5.19 FLUENSULFONE (265)

TOXICOLOGY

Fluensulfone is the ISO-approved name for 5-chloro-2-(3,4,4-trifluorobut-3-en-1-ylsulfonyl)-1,3-thiazole (IUPAC) (CAS No. 318290-98-1). Fluensulfone is a nematicide for use on a range of vegetable crops. Target pests are root knot, root lesion and cyst nematodes. Fluensulfone's mode of pesticidal activity has not been determined.

Fluensulfone has not been evaluated previously 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

Absorption of fluensulfone administered by gavage at 5 mg/kg bw is rapid, with maximal plasma concentrations achieved within 4 hours. At 5 and 500 mg/kg bw, the extent of oral absorption is high (> 80%). Fluensulfone is widely distributed in the body. High concentrations of both butene- and thiazole-labelled material were found in the liver and kidney, and the butene-labelled material was also found at high concentrations in the lung. Thiazole-labelled material partitioned readily to red blood cells. Two hours after administration at both high and low doses, the thyroid had a high concentration of thiazole-labelled material relative to whole blood, although levels in thyroid were comparable to the whole blood concentrations by the subsequent time point (51 hours). The labelled material was rapidly excreted via urine (> 70%), with faecal excretion accounting for no more than 5–13%. A small proportion (< 5% of the administered dose at 5 mg/kg bw) was exhaled as carbon dioxide. Absorbed fluensulfone was extensively metabolized, with almost no unmetabolized parent compound detected. Other than low amounts of thiazole sulfonic acid, no other faecal metabolites were present at levels above 5% of the administered dose. The parent compound probably reacts with glutathione and cleaves, giving rise to thiazole mercapturate and butene sulfinic acid, the major urinary metabolites. The excretion pattern, tissue distribution of radioactivity and metabolite profile were essentially unaltered when the administration of thiazole-labelled substance was preceded by 14 days of administration of the unlabelled material.

Fluensulfone has a molecular weight of 272 Da and contains three fluorine atoms. If all the available fluorine is released, this means a fluensulfone dose of 100 mg/kg bw could provide about 20 mg of free fluoride ion per kilogram body weight. Approximately 50% of the administered dose is excreted as fluoride-containing compounds.

Toxicological data

Fluensulfone was of moderate acute toxicity in rats via the oral route (LD_{50} = 671 mg/kg bw), but caused no mortality at limit doses after dermal (LD_{50} > 2000 mg/kg bw) or inhalation (LC_{50} > 5.1 mg/L air) exposure. Fluensulfone was slightly irritating to the skin of rabbits, but not irritating to the eyes of rabbits. It was a skin sensitizer in guinea-pigs.

In all species, the liver was a target organ, with increases in weight and hepatocellular hypertrophy. Investigations on liver enzyme activities in all species revealed that fluensulfone administration does not result in significant induction of cytochrome P450 subfamilies, but that the (conjugating) phase II enzymes were induced to a limited extent. Effects on kidney, observed in studies in rats conducted at high doses, were most prominent in males, but some renal effects were observed in female rats treated at high doses and in dogs treated for 1 year. Reduced body weight gain, often associated with reductions in feed consumption, was a consistent, sensitive end-point. Fluorosis leading to discolouration of the teeth was reported only in the 90-day rat study at 2000 ppm (equal to 139 mg/kg bw per day), but there were no other overt signs of fluorosis. Determinations of

fluoride content of bones and teeth showed significantly increased levels of total fluoride, even at low doses of fluensulfone. Examinations of tooth colour were performed and bones were examined histopathologically, but no specific investigations of bone density, thickness or bending resistance were performed. The Meeting concluded that the dietary intake of fluoride associated with the use of fluensulfone as a nematicide should be included in an overall assessment of fluoride intake from all sources. Upper levels for fluoride intake have been proposed by a number of organizations.

In mice, rats and dogs, decreases in alanine aminotransferase (ALT) activity in both plasma and liver were recorded. Mode of action investigations were performed in dogs, the most susceptible species for this effect. It was found that direct binding of fluensulfone itself did not cause the decrease in ALT activity and that there was no significant interaction with the cofactor pyridoxal 5′-phosphate. Messenger ribonucleic acid (mRNA) levels appeared slightly induced, whereas protein expression appeared stable. No effect on the general health of the dogs accompanied the reduction of hepatic ALT activity at low doses (50 ppm, equal to 1.5 mg/kg bw per day), even when the reduction was greater than 40%. The reduced ALT activity was reversible, and alternative metabolic pathways are available. The Meeting concluded that a reduction in hepatic ALT activity per se is not adverse.

