NOAEL of 3.5 mg/kg bw per day for a range of effects indicative of haemolytic anaemia in 13-week and 1-year dietary studies in dogs, using a safety factor of 100. This ADI was supported by a two-generation dietary reproductive toxicity study in rats, with a NOAEL for parental toxicity of 4.2 mg/kg bw per day, based on decreased body weight gain in males, and a NOAEL for offspring toxicity of 3.8 mg/kg bw per day, based on a reduction in average body weight gain during lactation of male and female pups of all generations. An additional safety factor to extrapolate to lifetime exposure was considered unnecessary, as the LOAELs in the 13-week and 1-year studies in dogs were both 500 ppm (equal to 18–19 mg/kg bw per day), and as the concentrations of flufenoxuron in blood and fat in dogs appear to reach steady state after 3 months of treatment, indicating that effects at lower doses with more prolonged exposure are not expected.

The Meeting concluded that it was not necessary to establish an ARfD for flufenoxuron in view of its low acute toxicity, the absence of developmental toxicity and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

A toxicological monograph was prepared.

Levels relevant to risk assessment of flufenoxuron

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day study of toxicity[a]	Toxicity	50 ppm, equal to 10 mg/kg bw per day	500 ppm, equal to 103 mg/kg bw per day
	Two-year studies of carcinogenicity[a,b]	Toxicity	1 000 ppm, equal to 187 mg/kg bw per day	5 000 ppm, equal to 559 mg/kg bw per day
		Carcinogenicity	50 000 ppm, equal to 7 356 mg/kg bw per day[c]	–
Rat	Two-year studies of toxicity and carcinogenicity[a,b]	Toxicity	500 ppm, equal to 25.9 mg/kg bw per day	5 000 ppm, equal to 230 mg/kg bw per day
		Carcinogenicity	50 000 ppm, equal to 2 470 mg/kg bw per day[c]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	10 000 ppm, equal to 771 mg/kg bw per day[c]	–
		Parental toxicity	50 ppm, equal to 4.2 mg/kg bw per day	190 ppm, equal to 16.1 mg/kg bw per

Species	Study	Effect	NOAEL	LOAEL
				day
		Offspring toxicity	50 ppm, equal to 3.8 mg/kg bw per day	190 ppm, equal to 14.3 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	967 mg/kg bw per day[c]	–
		Embryo and fetal toxicity	967 mg/kg bw per day[c]	–
Rabbit	Developmental toxicity study[d]	Maternal toxicity	1 000 mg/kg bw per day[c]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[c]	–
Dog	Ninety-day and 1-year studies of toxicity[a,b]	Toxicity	100 ppm, equal to 3.5 mg/kg bw per day	500 ppm, equal to 18 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.

Estimate of acceptable daily intake (ADI)

0–0.04 mg/kg bw

Estimate of acute reference dose (ARfD)

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to flufenoxuron

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rats: Rapid; > 80% in both sexes at 3.5 mg/kg bw; > 3% in males and 12% in females at 350 mg/kg bw
	Dogs: Rapid; > 30% in both sexes at 3.5 mg/kg bw
Dermal absorption	No data
Distribution	Rats and dogs: Widespread distribution, highest concentrations found in fat and, to a lesser extent, bone marrow
	Present in milk in lactating rats
Potential for accumulation	Fat/blood residue concentration ratios were 53 (rats) and 19 (dogs) 7 days post-dosing
	Rats: Half-life in various organs after repeated administration is 20–48 days
	Dogs: Half-life in blood is 33 days
	Potential for accumulation over repeated dosing

Rate and extent of excretion	Slow, mainly via faeces (biliary excretion) and urine
Metabolism in animals	Limited metabolism; major metabolite was 2,6-difluorobenzoic acid, accounting for 10–12% of the administered dose in 0- to 48-hour urine
Toxicologically significant compounds in animals and plants	Parent
Acute toxicity	
Rat, LD_{50}, oral	> 3 000 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.1 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Haemolytic anaemia
Lowest relevant oral NOAEL	3.5 mg/kg bw per day (dogs)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight gain, haemolytic anaemia
Lowest relevant NOAEL	25.9 mg/kg bw per day (rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans from the diet
Genotoxicity	
	Unlikely to be genotoxic in vivo
Reproductive toxicity	
Target/critical effect	None
Lowest relevant parental NOAEL	4.2 mg/kg bw per day
Lowest relevant offspring NOAEL	3.8 mg/kg bw per day
Lowest relevant reproductive NOAEL	771 mg/kg bw per day, highest dose tested
Developmental toxicity	
Target/critical effect	None
Lowest relevant maternal NOAEL	967 mg/kg bw per day, highest dose tested (rat)
Lowest relevant embryo/fetal NOAEL	967 mg/kg bw per day, highest dose tested (rat)
Neurotoxicity	
Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	1745 mg/kg bw per day, highest dose tested
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Studies on toxicologically relevant metabolites	Reg. No. 241208: Mouse LD_{50} oral = 1937 mg/kg bw Genotoxicity: unlikely to be genotoxic Reg. No. 406 4702: Mouse LD_{50} oral = 302 mg/kg bw Not mutagenic in Ames test

