5.15 FENAMIDONE (264)

TOXICOLOGY

Fenamidone is the ISO-approved common name for (5*S*)-5-methyl-2-(methylthio)-5-phenyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one (IUPAC), with CAS No. 161326-34-7. There is no conversion to the *R*-enantiomer in biological systems. Fenamidone is a foliar fungicide used on vegetables and ornamentals.

Fenamidone 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 experiments conducted in rats using [^{14}C]fenamidone labelled at either the C-phenyl or N-phenyl part of the molecule, the time to reach the maximum plasma concentration of radioactivity was 2 hours after a single oral dose of 3 mg/kg bw and 26 hours after a single oral dose of 300 mg/kg bw. Gastrointestinal absorption was greater than 80%. Radioactivity distributed to most tissues, with no evidence of accumulation. Relatively high concentrations of radioactivity (approximately 400 times higher than that in plasma) were detected in the thyroid following dosing with C-phenyl- but not N-phenyl-labelled fenamidone, suggesting the distribution of a radiolabelled metabolite to the thyroid. Fenamidone undergoes extensive metabolism in the rat by phase I (oxidation, reduction and hydrolysis) and phase II reactions (conjugation). More than 20 metabolites were detected in rat excreta. The plasma elimination half-life was at least 60 hours, with the majority of radioactivity excreted in the faeces (up to approximately 90% of the administered dose) and the remainder in urine. Mass balance data indicated that the majority of radioactivity (> 80%) was eliminated within 48 hours of dosing.

Toxicological data

The oral LD_{50} in rats was greater than 2000 mg/kg bw. The dermal LD_{50} in rats was greater than 2000 mg/kg bw, and the LC_{50} was greater than 2.1 mg/L. Fenamidone was neither a skin irritant nor an eye irritant in rabbits. In a guinea-pig maximization test, no skin sensitization occurred.

The target organs for fenamidone are the liver and thyroid. In rats, fenamidone was an inducer of cytochrome P450. Consistent with studies indicating a relatively high distribution of radiolabel to the rat thyroid, increased thyroid weight, follicular cell hypertrophy and hyperplasia were observed. This did not appear to be a secondary effect of liver enzyme induction, as thyroid hormone levels in plasma were not affected by treatment.

In a non-guideline 90-day toxicity study in mice that tested dietary concentrations of 0, 70, 700 and 7000 ppm (equal to 0, 11.4, 110 and 1102 mg/kg bw per day for males and 0, 14.5, 146 and 1468 mg/kg bw per day for females, respectively), the NOAEL was 700 ppm (equal to 110 mg/kg bw per day), based on clinical signs and deaths in males at 7000 ppm (equal to 1102 mg/kg bw per day). In a second 90-day study in mice that tested dietary concentrations of 0, 50, 200, 1000 and 5000 ppm (equal to 0, 11.3, 44.5, 220 and 1064 mg/kg bw per day for males and 0, 13.7, 54, 274 and 1375 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 220 mg/kg bw per day) for equivocal histopathological findings in the liver at 5000 ppm (equal to 1064 mg/kg bw per day). The overall NOAEL for the two 90-day studies in mice was 1000 ppm (equal to 220 mg/kg bw per day), with an overall LOAEL of 5000 ppm (equal to 1064 mg/kg bw per day).

A 28-day toxicity study in rats tested dietary concentrations of 0, 500, 5000 and 15 000 ppm (equal to 0, 39, 389 and 1203 mg/kg bw per day for males and 0, 42, 405 and 1194 mg/kg bw per day for females, respectively). The NOAEL was 500 ppm (equal to 39 mg/kg bw per day) for reduced red

cell parameters and increased spleen weights coincident with hyperplasia of the germinative follicle of the white pulp at and above 5000 ppm (equal to 389 mg/kg bw per day). The NOAEL in a second 28-day study in rats, which tested dietary concentrations of 0, 60, 150, 1000 and 5000 ppm (equal to 0, 4.9, 12.3, 82.7 and 418.5 mg/kg bw per day for males and 0, 5.3, 13.9, 90.6 and 450.1 mg/kg bw per day for females, respectively), was 1000 ppm (equal to 82.7 mg/kg bw per day) for thyroid follicular cell hypertrophy in males at 5000 ppm (equal to 418.5 mg/kg bw per day).