In a 28-day study of toxicity in mice, dietary concentrations were 0, 100, 500 and 2000 ppm (equal to 0, 30, 101 and 375 mg/kg bw per day for males and 0, 41, 155 and 353 mg/kg bw per day for females, respectively). The NOAEL was 500 ppm (equal to 101 mg/kg bw per day), based on body weight loss, changes in erythrocyte parameters (e.g. increased reticulocytes) and liver toxicity (altered cytoplasmic structure, single-cell necrosis, bile duct hyperplasia) at 2000 ppm (equal to 353 mg/kg bw per day).

In a 90-day study of toxicity in mice, dietary concentrations were 0, 60, 300 and 1500 ppm (equal to 0, 11, 51 and 229 mg/kg bw per day for males and 0, 18, 68 and 252 mg/kg bw per day for females, respectively). The NOAEL was 60 ppm (equal to 11 mg/kg bw per day), on the basis of haematological findings in males and hepatocyte hypertrophy observed at 300 ppm (equal to 51 mg/kg bw per day).

In a 28-day study of toxicity in rats, dietary concentrations were 0, 125, 500 and 2000 ppm (equal to 0, 10, 43 and 152 mg/kg bw per day for males and 0, 12, 37 and 166 mg/kg bw per day for females, respectively). The NOAEL was 125 ppm (equal to 10 mg/kg bw per day), based on reductions in body weight gain and kidney lesions in male rats at 500 ppm (equal to 43 mg/kg bw per day).

In a 90-day study of toxicity in rats, dietary concentrations were 0, 60, 120, 500 and 2000 ppm (equal to 0, 4, 8, 35 and 139 mg/kg bw per day for males and 0, 5, 12, 53 and 149 mg/kg bw per day for females, respectively). The NOAEL was 120 ppm (equal to 8 mg/kg bw per day), on the basis of forestomach hyperkeratosis, increased triglycerides and increased water consumption in females and decreased body weight/body weight gains in males at 500 ppm (equal to 35 mg/kg bw per day).

In a 28-day dietary study in which dogs were administered fluensulfone at 0, 50, 200 or 900 ppm (equal to 0, 1.9, 7 or 31 mg/kg bw per day for males and 0, 2, 8 or 30 mg/kg bw per day for females, respectively), the NOAEL was 200 ppm (equal to 7 mg/kg bw per day), based on reductions in feed consumption and body weight gain and increases in liver and thyroid weights at 900 ppm (equal to 30 mg/kg bw per day). In a 90-day study in which dogs received fluensulfone in the diet at 0, 5, 50 or 500 ppm (equal to 0, 0.2, 1.7 and 17 mg/kg bw per day for males and 0, 0.2, 1.8 and 18 mg/kg bw per day for females, respectively), the NOAEL was 50 ppm (equal to 1.7 mg/kg bw per day), based on increases in reticulocytes and liver weights at 500 ppm (equal to 17 mg/kg bw per day). In a 1-year study in dogs in which fluensulfone was administered in the diet at 0, 5, 50, 100 or 500 ppm (equal to 0, 0.1, 1.5, 3 and 16 mg/kg bw per day), the NOAEL was 100 ppm (equal to 3 mg/kg bw per day), on the basis of changes in erythrocyte parameters, liver weight increases and increased pigment deposition in the liver at 500 ppm (equal to 16 mg/kg bw per day). The overall NOAEL for the 90-day and 1-year studies was 100 ppm (equal to 3 mg/kg bw per day), and the overall LOAEL was 500 ppm (equal to 16 mg/kg bw per day).

In an 18-month toxicity and carcinogenicity study in mice, dietary concentrations were 0, 30, 200 and 1200 ppm (equal to 0, 4.2, 28 and 152 mg/kg bw per day for males and 0, 6.4, 39 and 188 mg/kg bw per day for females, respectively). The NOAEL was 30 ppm (equal to 4.2 mg/kg bw per day), based on decreased body weights and bronchiolization at 200 ppm (equal to 28 mg/kg bw per day). The NOAEL for tumours was 30 ppm (equal to 6.4 mg/kg bw per day), based on increased incidences of alveolar/bronchiolar adenomas and carcinomas in females receiving 200 ppm (equal to 39 mg/kg bw per day).

