5.15 FLUPYRADIFURONE (285)

TOXICOLOGY

Flupyradifurone is the ISO-approved common name for 4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan-2(5*H*)-one (IUPAC), with the CAS number 951659-40-8. Flupyradifurone is a butenolide insecticide that works by binding to and activating nicotinic acetylcholine receptors in insects.

Flupyradifurone 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.

Biochemical aspects

In studies conducted in rats using [^{14}C]flupyradifurone, maximum plasma concentrations of radioactivity were reached at 1 hour after a single oral dose of 2 mg/kg bw and 2–4 hours after a single oral dose of 200 mg/kg bw. Based on the level of radioactivity in urine and tissues following oral dosing, estimates of gastrointestinal absorption ranged from 79% in males to 91% in females. A comparison of the dose-normalized area under the plasma concentration–time curve (AUC) following equivalent oral and intravenous doses (2 mg/kg bw) in males indicated that gastrointestinal absorption was 93%. The plasma elimination half-life ranged from 3 to 8 hours. The majority (up to 90%) of radioactivity was excreted in urine within 24 hours. There was no evidence of tissue accumulation. Although flupyradifurone was the main compound detected in excreta (up to 50% of the radioactivity in males and 70% of the radioactivity in females), it undergoes hydroxylation, conjugation and cleavage reactions to generate eight identified metabolites and 19 unidentified metabolites.

Toxicological data

In rats, the oral LD_{50} was greater than 300 and less than 2000 mg/kg bw, the dermal LD_{50} was greater than 2000 mg/kg bw and the LC_{50} was greater than 4.67 mg/L. Flupyradifurone was not irritating to the skin or eyes of rabbits or a skin sensitizer in mice.

In mice, rats and dogs, the liver is the main target organ, with the thyroid an additional target in rats and the skeletal muscle an additional target in dogs. Liver enzyme induction (specifically CYP3A) and liver hypertrophy were noted in short-term repeated-dose studies in rats. A notable feature of this compound (and some of its metabolites) is its ability to reduce blood glucose levels.

In a 30-day range-finding study in mice, which tested dietary concentrations of 0, 300, 600 and 1200 ppm flupyradifurone (equal to 0, 40, 78 and 207 mg/kg bw per day for males and 0, 47, 98 and 192 mg/kg bw per day for females, respectively), reduced body weight gain occurred in males at 1200 ppm (equal to 207 mg/kg bw per day).

In a 90-day study in mice, which tested dietary concentrations of 0, 100, 500 and 2500 ppm flupyradifurone (equal to 0, 15.6, 80.6 and 407 mg/kg bw per day for males and 0, 18.8, 98.1 and 473 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 80.6 mg/kg bw per day), based on lower body weight and body weight gain, changes in clinical chemistry parameters, increased liver weights and an increase in the severity of hepatocellular vacuolation at 2500 ppm (equal to 407 mg/kg bw per day).

In a 30-day range-finding study in rats, which tested gavage flupyradifurone doses of 0, 75, 200 and 350 mg/kg bw per day, CYP3A was induced at every dose in males and at 200 and 350 mg/kg bw per day in females. Deaths and clinical signs (females), reduced glucose levels (males), increased triglyceride levels, increased alanine aminotransferase and alkaline phosphatase activities (females), increased liver weights, liver hypertrophy and thyroid follicular cell hypertrophy occurred at 200 and 350 mg/kg bw per day.

In a second 30-day range-finding study conducted only in male rats, which tested dietary flupyradifurone concentrations of 0, 410 and 5000 ppm (equal to 0, 33.6 and 385 mg/kg bw per day,

respectively), CYP3A was induced at 5000 ppm. Lower body weight, body weight gain, feed consumption and blood glucose levels, increased plasma urea and cholesterol levels, increased liver weights, liver hypertrophy and thyroid hypertrophy occurred at 5000 ppm (equal to 385 mg/kg bw per day).

In a 90-day study in rats that incorporated an assessment of neurotoxicity, dietary concentrations of 0, 100, 500 and 2500 ppm flupyradifurone (equal to 0, 6.0, 30.2 and 156 mg/kg bw per day for males and 0, 7.6, 38.3 and 186 mg/kg bw per day for females, respectively) were tested. The NOAEL was 500 ppm (equal to 30.2 mg/kg bw per day), based on lower body weight and body weight gain and thyroid follicular cell hypertrophy at 2500 ppm (equal to 156 mg/kg bw per day).