Medical data	
	One case of skin allergy possibly related to exposure to flufenoxuron

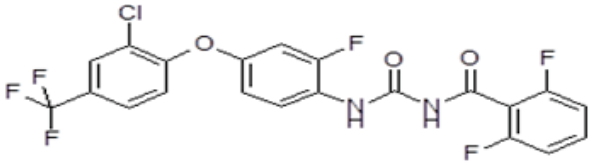
Summary

	Value	Study	Safety factor
ADI	0–0.04 mg/kg bw	Thirteen-week and 1-year studies of toxicity (dog)	100
ARfD	Unnecessary	–	–

RESIDUE AND ANALYTICAL ASPECTS

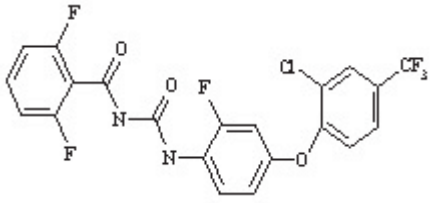
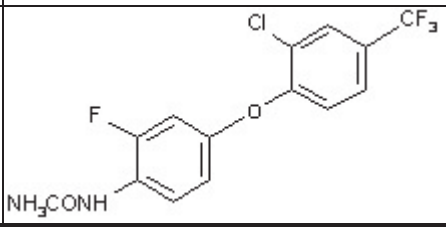
Flufenoxuron is a benzylurea insect growth regulator used to kill mites and insects, through interference with chitin production during cuticle development in mite and insect juvenile stages, on various orchard crops, fruiting vegetables and tea. It was considered for the first time by the 2014 JMPR for toxicology and residues.

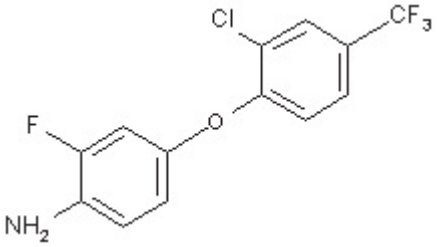
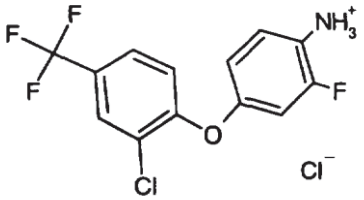
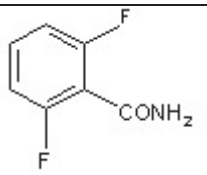
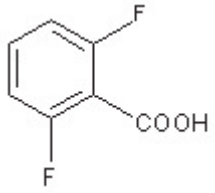
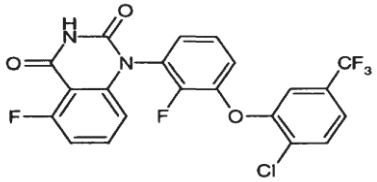
The Meeting received information on physical chemical properties, livestock and plant metabolism, environmental fate, analytical methods, storage stability, supervised residue trials, use patterns, processing and livestock feeding.



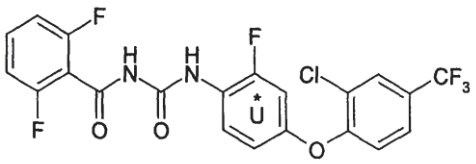
The IUPAC name of flufenoxuron is N-{4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl}-N'-(2,6-difluorobenzoyl)urea and the CA name is N-[[[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl]amino]carbonyl]-2,6-difluorobenzamide.