In a 90-day toxicity study in rats, which tested dietary concentrations of 0, 50, 150, 500 and 5000 ppm (equal to 0, 3, 9, 30 and 305 mg/kg bw per day for males and 0, 3, 11, 35 and 337 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 30 mg/kg bw per day) for reduced body weight gain, reduced feed consumption and changes in red cell parameters at 5000 ppm (equal to 305 mg/kg bw per day). The NOAEL in a second 90-day rat study, which tested dietary concentrations of 0, 60, 150, 1000 and 5000 ppm (equal to 0, 4.1, 10.4, 68.3 and 344 mg/kg bw per day for males and 0, 4.8, 12, 83.3 and 381 mg/kg bw per day for females, respectively), was 1000 ppm (equal to 68.3 mg/kg bw per day) for reduced body weight gain at 5000 ppm (equal to 344 mg/kg bw per day).

In a 28-day toxicity study in dogs, which tested doses of 0, 3, 10 and 100 mg/kg bw per day given by capsule, the NOAEL was 100 mg/kg bw per day, the highest dose tested. In a 13-week study in dogs, which tested doses of 0, 10, 100 and 500 mg/kg bw per day given by capsule, the NOAEL was 500 mg/kg bw per day, the highest dose tested. In a 52-week study in dogs, which tested doses of 0, 10, 100 and 1000 mg/kg bw per day given by capsule, the NOAEL was 100 mg/kg bw per day for clinical signs (hypersalivation and vomiting), increased AP activity, increased liver weight (males) and increased bile duct hyperplasia (males) at 1000 mg/kg bw per day. The overall NOAEL for the 13 and 52-week studies was 500 mg/kg bw per day, with an overall LOAEL of 1000 mg/kg bw per day.

In a long-term study of toxicity and carcinogenicity in mice, which tested dietary concentrations of 0, 70, 350, 3500 and 7000 ppm (equal to 0, 9.5, 47.5, 525.5 and 1100 mg/kg bw per day for males and 0, 12.6, 63.8, 690.5 and 1393 mg/kg bw per day for females, respectively), the NOAEL was 350 ppm (equal to 47.5 mg/kg bw per day) for lower body weight, increased liver weight and histopathological findings in the liver at 3500 ppm (equal to 525.5 mg/kg bw per day). No treatment-related neoplastic lesions were detected at dietary concentrations up to 7000 ppm (equal to 1100 mg/kg bw per day, respectively).

In a long-term toxicity and carcinogenicity study in rats that tested dietary concentrations of 0, 60, 150, 1000 and 5000 ppm (equal to 0, 2.8, 7.1, 47.7 and 260 mg/kg bw per day for males and 0, 3.6, 9.2, 60.9 and 330 mg/kg bw per day for females, respectively), the NOAEL was 60 ppm (equal to 2.8 mg/kg bw per day) for thyroid follicular cell hypertrophy and hyperplasia at 150 ppm (equal to 7.1 mg/kg bw per day). No treatment-related neoplastic lesions were detected at dietary concentrations up to 5000 ppm (equal to 260 mg/kg bw per day).

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

Fenamidone was negative in the Ames test and in an in vitro unscheduled DNA synthesis (UDS) assay in rat hepatocytes. Fenamidone was positive in a mouse lymphoma forward mutation assay and caused chromosomal aberrations in cultured human peripheral blood lymphocytes in the presence of metabolic activation. Negative responses were obtained in vivo in the mouse micronucleus test and UDS assay in rat hepatocytes.

The Meeting concluded that fenamidone is unlikely to be genotoxic in vivo.

Considering the lack of in vivo genotoxicity potential and the absence of a carcinogenic response in mice and rats, the Meeting concluded that fenamidone is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation study in rats, which tested dietary concentrations of 0, 60, 1000 and 5000 ppm (equal to 0, 3.9, 63.8 and 328.3 mg/kg bw per day for males and 0, 5.15, 84.4 and

459.6 mg/kg bw per day for females, respectively), there was no evidence of reproductive toxicity up to the highest tested dietary concentration of 5000 ppm (equal to 328.3 mg/kg bw per day). The NOAEL for parental toxicity was 60 ppm (equal to 3.9 mg/kg bw per day) for lower body weight, reduced body weight gain and reduced feed consumption at 1000 ppm (equal to 63.8 mg/kg bw per day). The NOAEL for offspring toxicity was 1000 ppm (equal to 63.8 mg/kg bw per day) for lower F_1 and F_2 pup weights at 5000 ppm (equal to 328.3 mg/kg bw per day).