In a 28-day range-finding study in dogs, which tested dietary flupyradifurone concentrations of 0, 500, 2000 and 4000 ppm (equal to 0, 16, 62 and 118 mg/kg bw per day for males and 0, 18, 77 and 131 mg/kg bw per day for females, respectively), body weight loss and reduced glycogen accumulation in hepatocytes occurred at 4000 ppm (equal to 118 mg/kg bw per day).

In a 90-day study in dogs, which tested dietary flupyradifurone concentrations of 0, 400, 1200 and 3600/2400 ppm (equal to 0, 12, 33 and 102/85 mg/kg bw per day for males and 0, 12, 41 and 107/78 mg/kg bw per day for females, respectively), the NOAEL was 400 ppm (equal to 12 mg/kg bw per day), based on increased creatine phosphokinase, aspartate aminotransferase and alanine aminotransferase activities and skeletal muscle degeneration at 1200 ppm (equal to 33 mg/kg bw per day).

In a 1-year toxicity study in dogs, which tested dietary flupyradifurone concentrations of 0, 150, 300 and 1000 ppm (equal to 0, 4.6, 7.8 and 28.1 mg/kg bw per day for males and 0, 4.1, 7.8 and 28.2 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 7.8 mg/kg bw per day), based on reduced body weight gain (females) and skeletal muscle degeneration (both sexes) at 1000 ppm (equal to 28.1 mg/kg bw per day).

The overall NOAEL in the 90-day and 1-year dog studies was 400 ppm (equal to 12 mg/kg bw per day), with an overall LOAEL of 1000 ppm (equal to 28.1 mg/kg bw per day).

In a 24-month chronic toxicity and carcinogenicity study in mice, which tested dietary flupyradifurone concentrations of 0, 70, 300 and 1500 ppm (equal to 0, 10, 43 and 224 mg/kg bw per day for males and 0, 12.2, 53 and 263 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 43 mg/kg bw per day), based on reduced body weight and body weight gain at 1500 ppm (equal to 224 mg/kg bw per day). No treatment-related tumours were observed up to the highest dietary concentration of 1500 ppm (equal to 224 mg/kg bw per day).

In a 2-year chronic toxicity and carcinogenicity study in rats, which tested dietary flupyradifurone concentrations of 0, 80, 400 and 2000 ppm (equal to 0, 3.16, 15.8 and 80.8 mg/kg bw per day for males and 0, 4.48, 22.5 and 120 mg/kg bw per day for females, respectively), the NOAEL was 400 ppm (equal to 15.8 mg/kg bw per day), based on reduced body weight and body weight gain, liver enlargement with accompanying histopathological changes, and histopathological changes in the lungs and thyroid at 2000 ppm (equal to 80.8 mg/kg bw per day). No treatment-related tumours were observed up to the highest dietary concentration of 2000 ppm (equal to 80.8 mg/kg bw per day).

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

Flupyradifurone was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Meeting concluded that flupyradifurone is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that flupyradifurone is unlikely to pose a carcinogenic risk to humans.

In a one-generation range-finding reproductive toxicity study in rats, which tested dietary flupyradifurone concentrations of 0, 200, 700 and 2000 ppm (equal to 0, 14.5, 50.1 and 147.5 mg/kg bw per day for males and 0, 17.5, 60.0 and 168.9 mg/kg bw per day for females, respectively), the NOAEL for reproductive toxicity was 2000 ppm (equal to 147.5 mg/kg bw per day), the highest dose

tested. The NOAEL for both parental toxicity and offspring toxicity was 700 ppm (equal to 50.1 mg/kg bw per day), based on reduced body weight at 2000 ppm (equal to 147.5 mg/kg bw per day).

In a two-generation reproductive toxicity study, which tested dietary flupyradifurone concentrations of 0, 100, 500 and 1800 ppm (equal to 0, 6.5, 32.3 and 119.8 mg/kg bw per day for males and 0, 7.8, 39.2 and 140.2 mg/kg bw per day for females, respectively), the NOAEL for reproductive toxicity was 500 ppm (equal to 39.2 mg/kg bw per day), based on decreases in estrous cycle length, the number of implantation sites and litter size at 1800 ppm (equal to 140.2 mg/kg bw per day). The NOAEL for parental toxicity was 100 ppm (equal to 7.8 mg kg bw per day), based on decreased body weight in females at 500 ppm (equal to 39.2 mg/kg bw per day). The NOAEL for offspring toxicity was 100 ppm (equal to 7.8 mg kg bw per day), based on decreased female pup weight and weight gain at 500 ppm (equal to 39.2 mg/kg bw per day).