Common chemical names, code names and structures of the parent and metabolites are captured below:

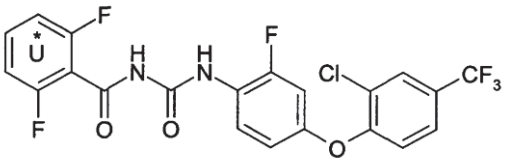
Code	Structure	Occurrence
Flufenoxuron WL 115110		Rat Lactating goat Laying hen Grape Apple Tomato Chinese cabbage Soil Hydrolysis study
Reg. No. 4064702		Rat Laying hen Soil Hydrolysis study

Code	Structure	Occurrence
Reg. No. 241208		Rat Laying hen Hydrolysis study
Reg. No. 4064703 (chloride salt of Reg. No. 241208)		Hydrolysis study
Reg. No. 102719		Rat Hydrolysis study
Reg. No. 206925		Rat Hydrolysis study
Reg. No. 4964847		Hydrolysis study

Flufenoxuron uniformly labelled in either the fluoroaniline or difluoroamide rings was used in the metabolism and environmental fate studies.



Fluoroaniline U-^{14}C



Difluoroamide U-^{14}C

Animal metabolism

Information was available on the metabolism of flufenoxuron in laboratory animals, lactating goats and laying hens.

Metabolism studies in rats demonstrated that unchanged flufenoxuron accounted for the majority of the total applied radioactivity (TAR) in faeces, with minor metabolites (less than 1% of the TAR) identified as 2-amino-5-(2-chloro-α,α,α-trifluoro-p-tolyoxy)-3-fluorophenol (Reg. No.

4110959), Reg. No. 4064702, Reg. No 241208, Reg. No 102719 and Reg. No. 206925. For organs and tissues, parent flufenoxuron was the main component observed.

In the lactating goat metabolism study, one goat received four daily doses of 2-fluoroaniline-[U-^{14}C]-ring-labelled flufenoxuron at a rate equivalent to 10 ppm in the diet (10 mg/day). The animal was sacrificed 24 hours after administration of the last dose. While the majority of the radioactivity was excreted via the faeces (18% of the TAR) and urine (2.5% of the TAR), milk and tissues accounted for ≤ 10% of the TAR. Total recovered radioactivity was low (33%). No explanation for the low recovery was evident.

The total radioactive residues (TRRs) were highest in fat (1.6 mg eq/kg), followed by liver (0.37 mg eq/kg), kidney (0.13 mg eq/kg) and muscle (0.076 to 0.1 mg eq/kg). Milk residues peaked on day 4 (average of 0.27 mg eq/L) with the highest concentrations of radioactivity detected in the cream fraction (accounting for 82–93% of the TRR in whole milk) and the lowest found in whey (1.3–5.7% of the TRR). Following solvent extraction, residue extractabilities were 66–100%.In milk and all tissues sampled, the flufenoxuron molecule remained intact with no other metabolites being detected.

Two studies on metabolism in laying hens were available. In the first study, the laying hens received 14 daily doses of flufenoxuron, uniformly labelled in the difluoroamide or fluoroaniline rings, at 13–14 ppm in the feed. The animals were sacrificed approximately 23 h after the last dose. Excreta accounted for 72–78% of the TAR. No plateau was reached in eggs during the dosing period (14 days); however, the radioactivity in eggs and tissues amounted to 1.0–1.3% and 2.6–3.6% of the TAR, respectively. Among all the tissues analysed, radioactive residues were highest in fat (5.0–5.3 mg eq/kg) followed by liver (0.6–1.1 mg eq/kg) and muscle (0.3–0.4 mg eq/kg). The total recovery of radioactivity was 82% and 77% in the groups administered the difluoroamide- and fluoroaniline-labelled flufenoxuron, respectively.

Solvent extraction released 91–102% and 88–99% of the TRRs for the difluoroamide- and fluoroaniline-label, respectively. While the lowest extractability occurred in the liver of the fluoroaniline-labelled study (88% of the TRR), microwave extraction of the liver post-extraction solid (PES) sample released another 8% of the TRR. For the difluoroamide-label, the parent was the only analyte identified in eggs, muscle, fat and liver ranging from 0.28 mg eq/kg (86.5% of the TRR, muscle) to 4.6 mg eq/kg (91.4%, fat).

For the fluoroaniline-label, the parent compound accounted for the majority of the TRRs (70–91%) in the eggs, muscle, fat and liver. The lowest level of parent was found in muscle (0.30 mg eq/kg) with the highest observed in fat (4.8 mg eq/kg). In eggs and liver, Reg. No. 4064702 was present at 0.10 mg eq/kg and 0.13 mg eq/kg (12.0% and 12.6% of the TRR, respectively) while in muscle and fat, Reg. No. 4064702 was a minor metabolite amounting to 0.02 mg eq/kg and 0.05 mg/kg, respectively (5.5% and 1.0% of the TRR). The formate derivative of Reg. No. 241208 was released from the PES of liver after microwave treatment in the presence of formic acid/acetonitrile. The radioactivity associated with this derivative amounted to 0.04 mg eq/kg (3.3% TRR). The Meeting could not confirm whether the metabolite Reg. No. 241208 is an actual in-vivo metabolite or an artefact formed during microwave treatment.