In a rat developmental toxicity study that tested doses of 0, 25, 150 and 1000 mg/kg bw per day, the NOAEL for maternal toxicity was 150 mg/kg bw per day for reduced body weight gain and feed consumption at 1000 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 150 mg/kg bw per day for lower fetal weight at 1000 mg/kg bw per day.

In a rabbit developmental toxicity study that tested doses of 0, 10, 30 and 100 mg/kg bw per day, the NOAEL for maternal toxicity was 30 mg/kg bw per day for decreased body weight gain at 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, the highest dose tested.

The Meeting concluded that fenamidone is not teratogenic in rats or rabbits.

Acute and subchronic neurotoxicity studies were conducted in rats. In the acute gavage study, which tested doses of 0, 125, 500 and 2000 mg/kg bw per day, the NOAEL was 125 mg/kg bw for nonspecific findings in the functional observational battery at 4 hours after dosing at 500 mg/kg bw per day and above. In the subchronic study, which tested dietary concentrations of 0, 150, 1000 and 5000 ppm (equal to 0, 11.2, 73.5 and 392.3 mg/kg bw per day for males and 0, 12.7, 83.4 and 414.2 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 73.5 mg/kg bw per day) for reduced body weight gain at 5000 ppm (equal to 392.3 mg/kg bw per day).

There was no evidence of developmental neurotoxicity in a dietary study conducted in female rats, which tested dietary concentrations of 0, 60, 250, 1000 and 4700 ppm (equal to 0, 5.5, 23, 92.3 and 429 mg/kg bw per day, respectively). The NOAEL for maternal toxicity was 1000 ppm (equal to 92.3 mg/kg bw per day) for reduced body weight gain at 4700 ppm (equal to 429 mg/kg bw per day). The NOAEL for offspring toxicity was also 1000 ppm (equal to 92.3 mg/kg bw per day), for lower absolute body weight and reduced body weight gain at 4700 ppm (equal to 429 mg/kg bw per day).

The Meeting concluded that fenamidone is not neurotoxic.

Toxicological data on metabolites and/or degradates

Toxicity studies were conducted on three fenamidone metabolites: (1) (*S*)-5-methyl-5-phenyl-2,4-imidazolidine-dione (RPA 412636), which is a metabolite in rat urine present at less than 1% of an administered dose; (2) (5*S*)-5-methyl-5-phenyl-3-(phenylamino)-2,4-imidazolidine-dione (RPA 410193), which is a novel plant metabolite; and (3) (5*S*)-5-methyl-2-(methylthio)-5-phenyl-3,5-dihydro-4H-imidazol-4-one (RPA 412708), which is a metabolite in rat bile present at greater than 10% of an administered dose.

The oral LD_{50} in rats was 1520 mg/kg bw for RPA 412636, greater than 2000 mg/kg bw for RPA 410193 and greater than 100 mg/kg bw for RPA 412708.

Short-term studies of toxicity were performed on RPA 412636, RPA 410193 and RPA 412708 in rats. As with the parent compound, liver enzyme induction and liver hypertrophy occurred with RPA 412636 and RPA 410193, but not RPA 412708. In a non-guideline 14-day study on RPA 412636 that tested dietary concentrations of 0, 300, 1200 and 300 ppm (equal to 0, 23, 90 and 215 mg/kg bw per day for males and 0, 24.5, 96.7 and 233 mg/kg bw per day for females, respectively), the NOAEL was 1200 ppm (equal to 90 mg/kg bw per day) for reduced body weight gain and feed consumption at 3000 ppm (equal to 215 mg/kg bw per day). In a similar non-guideline study on RPA 410193, which tested dietary concentrations of 0, 450, 4500 and 15 000 ppm (equal to 0, 30, 299 and 1098 mg/kg bw per day for males and 0, 37, 374 and 1133 mg/kg bw per day for females, respectively), the NOAEL was 450 ppm (equal to 30 mg/kg bw per day) for histopathological