In a developmental toxicity study in rats, which tested gavage flupyradifurone doses of 0, 15, 50 and 150 mg/kg bw per day from days 6 to 20 of gestation, the NOAEL for maternal toxicity was 50 mg/kg bw per day, for clinical signs (salivation) and body weight loss at 150 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 50 mg/kg bw per day, for slightly delayed ossification at 150 mg/kg bw per day.

In a follow-up developmental toxicity study in rats, which tested doses of 0, 20 and 30 mg/kg bw per day, the NOAEL for maternal toxicity and embryo and fetal toxicity was 30 mg/kg bw per day, the highest dose tested.

The overall NOAEL for both maternal and embryo/fetal toxicity from both developmental toxicity studies was 50 mg/kg bw per day.

In a developmental toxicity study in rabbits, which tested gavage flupyradifurone doses of 0, 7.5, 15 and 40 mg/kg bw per day, the NOAEL for maternal toxicity was 15 mg/kg bw per day, based on body weight loss and reduced feed consumption over the first few days of dosing at 40 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 40 mg/kg bw per day, the highest dose tested.

The Meeting concluded that flupyradifurone is not teratogenic.

In an acute neurotoxicity study in rats, which tested flupyradifurone doses of 0, 50, 200 and 800 mg/kg bw per day, piloerection occurred at every dose. In a follow-up study, which tested flupyradifurone doses of 0, 20 and 35 mg/kg bw, no systemic toxicity or neurotoxicity was observed at any dose.

In a 90-day neurotoxicity study in rats, which tested dietary flupyradifurone concentrations of 0, 100, 500 and 2500 ppm (equal to 0, 5.7, 29.4 and 143 mg/kg bw per day for males and 0, 6.9, 34.8 and 173 mg/kg bw per day for females, respectively), reduced body weight, body weight gain and feed consumption were observed at 2500 ppm (equal to 143 mg/kg bw per day). No neurotoxicity was observed at any dose.

In a developmental neurotoxicity study in rats, which tested dietary flupyradifurone concentrations of 0, 120, 500 and 1200 ppm (equal to 0, 10.3, 42.4 and 102 mg/kg bw per day, respectively), the NOAEL for maternal toxicity and offspring toxicity was 500 ppm (equal to 42.4 mg/kg bw per day), based on lower body weight and body weight gain in dams, reduced body weight gain in pups during lactation, and an increase in the auditory startle reflex in pups at 1200 ppm (equal to 102 mg/kg bw per day). No neurotoxicity was observed at any dose.

The Meeting concluded that flupyradifurone is not neurotoxic.

In a 28-day immunotoxicity study in rats, which tested dietary flupyradifurone concentrations of 0, 125, 600 and 3000 ppm (equal to 0, 10, 50 and 230 mg/kg bw per day, respectively), no effects on the immune system were noted up to the highest dose tested. Reduced body weight gain and feed consumption were observed at 3000 ppm (equal to 230 mg/kg bw per day).

The Meeting concluded that flupyradifurone is not immunotoxic.

Toxicological data on metabolites and/or degradates

Toxicity tests were conducted on six flupyradifurone metabolites: (1) difluoroacetic acid (DFA), which is a major soil, water and plant metabolite and is also detected in rat urine at approximately 6% of the administered dose; (2) difluoroethyl-amino-furanone, which is a minor plant metabolite also present in rat urine at approximately 10% of the administered dose; (3) (6-chloro-3-pyridyl)methanol and (4) 6-chloronicotinic acid, which are plant metabolites not detected in rat metabolism studies and metabolites common to other pesticides; and (5) amino-furanone and (6) flupyradifurone-acetic acid, which are plant metabolites not detected in rat metabolism studies.

The LD_{50}s in rats were greater than 300 and less than 2000 mg/kg bw for DFA, greater than 2000 mg/kg bw for difluoroethyl-amino-furanone, 1483 mg/kg bw for (6-chloro-3-pyridyl)methanol and greater than 5000 mg/kg bw for 6-chloronicotinic acid. Amino-furanone and flupyradifurone-acetic acid were not tested for acute toxicity.

In a 14-day range-finding study in rats, which tested dietary DFA concentrations of 0, 500, 2000 and 8000 ppm (equal to 0, 48, 187 and 745 mg/kg bw per day for males and 0, 51, 201 and 800 mg/kg bw per day for females, respectively), reduced blood glucose levels were observed at every dose.