In the second study, laying hens received seven consecutive daily doses of flufenoxuron uniformly labelled in the fluoroaniline ring at a rate equivalent to 10 ppm in the diet. Hens were sacrificed 22 hours following administration of the last dose. To investigate the depuration behaviour of flufenoxuron, four groups of three laying hens each were sacrificed at 2, 9, 16, and 34 days after the last administration.

On average, 26% of the TAR was excreta-related with eggs, sampled from 0–166 h after the first administration, accounting for 5% of the TAR. At sacrifice, the highest amount of radioactive residues was detected in fat (47% of the TAR), followed by skin (12% of the TAR), muscle (4% of the TAR), liver (2% of the TAR), kidney (0.3% of the TAR), heart and gizzard (combined 0.2% TAR). The recovery of radioactivity amounted to 96% of the TAR.

Solvent extraction (including incubation of liver and kidney samples at 37 °C) released 91–102% of the TRRs). The parent compound was the predominant analyte detected in yolks, liver, kidney, muscle, gizzard and heart while it was the only compound detected in fat and skin. The metabolite Reg. No. 4064702 was detected in yolks, liver, kidney, muscle, gizzard, and heart at 6–22% of the TRR, while the minor metabolite Reg. No. 241208 was only detected in liver and kidney at ≤ 4% of the TRR, the only matrices that were incubated for 16 hours at 37 °C in 0.07M phosphate buffer at pH 7.5 prior to extraction

In the depuration study, radioactivity in egg yolks decreased steadily from a mean of 0.02 mg eq./kg, 2 days after cessation of dosing to 0.006 mg eq.kg on depuration day 34. Similarly, radioactivity in muscle decreased from 0.28 mg eg/kg to 0.06 mg eq/kg, during this same interval In kidney and liver, the decrease in radioactivity was more prominent from day 16 to day 34 of the depuration phase (kidney; 0.48 mg eq/kg to 0.17 mg eq/kg and liver; 0.89 mg eq/kg to 0.42 mg eq/kg) yet in fat, the decrease in radioactivity occurred most rapidly from day 2 to day 9 (13.18 mg eq/kg to 6.00 mg eq/kg) and from day 16 to day 34 (4.6 mg eq/kg to 1.97 mg eq/kg). These results demonstrate that radioactive residues are not retained in eggs, organs and tissues after cessation of dosing.

In both laying hen studies, the metabolic pattern was comparable with unchanged flufenoxuron accounting for the majority of the radioactivity, representing ≥ 60% of the TRRs in eggs and tissues. The minor metabolites Reg. No. 4064702 (eggs and tissues) and Reg. No. 241208 (liver and kidney), resulting from the cleavage of the benzoyl urea bond, were also observed to a limited extent (≤ 12% of the TRRs; except in the kidney where Reg. No. 4064702 represented 22% of the TRRs).

The Meeting concluded that in the lactating goat metabolism study, the parent flufenoxuron remained intact and was the only residue identified in milk and all tissues. In the laying hen metabolism studies, while flufenoxuron was the predominant residue in eggs and tissues, cleavage of the benzoyl urea bond was observed to a limited extent resulting in the formation of the metabolites Reg. No. 4064702 (eggs and tissues) and Reg. No. 241208 (liver and kidney).which were also identified in the rats.

Plant metabolism

The Meeting received metabolism studies for flufenoxuron following foliar applications of either [difluorobenzamide-U-^{14}C]- or 2-fluoroaniline-[U-^{14}C]-ring-flufenoxuron to grape, apple, tomato and Chinese cabbage.

Two foliar sprays were made to grape vines, grown outdoor and protected with plastic covers after application, during fruit development; at a rate of 0.04 kg ai/ha/application, with a 40-day retreatment interval, resulting in a total rate of 0.08 kg ai/ha. Immature leaves were collected at 15 DAT (days after last treatment) while mature leaves, stalks and fruit (from grape clusters) were harvested 28–29 DAT. TRRs in leaves declined from 2.3–2.7 mg eq/kg at 15 DAT to 1.4–1.8 mg eq/kg at 29 DAT. TRRs in mature fruit and stalks were 0.012–0.014 mg eq/kg and 0.11–0.16 mg eq/kg, respectively. Solvent extraction released approximately 95–97% of the TRR (0.012–2.6 mg eq/kg) from the grape matrices. Flufenoxuron was the only compound identified in all fruit, leaf and stalk samples (50–97% of the TRR; 0.007–2.2 mg eq/kg). Polar unknowns comprised up to 40–46% of the TRR in mature grape samples (0.005–0.006 mg eq/kg) with unextracted residues in all leaf, fruit and stalk samples accounting for ≤ 5% of the TRR (< 0.11 mg eq/kg).