findings in the thyroid at 4500 ppm (equal to 299 mg/kg bw per day). In the non-guideline 14-day study on RPA 412708, which tested dietary concentrations of 0, 200, 500 and 2000 ppm (equal to 0, 15.1, 38.5 and 150.5 mg/kg bw per day for males and 0, 16.1, 37.8 and 147.3 mg/kg bw per day for females, respectively), the NOAEL was 2000 ppm (equal to 147.3 mg/kg bw per day), the highest dietary concentration tested.

Ninety-day dietary studies were conducted on RPA 412636 and RPA 410193 in rats. The study on RPA 412636 tested dietary concentrations of 0, 100, 500 and 2500 ppm (equal to 0, 6.4, 33 and 162 mg/kg bw per day for males and 0, 7.7, 39 and 196 mg/kg bw per day for females, respectively); the NOAEL was 100 ppm (equal to 6.4 mg/kg bw per day) for vacuolation of centrilobular hepatocytes at 500 ppm (equal to 33 mg/kg bw per day) in males. In the study conducted on RPA 410193, which tested dietary concentrations of 0, 150, 1500 and 15 000 ppm (equal to 0, 9.3, 93.3 and 978 mg/kg bw per day for males and 0, 11.5, 115 and 1090 mg/kg bw per day for females), the NOAEL was 1500 ppm (equal to 93.3 mg/kg bw per day), based on perturbations in red cell parameters at 15 000 ppm (equal to 978 mg/kg bw per day).

There was no evidence of genotoxicity in vitro and in vivo for RPA 412636, RPA 410193 and RPA 412708.

Human data

In a cohort of 15 workers involved in the processing of fenamidone over a 5-year period, no adverse medical events were reported, and there were no detectable effects following physical and biochemical examinations.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.03 mg/kg bw per day, based on the NOAEL of 2.8 mg/kg bw per day for a dose-related increase in thyroid follicular cell hypertrophy and hyperplasia in both sexes after 52 weeks in the 2-year rat study. A 100-fold safety factor was applied.

The Meeting established an ARfD of 1 mg/kg bw, based on the NOAEL of 125 mg/kg bw per day for nonspecific findings in the functional observational battery in the acute neurotoxicity study in rats, and using a 100-fold safety factor. The Meeting noted that decreased maternal body weight gain in the developmental toxicity study in rabbits with a lower NOAEL of 30 mg/kg bw per day was not an acute effect and was therefore not a suitable basis for establishing an ARfD for fenamidone.

A toxicological monograph was prepared.

Levels relevant to risk assessment of fenamidone

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day studies of toxicity[a,b]	Toxicity	1000 ppm, equal to 220 mg/kg bw per day	5000 ppm, equal to 1064 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	350 ppm, equal to 47.5 mg/kg bw per day	3500 ppm, equal to 525.5 mg/kg bw per day
		Carcinogenicity	7000 ppm, equal to 1100 mg/kg bw per	—

Species	Study	Effect	NOAEL	LOAEL
			day[c]	
Rat	Acute neurotoxicity study[d]	Toxicity	125 mg/kg bw per day	500 mg/kg bw per day
	Thirteen-week studies of toxicity or neurotoxicity[a,b]	Toxicity	1000 ppm, equal to 73.5 mg/kg bw per day	5000 ppm, equal to 305 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	60 ppm, equal to 2.8 mg/kg bw per day	150 ppm, equal to 7.1 mg/kg bw per day
		Carcinogenicity	5000 ppm, equal to 260 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	5000 ppm, equal to 328.3 mg/kg bw per day[c]	—
		Parental toxicity	60 ppm, equal to 3.9 mg/kg bw per day	1000 ppm, equal to 63.8 mg/kg bw per day
		Offspring toxicity	1000 ppm, equal to 63.8 mg/kg bw per day	5000 ppm, equal to 328.3 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	150 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	150 mg/kg bw per day	1000 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	100 mg/kg bw per day[c]	—
Dog	Thirteen- and 52-week studies of toxicity[b,e]	Toxicity	500 mg/kg bw per day	1000 mg/kg bw per day

[a] Dietary administration.