In a 2-year chronic toxicity and carcinogenicity study in rats, dietary concentrations were 0, 30, 200 and 1200 ppm (equal to 0, 1.4, 9.6 and 58 mg/kg bw per day for males and 0, 1.7, 12 and 69 mg/kg bw per day for females, respectively). Fluensulfone showed no carcinogenic potential in rats. The NOAEL for non-neoplastic effects was 30 ppm (equal to 1.4 mg/kg bw per day), on the basis of chronic interstitial inflammation in the lungs of females, oesophageal hyperkeratosis and decreased body weight gain in males at 200 ppm (equal to 9.6 mg/kg bw per day). Dose-related increases in plasma sodium levels were seen in both sexes from weeks 13 to 52. The sodium levels in the fluensulfone-treated groups were within the control ranges in the study, and this finding was not consistent with the results of the 90-day rat study, in which plasma sodium levels were unaltered by treatment at week 13. The Meeting concluded that the increases in plasma sodium levels were not an adverse effect of fluensulfone administration.

The Meeting concluded that fluensulfone is carcinogenic in female mice but not male mice or rats.

An adequate battery of in vitro and in vivo mutagenicity studies has been performed with fluensulfone. Weak positive results were seen with one strain of *Salmonella typhimurium* in one Ames test but not in two other Ames tests using the same strains. An equivocal finding was noted at high concentrations in an assay for chromosome damage. No evidence of genotoxicity was seen in an adequately performed bone marrow micronucleus assay in mice.

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

Mechanistic studies were carried out to determine the relevance for humans of the lung tumour findings in mice. Species-specific lung tumours in the mouse have been induced by a number of chemicals. The underlying cause is attributed to a high metabolic activity of the mouse lung, due to a relatively high abundance of Clara cells in the mouse compared with humans and the mouse-specific cytochrome P450 (Cyp) enzyme 2f2 in the Clara cells. The compounds are activated to reactive intermediates, leading to local cytotoxicity that promotes sustained cell proliferation, leading finally to tumour formation. Rats have lower metabolic activity in the lungs compared with mice (below the threshold needed to cause lung tumours upon lifetime exposure), and this metabolic activity in humans is reported to be significantly lower than in rats. A limited package of data specific for fluensulfone showed it to be extensively metabolized by male and female mouse lung microsomes, whereas essentially no metabolic activity was seen in human lung microsomes. Mouse-specific Cyp2f2 was shown to be a significant contributor to fluensulfone's metabolism. Administration of fluensulfone to mice led to an early increase in bronchiolar epithelial cell proliferation; however, no equivalent data are available for human or rat lung preparations. Bronchiolization indicative of a chronic inflammatory response was noted in male as well as female mice. These data do not address the fact that no increases in lung tumours were seen in male mice.

Considering the submitted mode of action, the Meeting concluded that the work undertaken is not extensive enough to conclusively identify the mode of action or to entirely exclude human relevance. However, the mode of action for mouse lung tumours in female mice administered fluensulfone is likely to be non-genotoxic and threshold dependent.

The Meeting concluded that fluensulfone is unlikely to be genotoxic in vivo and that there is a clear threshold for lung tumours in female mice. Therefore, fluensulfone is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation study of reproductive toxicity in rats, dietary concentrations were 0, 30, 250 and 1800 ppm (equal to mean intakes of 0, 2.0, 16 and 122 mg/kg bw per day for males and 0, 2.8, 23 and 169 mg/kg bw per day for females, respectively). The NOAEL for reproductive effects was 1800 ppm (equal to 122 mg/kg bw per day), the highest dose tested. The parental NOAEL was 250 ppm (equal to 16 mg/kg bw per day), based on effects on body weight throughout the study in males and increased liver and kidney weights at 1800 ppm (equal to 122 mg/kg bw per day). The NOAEL for effects on offspring was 250 ppm (equal to 16 mg/kg bw per day), based on reduced pup weight at 1800 ppm (equal to 122 mg/kg bw per day).

In a study of developmental toxicity in rats dosed at 0, 7.7, 49 or 292 mg/kg bw per day, there was no evidence of teratogenicity. The Meeting noted a decrease in the incidence of some variations of the skull bones but considered that this was not toxicologically relevant. The NOAEL for maternal toxicity was 49 mg/kg bw per day, on the basis of decreased body weight gain and clinical signs at 292 mg/kg bw per day. The NOAEL for embyo and fetal toxicity was 49 mg/kg bw per day, based on four non-viable fetuses at 292 mg/kg bw per day.