Ten apple trees, maintained in glasshouses, were sprayed with flufenoxuron, uniformly labelled in the fluoroaniline ring. A single application of the dispersible concentrate was made to trees, during fruit development, at a rate of 0.01 kg ai/hL. Samples of immature fruit were harvested 0 days (4 h post-treatment) and 46 days after treatment (DAT), and mature fruit samples were collected at 99 DAT. TRRs in immature fruit were 2.6 mg eq/kg (0 DAT) and declined to 0.16 mg eq/kg (46 DAT) and 0.06 mg eq/kg (99 DAT). The radioactivity in the combined acetonitrile and hexane

surface washes decreased with increasing DAT, from 96% of the TRRs at 0 DAT to 77% of the TRRs at 99 DAT, with a corresponding increase in TRRs in fruit extracts (3.7% TRR at 0 DAT to 23% of the TRRs at 99 DAT), demonstrating limited translocation. The parent flufenoxuron accounted for the majority of the TRRs in surface washes (74–93%; 0.043–2.4 mg eq/kg) and in fruit extracts (3–16% of the TRRs; (0.01–0.08 mg eq/kg).

A single broadcast foliar application of 2-fluoroaniline-[U-^{14}C]-ring-flufenoxuron, formulated as an emulsifiable concentrate, was made to tomato plants, maintained outdoor, during fruit development at a rate of 0.125 kg ai/ha. Tomato fruit was harvested at 0 and 28 DAT. TRRs in/on tomato fruit declined from 0.38 mg eq/kg on day 0, to 0.2 mg eq/kg by day 28. The total extracted residues (ACN:water surface washes and fruit extracts) from 0 DAT to 28 DAT, accounted for 94–99% of the TRR (0.16–0.38 mg eq/kg), mainly from the surface wash (≥ 94% of the TRRs). Flufenoxuron was the only identified residue in the mature tomato sample (91% of the TRRs).

2-Fluoroaniline-[U-^{14}C]-ring-flufenoxuron, formulated as an emulsifiable concentrate, was applied once to Chinese cabbage plants, grown outdoor, during leaf development, as a foliar application, at a rate equivalent to 0.10 kg ai/ha. Cabbage plants were harvested at 0 and 28 DAT. TRRs in/on cabbage wrapper leaves declined from 6.3 mg eq/kg on day 0 to 0.35 mg eq/kg by day 28. At 0 DAT, the surface wash represented the majority of the extracted residues (84% of the TRRs; 5.3 mg eq/kg) while at the 28 DAT, the leaf extracts accounted for a greater fraction of the extractable radioactivity (76% of the TRRs; 0.27 mg eq/kg). The parent flufenoxuron was the only identified residue in mature cabbage leaves (93% of the TRRs).

The Meeting concluded that the metabolism of flufenoxuron in grape, apple, tomato and Chinese cabbage is consistent among all crops, where parent flufenoxuron remained intact. No other metabolites were identified and no other residues were characterized (other than polar unknowns). The Meeting agreed that the majority of radioactivity remained on the leaves or surface of the fruit, with limited translocation.

Environmental fate in soil

The Meeting received information on aerobic degradation in soil.

In these studies, the fluoroaniline-specific metabolite, Reg. No. 4064702, was the only metabolite identified, reaching a maximum concentration after 30 days of incubation (4.1–8.3% TAR). The predominant residue, flufenoxuron, decreased to 45.8–51.0% TAR in the soil after 119 days, resulting in a calculated DT_{50} for flufenoxuron of 115–122 days. Considering the persistence of flufenoxuron, it is desirable that confined rotational crop and field accumulation studies be submitted.

Methods of residue analysis

The Meeting received analytical methods for the analysis of flufenoxuron in plant and animal commodities. The basic principle for plant commodities employs extraction by homogenisation with dichloromethane, methanol/water/HCl or acetone followed by partitioning with water/cyclohexane. For animal matrices, flufenoxuron residues are extracted by homogenization with various non-polar organic solvents followed by liquid partitioning and/or clean-up by normal-phase or reverse-phase HPLC prior to analysis. Residues of flufenoxuron are measured by HPLC-MS/MS with two specific mass transitions or with HPLC-UV at 254–260 nm. The applicability of the proposed enforcement methods was confirmed in various independent laboratories where parent flufenoxuron was analyzed with validated LOQs of 0.05 mg/kg for plant and animal commodities and eggs, and 0.01 mg/kg for milk.