In a 90-day toxicity study in rats, which tested dietary DFA concentrations of 0, 200, 1000 and 6000 ppm (equal to 0, 12.7, 66.2 and 380 mg/kg bw per day for males and 0, 15.6, 78.7 and 472 mg/kg bw per day for females, respectively), the NOAEL was 200 ppm (equal to 12.7 mg/kg bw per day), based on reduced body weight, reduced blood glucose levels associated with increased urine volume and ketones, and the presence of black foci and glandular erosion in the stomach at 1000 ppm (equal to 66.2 mg/kg bw per day).

In a 14-day range-finding study in rats, which tested dietary difluoroethyl-amino-furanone concentrations of 0, 1280, 3200, 8000 and 20 000 ppm (equal to 0, 135, 339, 736 and 1226 mg/kg bw per day for males and 0, 135, 335, 741 and 2254 mg/kg bw per day for females, respectively), reduced body weight gain, feed conversion efficiency and blood glucose levels occurred at and above 3200 ppm (equal to 335 mg/kg bw per day).

In a 28-day follow-up study in rats, which tested dietary difluoroethyl-amino-furanone concentrations of 0, 200, 800 and 3000 ppm (equal to 0, 17, 68 and 243 mg/kg bw per day for males and 0, 19, 76 and 273 mg/kg bw per day for females, respectively), the NOAEL was 3000 ppm (equal to 243 mg/kg bw per day), the highest dose tested.

In a 13-week toxicity study in rats, which tested dietary (6-chloro-3-pyridyl)methanol concentrations of 0, 160, 800, 4000 and 20 000 ppm (equal to 0, 9.9, 48.9, 250.1 and 1246.6 mg/kg bw per day for males and 0, 11.1, 55.9, 275.9 and 1173.7 mg/kg bw per day for females, respectively), the NOAEL was 800 ppm (equal to 48.9 mg/kg bw per day), based on decreased body weight gain and feed consumption, increased alkaline phosphatase activity and the presence of eosinophilic intranuclear inclusions in the proximal tubular epithelium of the kidney at 4000 ppm (equal to 250.1 mg/kg bw per day).

The six flupyradifurone metabolites were tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found, with the exception of difluoroethyl-amino-furanone, which was clastogenic in vitro in the absence of metabolic activation. However, when further in vivo testing was undertaken, no evidence of genotoxicity was found. On this basis, none of the six metabolites tested is likely to be genotoxic in vivo.

As limited toxicological data were provided for two of the metabolites, amino-furanone and flupyradifurone-acetic acid, supplementary analysis was undertaken using JMPR's Plant and Animal Metabolite Assessment Scheme. On the basis of this assessment and the fact that negligible levels of these metabolites are present in plant commodities, the Meeting concluded that neither of these metabolites poses a safety concern.

The Meeting concluded that difluoroethyl-amino-furanone, (6-chloro-3-pyridyl)methanol, 6-chloronicotinic acid, amino-furanone and flupyradifurone-acetic acid are of no greater toxicity than

flupyradifurone, and therefore the ADI and ARfD established for flupyradifurone would adequately cover dietary exposure to these metabolites.

Based on a comparison of the NOAELs in rats over 90 days of dietary exposure (12.7 mg/kg bw per day for DFA versus 30.2 mg/kg bw per day for flupyradifurone), the Meeting concluded that DFA is approximately 2.5-fold more potent than flupyradifurone.

Human data

No information was provided on adverse health effects in workers involved in the manufacture or use of flupyradifurone. No information on accidental or intentional poisoning in humans is available.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.08 mg/kg bw based on a NOAEL of 7.8 mg/kg bw per day for decreased maternal body weight, reduced female pup weight and pup weight gain at 39.2 mg/kg bw per day in a two-generation reproductive toxicity study in rats, with the application of a 100-fold safety factor. This NOAEL is supported by the NOAEL of 12 mg/kg bw per day for skeletal muscle degeneration in repeated-dose studies in the dog.

The Meeting established an ARfD of 0.2 mg/kg bw based on the maternal toxicity NOAEL of 15 mg/kg bw for body weight loss and reduced feed consumption over the first few days of exposure in a rabbit developmental toxicity study, with the application of a 100-fold safety factor.