In a study of developmental toxicity in rabbits dosed at 0, 2.5, 10 or 40 mg/kg bw per day, the NOAEL for maternal toxicity was 10 mg/kg bw per day, based on body weight loss, and the NOAEL for embryo and fetal toxicity was 40 mg/kg bw per day, the highest dose tested.

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

The acute neurotoxicity of fluensulfone was investigated in rats at dose levels of 0, 100, 400 and 1200 mg/kg bw. Clinical signs and reduced activity in a functional observational battery on day 1, but not subsequently, were seen at all dose levels, with females being more sensitive than males. Benchmark dose modelling gave reliable $BMDL_{SD}$ values ($BMDL_{SD}$ is the 95% lower confidence limit of the dose corresponding to a change equivalent to one standard deviation in the response for unexposed animals) in the range 19–33 mg/kg bw for females, and the Meeting identified 25 mg/kg bw as a reference point or point of departure (POD). There were no indications of neuropathy.

In a subchronic (90-day) neurotoxicity study in rats, dietary concentrations were 0, 100, 500 and 2500 ppm (equal to 0, 6, 31 and 153 mg/kg bw per day for males and 0, 7, 34 and 162 mg/kg bw per day for females, respectively). The NOAEL for neurotoxicity was 2500 ppm (equal to 153 mg/kg bw per day), the highest dose tested, with a NOAEL for general toxicity of 500 ppm (equal to 31 mg/kg bw per day), based on reduced body weight gain in males at 2500 ppm (equal to 153 mg/kg bw per day).

In a 28-day immunotoxicity study in female mice, dietary concentrations were 0, 100, 500 and 2500/1500 ppm (equal to 0, 17, 86 and 204 mg/kg bw per day). There were no significant effects on anti–sheep red blood cell IgM titres at any dose level. The NOAEL for general toxicity was 500 ppm (equal to 86 mg/kg bw per day), based on deaths and clinical signs at 2500/1500 ppm (equal to 204 mg/kg bw per day). Fluensulfone did not affect splenic or bone marrow cell counts or IgA, IgG or IgM titres in rats exposed at concentrations up to 2000 ppm (equal to 152 mg/kg bw per day) in the diet in the 28-day study of toxicity in rats.

Toxicological data on metabolites and/or degradates

Acute oral toxicity and genotoxicity studies were performed on the following metabolites: thiazole sulfonic acid (M-3625), methyl sulfone derivative (M-3626) and butene sulfonic acid (M-3627). Thiazole sulfonic acid and butene sulfonic acid were of low acute oral toxicity (LD_{50} > 2000 mg/kg bw) and were not genotoxic in vitro or in vivo. The methyl sulfone derivative had an acute oral LD_{50} of ≥ 300 mg/kg bw. It was weakly positive in the Ames test for test strain *S. typhimurium* TA100 at the highest concentration tested (5000 μg/plate) in the absence of metabolic activation and equivocal in a reverse mutation assay in Chinese hamster cells. Two in vivo genotoxicity studies, for bone marrow micronuclei and liver UDS, were negative.

Human data

No adverse effects have been reported in a group of over 20 individuals involved in the manufacturing, handling and testing of fluensulfone.

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

Toxicological evaluation

The Meeting established an ADI for fluensulfone of 0–0.01 mg/kg bw on the basis of the NOAEL of 1.4 mg/kg bw per day for chronic interstitial inflammation in the lungs, oesophageal hyperkeratosis and decreased body weight from the rat chronic toxicity and carcinogenicity study. A safety factor of 100 was applied. This provides a margin of exposure of 3900 between the upper bound of the ADI and the LOAEL for tumours in female mice.

The Meeting established an ARfD for fluensulfone of 0.3 mg/kg bw, on the basis of the POD of 25 mg/kg bw for changes in the functional observational battery in the acute neurotoxicity study in rats. A safety factor of 100 was applied.

A toxicological monograph was prepared.