The multiresidue method DFG S-19 was tested, for the analysis of flufenoxuron in animal matrices only, and found to be unsuitable.

A number of scientific papers report the validation of the QuEChERS multi-residue method using GC-MS/MS for flufenoxuron in various plant commodities.

The Meeting concluded that the available enforcement analytical methods are suitable for determining residues of flufenoxuron in plant and animal commodities with LOQs, ranging from 0.01–0.05 mg/kg depending on the matrix.

Stability of residues in stored analytical samples

Based on the storage stability data submitted, the Meeting concluded that no significant dissipation of flufenoxuron residues was observed in cottonseed, orange, grape, and apple after 36 months of storage, in lettuce after 27 months and in watermelon (pulp and peel) after 26 months.

The Meeting agreed that no degradation of flufenoxuron residues was observed in animal matrices stored for up to 53 months of storage, except egg whites, where flufenoxuron residues were determined to be stable for up to 4 months.

Definition of the Residue

In the lactating goat metabolism study, flufenoxuron was the only residue identified in tissues and milk with no other metabolites detected. Similarly, in the laying hen metabolism studies, flufenoxuron accounted for the majority of the radioactivity in eggs, muscle, fat, liver and kidney (60–104% of the TRRs).

Therefore, the Meeting recommends the residue definition for compliance with MRL for animal commodities as flufenoxuron.

The Log K_{ow} of flufenoxuron is 4. In the goat metabolism study, highest levels of the parent compound were observed in fat and cream, while in the laying hen metabolism studies, the highest concentrations of flufenoxuron were observed in the fat (≤ 98% of the TRRs). These findings were supported by the livestock feeding studies, where the average ratio for cream/skim milk was ≥ 155 and ≥ 112 for egg yolks/egg whites. Further to this, residues in fat were 24–30-fold higher than those in muscle.

In light of this, the Meeting concluded that the residue is fat soluble.

The metabolite Reg. No. 4064702 was also identified in laying hen muscle, fat, liver, kidney and eggs (1–22% of the TRRs) with the highest levels observed in liver and kidney. The minor metabolite Reg. No. 241208 was also observed but only in liver and kidney (2.5–3.7% of the TRRs) which were the only matrices that were subject to microwave extraction (liver only) or incubation at 37 °C in 0.07M phosphate buffer at pH 7.5 prior to extraction (liver and kidney), and hence considered a potential artefact of the analytical procedure.

The toxicity of the minor metabolite Reg. No. 241208, found in laying hen matrices, was considered to be covered by toxicity studies on flufenoxuron since this metabolite was seen in the rat. The metabolite Reg. No. 4064702, also observed in eggs and tissues of laying hens, and observed in the rat, was determined to be more acutely toxic than the parent flufenoxuron based on the LD_{50}. However, according to the poultry feeding study, residues of this metabolite in liver are not expected to exceed 0.04 mg/kg at the lowest feeding level of 1 ppm. Hence, as there are no poultry feed items derived from the proposed crops, the dietary exposure to this metabolite from poultry matrices is unlikely.

The Meeting recommends the residue for dietary intake for animal commodities as parent only.

The fate of flufenoxuron in plants was investigated following foliar application to tomato, apple, grape and Chinese cabbage. In all plant commodities tested, flufenoxuron was the predominant residue accounting for > 90% of the TRR, with the exception of grape, where flufenoxuron accounted

for 50% of the TRR. No other metabolites were identified and no other residues were characterized (other than polar unknowns).

According to the hydrolysis study, simulating typical processing conditions (pH 4, 5 and 6 with 90 °C, 100 °C and 120 °C for 20, 60 and 20 minutes), flufenoxuron was degraded to various metabolites including: Reg. No. 102719 (8–32%), Reg. No. 4064702 (4%) and Reg. No. 4964847 (4–9%). All metabolites, except Reg. No. 4064702 and Reg. No. 4964847 are considered to be covered by toxicity studies on flufenoxuron, since they were seen in the rat. The absorption, distribution, metabolism and excretion studies in rat demonstrated that Reg. No. 4064702 was more acutely toxic than the parent flufenoxuron. Conversely, no toxicity information is available on metabolite Reg. No. 4964847, Reg. No. 4064702. Nevertheless, in the tomato processing study where these metabolites were measured in juice, purée and canned tomatoes, none were detected (< 0.01 mg/kg)

The Meeting recommended the following residue definition for flufenoxuron:

Definition of the residue for compliance with MRL and for estimation of dietary intake for plants and animal commodities: *flufenoxuron*

The Meeting considers the residue fat soluble.