The Meeting concluded that the metabolite DFA is 3-fold (rounded) more toxic than flupyradifurone over 90 days of dietary exposure in rats. On this basis, it was concluded that a 3-fold potency factor should be applied to the residue levels for use in both the acute and chronic dietary exposure estimates for DFA and that these should be added to the dietary exposures for flupyradifurone and compared with the ARfD and ADI for flupyradifurone, respectively.

Both the ADI and ARfD are established for the sum of flupyradifurone and its metabolites (difluoroethyl-amino-furanone, (6-chloro-3-pyridyl)methanol, 6-chloronicotinic acid, amino-furanone, flupyradifurone-acetic acid and 3× DFA) and expressed as the parent flupyradifurone.

A toxicological monograph was prepared.

Levels relevant to risk assessment of flupyradifurone

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 43 mg/kg bw per day	1 500 ppm, equal to 224 mg/kg bw per day
		Carcinogenicity	1 500 ppm, equal to 224 mg/kg bw per day[b]	–
Rat	Acute neurotoxicity studies[c,d]	Toxicity	35 mg/kg bw per day	50 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	400 ppm, equal to 15.8 mg/kg bw per day	2 000 ppm, equal to 80.8 mg/kg bw per day
		Carcinogenicity	2 000 ppm, equal to 80.8 mg/kg bw per day[b]	–

Species	Study	Effect	NOAEL	LOAEL
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	500 ppm, equal to 39.2 mg/kg bw per day	1 800 ppm, equal to 140.2 mg/kg bw per day
		Parental toxicity	100 ppm, equal to 7.8 mg/kg bw per day	500 ppm, equal to 39.2 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 7.8 mg/kg bw per day	500 ppm, equal to 39.2 mg/kg bw per day
	Developmental toxicity studies[c,d]	Maternal toxicity	50 mg/kg bw per day	150 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day	150 mg/kg bw per day
	Developmental neurotoxicity study[a]	Maternal toxicity	500 ppm, equal to 42.4 mg/kg bw per day	1 200 ppm, equal to 102 mg/kg bw per day
		Embryo and fetal toxicity	500 ppm, equal to 42.4 mg/kg bw per day	1 200 ppm, equal to 102 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	15 mg/kg bw per day	40 mg/kg bw per day
		Embryo and fetal toxicity	40 mg/kg bw per day[b]	–
Dog	Ninety-day and 1-year studies of toxicity[a,d]	Toxicity	400 ppm, equal to 12 mg/kg bw per day	1 000 ppm, equal to 28.1 mg/kg bw per day

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

Estimate of acceptable daily intake (ADI) (for sum of flupyradifurone and metabolites,[1] expressed as flupyradifurone)

0–0.08 mg/kg bw

Estimate of acute reference dose (ARfD)

0.2 mg/kg bw

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 flupyradifurone

Absorption, distribution, excretion and metabolism in mammals

[1] Difluoroethyl-amino-furanone, (6-chloro-3-pyridyl)methanol, 6-chloronicotinic acid, amino-furanone, flupyradifurone-acetic acid and 3× DFA.

Rate and extent of oral absorption	Rapid; > 80%
Dermal absorption	No data
Distribution	Rapid tissue distribution
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid and complete
Metabolism in animals	Extensive; hydroxylation, conjugation and cleavage reactions
Toxicologically significant compounds in animals and plants	Flupyradifurone, DFA, difluoroethyl-amino-furanone
Acute toxicity	
Rat, LD_{50}, oral	> 300 and < 2 000 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.67 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Mouse, dermal sensitization	Not sensitizing (local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Muscle degeneration
Lowest relevant oral NOAEL	12 mg/kg bw per day (dog)
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 and body weight gain, liver toxicity
Lowest relevant NOAEL	15.8 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in mice or rats[a]
Genotoxicity	
	No evidence of genotoxicity[a]
Reproductive toxicity	
Reproduction target/critical effect	Reduced body weight, length of estrous cycle, implantation sites and litter size
Lowest relevant parental NOAEL	7.8 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	7.8 mg/kg bw per day (rat)
Lowest relevant reproduction NOAEL	39.2 mg/kg bw per day (rat)
Developmental toxicity	
Developmental target/critical effect	Slightly delayed ossification at maternally toxic doses
Lowest maternal NOAEL	15 mg/kg bw per day (rabbit)
Lowest embryo/fetal NOAEL	30 mg/kg bw per day (rat)
Neurotoxicity	
Acute neurotoxicity NOAEL	800 mg/kg bw (highest dose tested; rat)
Subchronic neurotoxicity NOAEL	143 mg/kg bw per day (highest dose tested; rat)