Levels relevant to risk assessment of fluensulfone

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day study of toxicity[a]	Toxicity	60 ppm, equal to 11 mg/kg bw per day	300 ppm, equal to 51 mg/kg bw per day
	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	30 ppm, equal to 4.2 mg/kg bw per day (m)	200 ppm, equal to 28 mg/kg bw per day (m)
		Carcinogenicity	30 ppm, equal to 6.4 mg/kg bw per day (f)	200 ppm, equal to 39 mg/kg bw per day (f)
Rat	Acute neurotoxicity study[b]	Neurotoxicity	25 mg/kg bw[c]	100 mg/kg bw[d]
	Ninety-day study of toxicity[a]	Toxicity	120 ppm, equal to 8 mg/kg bw per day	500 ppm, equal to 35 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	30 ppm, equal to 1.4 mg/kg bw per day	200 ppm, equal to 9.6 mg/kg bw per day
		Carcinogenicity	1200 ppm, equal to 69 mg/kg bw per day[e]	—
	Multigeneration reproductive toxicity study[a]	Reproductive toxicity	1800 ppm, equal to 122 mg/kg bw per day[e]	—
		Parental toxicity	250 ppm, equal to 16 mg/kg bw per day	1800 ppm, equal to 122 mg/kg bw per day
		Offspring toxicity	250 ppm, equal to 16 mg/kg bw per day	1800 ppm, equal to 122 mg/kg bw per day
	Developmental toxicity study[b]	Maternal toxicity	49 mg/kg bw per day	292 mg/kg bw per day
		Embryo and fetal toxicity	49 mg/kg bw per day	292 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
Rabbit	Developmental toxicity study[b]	Maternal toxicity	10 mg/kg bw per day	40 mg/kg bw per day
		Embryo and fetal toxicity	40 mg/kg bw per day[e]	—
Dog	Ninety-day and 1-year studies of toxicity[a,f]	Toxicity	100 ppm, equal to 3 mg/kg bw per day	500 ppm, equal to 16 mg/kg bw per day

f, female; m, male

[a] Dietary administration.

[b] Gavage administration.

[c] POD based on $BMDL_{SD}$ values of 19–33 mg/kg bw in females.

[d] Lowest dose tested.

[e] Highest dose tested.

[f] Two or more studies combined.

Estimate of acceptable daily intake

0–0.01 mg/kg bw

Estimate of acute reference dose

0.3 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 fluensulfone

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, plasma T_{max} is 4 h; > 80%
Dermal absorption	No data
Distribution	Widely distributed; highest concentrations in liver, kidney, red blood cells and thyroid
Potential for accumulation	Potential accumulation in erythrocytes
Rate and extent of excretion	Largely cleared within 48 h at low dose; primarily via urine (> 70%) and, to a lesser extent, faeces (5–13%)
Metabolism in animals	Extensive; mainly by cleavage to yield the thiazole glutathione conjugate and butene sulfinic acid, which are further metabolized
Toxicologically significant compounds in animals, plants and the environment	Fluensulfone; fluoride ion
Acute toxicity	
Rat, LD_{50}, oral	671 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw

Rat, LC_{50}, inhalation	> 5.1 mg/L
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Body weight gain, haematology; liver and kidney weights
Lowest relevant oral NOAEL	3 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	Lung, oesophagus, body weight
Lowest relevant NOAEL	1.4 mg/kg bw per day (rat)
Carcinogenicity	Unlikely to be carcinogenic to humans from the diet
Genotoxicity	
	Not genotoxic in vivo
Reproductive toxicity	
Target/critical effect	Lower pup weight at parentally toxic dose
Lowest relevant parental NOAEL	16 mg/kg bw per day
Lowest relevant offspring NOAEL	16 mg/kg bw per day
Lowest relevant reproductive NOAEL	122 mg/kg bw per day, the highest dose tested
Developmental toxicity	
Target/critical effect	Pup viability at maternally toxic dose (rat)
Lowest relevant maternal NOAEL	10 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	40 mg/kg bw per day, the highest dose tested (rabbit); 49 mg/kg bw per day (rat)
Neurotoxicity	
Acute neurotoxicity NOAEL	POD 25 mg/kg bw (based on $BMDL_{SD}$) (rat)
Subchronic neurotoxicity NOAEL	153 mg/kg bw per day, the highest dose tested (rat)
Other toxicological studies	
Immunotoxicity	Not immunotoxic (mouse and rat)
Studies on metabolites	Thiazole sulfinic acid (M-3625): Oral LD_{50} > 2000 mg/kg bw; not genotoxic
	Methyl sulfone derivative (M-3626): Oral $LD_{50} \geq$ 300 mg/kg bw; not genotoxic in vivo
	Butene sulfonic acid (M-3627): Oral LD_{50} > 2000 mg/kg bw; not genotoxic
Medical data	
	No adverse effects reported

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Two-year toxicity/carcinogenicity study in rats	100
ARfD	0.3 mg/kg bw	Acute neurotoxicity study in rats	100