Results of supervised residue trials on crops

The Meeting received supervised residue trials from Brazil, Europe and South Africa where flufenoxuron was applied to oranges, apples, pears, melons and tomatoes and Japanese trials on tea.

Oranges

The critical GAP in Brazil for flufenoxuron on oranges is up to two foliar applications of 0.005 kg ai/hL, with a re-treatment interval of 30 days and a PHI of 15 days. Sixteen supervised field trials were conducted in Greece, Italy, South Africa and Brazil.

Four of the trials were conducted in Brazil according to the critical GAP. Residues in whole oranges at the 15-day PHI were: 0.09, 0.11, 0.13 and 0.16 mg/kg.

Five trials in Brazil were conducted at 2× 0.002–0.003 kg ai/hL with a PHI of 15 days, representing 0.4–0.6× the critical GAP in Brazil. The residues in whole fruit were 0.03, 0.05, 0.07, 0.08 and 0.10 mg/kg. The Meeting agreed to use the proportionality approach to scale the residues at the 15-day PHI according to an application rate of 0.005 kg ai/hL. The rank order of scaled residues in whole fruit was (n=5): 0.08 (2), 0.13 (2), and 0.17 mg/kg.

When combining all the residue data, residues in whole oranges were 0.08 (2), 0.09, 0.11, 0.13 (3), 0.16 and 0.17 mg/kg and residues in pulp were 0.03, 0.04, 0.05 (3), 0.06, 0.08 (2) and 0.10 mg/kg.

The Meeting estimated a maximum residue level of 0.4 mg/kg, and a median residue of 0.13 mg/kg for residues of flufenoxuron in whole oranges. For orange pulp, the Meeting estimated an STMR of 0.05 mg/kg.

Apples

The Brazil critical GAP for flufenoxuron on apples is a single foliar application at 0.01 kg ai/hL and a PHI of 35 days.

Three trials on apples were available from Chile where trees were treated at 0.015–0.02 kg ai/hL with PHIs of 35–36 days, representing 1.5–2× the critical GAP in Brazil. The residues in the fruit were 0.17, 0.43 and 0.52 mg/kg. The Meeting agreed to use the proportionality approach to scale the residues at the PHIs of 35–36 days according to the application rate of 0.01 kg ai/hL. The scaled residues, in ranked order, were: 0.08, 0.22 and 0.35 mg/kg (n=3).

In two trials conducted in Chile in accordance with Brazilian GAP, flufenoxuron residues were 0.07 and 0.45 mg/kg.

The Meeting concluded that the number of trials available was insufficient to estimate a maximum residue level for residues of flufenoxuron in apples.

Melons

There is no GAP in Brazil for flufenoxuron on melons; therefore, the Meeting could not recommend a maximum residue level.

Tomatoes

There is no GAP in Brazil for flufenoxuron on tomatoes; therefore, the Meeting could not recommend a maximum residue level.

Tea

The critical GAP for flufenoxuron in Japan for tea is up to two foliar applications of 0.025 kg ai/hL at a re-treatment interval of 7–14 days and a PHI of 7 days.

In seven of the eleven trials conducted in Japan and matching the critical GAP, residue levels in tea (green) were: 2.37, 2.48, 3.95, 4.58, 6.02, 6.23 and 11.8 mg/kg.

The Meeting estimated a maximum residue level of 20 mg/kg and a STMR of 4.58 mg/kg for residues of flufenoxuron in tea, green, black (black, fermented and dried).

Fate of residues during processing

Nature of residues

The Meeting received information on the hydrolysis of flufenoxuron uniformly labelled in the fluoroaniline and difluoroamide rings where typical processing conditions were simulated (pH 4,5 and 6 with 90 °C, 100 °C and 120 °C for 20, 60 and 20 minutes).

In duplicate samples of sterile buffer solution flufenoxuron (accounting for 63–93% of the TAR) was seen to hydrolyse to various metabolites, however, none accounted for greater than 10%of the TAR, with the exception of Reg. No. 102719, present at 32% of the TAR, following hydrolysis conditions simulating baking, boiling and brewing procedures.