[b] Two or more studies combined. For the rat, two 90-day toxicity studies and a subchronic neurotoxicity study were combined.

[c] Highest dose tested.

[d] Gavage administration.

[e] Capsule administration.

Levels relevant to risk assessment of fenamidone metabolites based on studies conducted in rats

Metabolite	Study	Effect	NOAEL	LOAEL
RPA 412636[a]	Ninety-day study of toxicity[b]	Toxicity	100 ppm, equal to 6.4 mg/kg bw per day	500 ppm, equal to 33 mg/kg bw per day
RPA 410193[c]	Ninety-day study of toxicity[b]	Toxicity	1500 ppm, equal to 93.3 mg/kg bw per	15 000 ppm, equal to 978 mg/kg bw per

Metabolite	Study	Effect	NOAEL	LOAEL
			day	day
RPA 412708[d]	Fourteen-day study of toxicity[b]	Toxicity	2000 ppm, equal to 147.3 mg/kg bw per day[e]	—

[a] (*S*)-5-Methyl-5-phenyl-2,4-imidazolidine-dione.

[b] Dietary administration.

[c] (5*S*)-5-Methyl-5-phenyl-3-(phenylamino)-2,4-imidazolidine-dione.

[d] (5*S*)-5-methyl-2-(methylthio)-5-phenyl-3,5-dihydro-4H-imidazol-4-one.

[e] Highest dose tested.

Estimate of acceptable daily intake

0–0.03 mg/kg bw

Estimate of acute reference dose

1 mg/kg bw

Information that would be useful for the continued evaluation of fenamidone

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rats: T_{max} = 2–26 h; extensive, > 80%
Distribution	Widespread tissue distribution; preferential distribution of radiolabel to the thyroid
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Excretion via faeces and urine; half-life > 60 h
Metabolism in animals	Extensive
Toxicologically significant compounds in animals, plants and the environment	Fenamidone, RPA 412636, RPA 410193, RPA 412708
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 2.1 mg/L
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Non-irritating
Dermal sensitization	Non-sensitizing (guinea-pigs)
Short-term studies of toxicity	
Target/critical effect	Liver, thyroid

Lowest relevant oral NOAEL	68.3 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Thyroid
Lowest relevant NOAEL	2.8 mg/kg bw per day (rat)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans from the diet
Genotoxicity	
	Not genotoxic in vivo
Reproductive toxicity	
Reproduction target/critical effect	No evidence of reproductive toxicity (rat)
Lowest relevant parental NOAEL	3.9 mg/kg bw per day
Lowest relevant offspring NOAEL	63.8 mg/kg bw per day
Lowest relevant reproduction NOAEL	328.3 mg/kg bw per day, the highest dose tested
Developmental toxicity	
Developmental target/critical effect	Decreased pup weights at maternally toxic doses (rat)
Lowest maternal NOAEL	30 mg/kg bw per day (rabbit)
Lowest embryo/fetal NOAEL	100 mg/kg bw per day, the highest dose tested (rabbit); 150 mg/kg bw per day (rat)
Neurotoxicity	
Neurotoxicity	Not neurotoxic
Developmental neurotoxicity	No developmental neurotoxicity
Toxicological studies on RPA 412636	
Rat, LD_{50}, oral	1520 mg/kg bw
Lowest relevant short-term oral NOAEL	6.4 mg/kg bw per day (90 days, rat)
Genotoxicity	Not genotoxic
Toxicological studies on RPA 410193	
Rat, LD_{50}, oral	> 2000 mg/kg bw
Lowest relevant short-term oral NOAEL	30 mg/kg bw per day (90 days, rat)
Genotoxicity	Not genotoxic
Toxicological studies on RPA 412708	
Rat, LD_{50}, oral	100 mg/kg bw
Lowest relevant short-term oral NOAEL	147.3 mg/kg bw per day (14 days, rat)
Genotoxicity	Not genotoxic
Medical data	
	No adverse effects in workers involved with manufacturing fenamidone

Summary

	Value	Studies	Safety factor
ADI	0–0.03 mg/kg bw	Two-year dietary study in rats	100
ARfD	1 mg/kg bw	Acute neurotoxicity study in rats	100