Level of residues

The Meeting also received information on the fate of flufenoxuron residues during the processing of the raw agricultural commodities like tomato to juice, pomace, puree and canned tomatoes and tea to infusion. While the magnitude of the residues of Reg. No. 102719in tea infusion was not elucidated, in the tomato processing study, the residues of flufenoxuron metabolites, including Reg. No. 102719, Reg. No. 4064702 and Reg. No. 4964847 in all processed tomato commodities were below the LOQ (0.01 mg/kg). However, in the absence of a critical GAP in Brazil for flufenoxuron on tomatoes, the tomato processing study was not relied upon to derive processing factors, STMR-P values and to estimate maximum residue levels for tomato processed commodities.

The processing factor obtained in the tea processing study and the estimated STMR-P value for the dietary intake calculation is summarized below:

Raw agricultural commodity	STMR, mg/kg	Processed commodity (food)	Processing factor	STMR-P (mg/kg)
Tea (green)	6.02	Infusion	0.0065 (median)	0.04

Residues in animal commodities

Farm animal feeding studies

The Meeting received information on the residue levels arising in animal tissues and milk when dairy cows were fed flufenoxuron for 90 days at levels equivalent of 1.75, 5.25 and 17.5 ppm in the diet. Three animals from the highest dose level group were monitored for flufenoxuron residues over a 40-day depuration phase.

At the lowest dose tested, flufenoxuron residues in milk increased steadily over the 90-day period, from 0.0243 mg/kg on day 2 to 0.64 mg/kg on day 90, however, in the mid and high dose groups, residues seemed to plateau on day 56 (at 1.6 mg/kg) and day 70 (at 5.5 mg/kg), respectively. Analysis of milk obtained from the high dose group showed that pasteurization had no effect on the levels of flufenoxuron in milk. Residues in cream were concentrated by a factor of 1.5. In skimmed milk and acid whey, residues were lower than those of raw milk.

In all tissues tested, except subcutaneous fat, residues of flufenoxuron were dose dependant, increasing with increasing dose. In subcutaneous fat, residues were 0.8 mg/kg, 9.3 mg/kg and 8.7 mg/kg, in the low, mid and high dose groups, respectively.

The residue depuration study demonstrated that flufenoxuron residues in milk decreased slowly within the 40 days of cessation of dosing, from 5.1 mg/kg on day 91 to 0.9 mg/kg on day 130. In tissues, flufenoxuron residues decreased on average by 80% by day 130.

The Meeting also received information on the residues in tissues and eggs of laying hens when dosed with flufenoxuron for 50 days at levels equivalent to 1, 3, 10 ppm in the diet. Fifteen animals from the top dose group were monitored for flufenoxuron residues over a 40-day depuration phase.

At all feeding levels, no residues of flufenoxuron in egg white exceeded 0.05 mg/kg. However, residues in egg yolks did not reach a plateau but rather increased steadily over the 50-day period.

Residues of flufenoxuron in liver, muscle, skin and fat increased with increasing feeding level.

During the depuration study, flufenoxuron residues in egg yolks decreased more rapidly than in cattle milk over the same duration, from 32.5 mg/kg on day 51 to 2.3 mg/kg on day 90.

Farm animal dietary burden

As there is no information on citrus dry pulp, the only potential cattle feed item derived from the proposed crops and there are no poultry feed items, the Meeting did not calculate farm animal dietary burdens.

Therefore, the Meeting estimated maximum residue levels of 0.05* mg/kg for flufenoxuron in meat (from mammals other than marine mammals), edible offal (mammalian), mammalian fat (except milk fats) and 0.01* mg/kg for milks. STMRs and HRs for dietary intake estimation are 0 mg/kg for meat (from mammals other than marine mammals), edible offal (mammalian), mammalian fat (except milk fats) and milks.

The Meeting did not estimate maximum residue levels, STMRs or HRs for poultry matrices.

The residue in animal commodities is considered fat soluble.

RECOMMENDATIONS

On the basis of the data from supervised trials, the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits and for IEDI assessment.

Definition of the residue (for compliance with the MRL and for estimation of dietary intake, animal and plant commodities): *flufenoxuron*

The residue is fat soluble.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intake (IEDI) for flufenoxuron was calculated based on the recommendation for STMRs for raw and processed commodities (tea infusion) in combination with consumption data for corresponding food commodities. These results are shown in Annexe 3.

The IEDI of the 17 GEMS/Food cluster diets, based on the estimated STMRs represented 0% of the maximum ADI of 0.04 mg/kg bw, expressed as flufenoxuron. The Meeting concluded that the long-term intake of flufenoxuron residues from uses considered by the Meeting is unlikely to present a public health concern.

Short-term intake

No ARfD was considered necessary. The Meeting concluded that the short-term intake of flufenoxuron residues from uses considered by the Meeting is unlikely to present a public health concern.