FLUENSULFONE

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Explanation

Fluensulfone is the International Organization for Standardization–approved name for 5-chloro-2-(3,4,4-trifluorobut-3-en-1-ylsulfonyl)-1,3-thiazole (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service 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 been known under the development codes MCW-2 and BYI 01921. Most toxicological studies were performed with pilot plant–produced material (batch 36372130-291-PF1), produced by Makhteshim Chemical Works Ltd. A small number of initial toxicological studies were conducted with the test batches produced by Bayer CropScience.

Fluensulfone has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

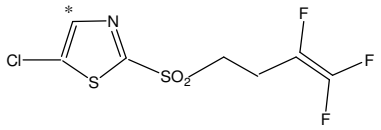
All critical studies contained statements of compliance with good laboratory practice (GLP) and met the minimum requirements of the applicable Organisation for Economic Co-operation and Development (OECD) or national test guidelines.

Evaluation for acceptable daily intake

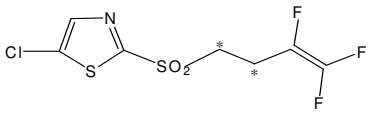
1. Biochemical aspects

The metabolism and excretion of [^{14}C]fluensulfone were determined using fluensulfone radiolabelled at the thiazole and butene positions (Fig. 1).

Fig. 1. Structure and radiolabel positions for fluensulfone



[thiazole-^{14}C]fluensulfone



[butene-^{14}C]fluensulfone

* Indicates position of ^{14}C label

1.1 Absorption, distribution and excretion

Rats

The absorption and excretion of [^{14}C]fluensulfone (thiazole and butene labels), prepared as a suspension in polyethylene glycol (PEG) 300, were investigated using single oral low (5 mg/kg body weight [bw]) or high (500 mg/kg bw) doses administered to groups of 4–8 male and 4–8 female Wistar rats. For the excretion segment, rats were housed in individual glass metabolism cages equipped for collection of $^{14}CO_2$, urine and faeces over 120 hours and were terminated thereafter. Kinetic parameters were determined from blood samples obtained from jugular vein cannulae over a period of up to 336 hours.

The recovery of radioactivity was 91.3–97.3% of the administered dose for all groups of rats for the excretion segment. The excretion of radiolabel was generally similar in males and females (Table 1). There was no indication that elimination processes were saturated by the 100-fold increase in dose level, as levels of radiolabel in urine, cage wash, faeces, carcass and gastrointestinal tract were similar. Urine was the major route of excretion (> 70%) for both radiolabel positions. Analysis showed that the metabolites in cage wash and urine were similar, thus indicating that the cage wash mainly derived from urine.

Table 1. Summary of excretion (to 72 hours) for rats administered [^{14}C]fluensulfone

	Excretion (% of administered dose; mean ± standard deviation)							
	[thiazole-^{14}C]fluensulfone				[butene-^{14}C]fluensulfone			
	Low dose (5 mg/kg bw)		High dose (500 mg/kg bw)		Low dose (5 mg/kg bw)		High dose (500 mg/kg bw)	
	Males	Females	Males	Females	Males	Females	Males	Females
CO_2 traps	0.36 ± 0.13	0.55 ± 0.13	0.06 ± 0.03	0.07 ±0.05	4.0 ± 1.8	4.4 ± 1.7	1.9 ± 1.0	1.3 ± 0.7
Urine	77.8 ± 1.6	71.7 ± 9.9	76.0 ± 7.6	76.0 ± 9.6	63.8 ± 4.5	63.2 ± 6.0	71.7 ± 8.1	71.8 ± 4.9
Cage wash	6.6 ± 0.9	14.2 ± 4.0	9.1 ± 3.3	12.2 ± 5.2	11.9 ± 2.2	11.9 ± 6.6	8.9 ± 3.7	10.9 ± 3.2
Faeces	11.0 ± 1.1	8.3 ± 0.7	7.2 ± 0.9	5.1 ± 1.3	13.3 ± 1.2	10.0 ± 1.1	11.4 ± 3.0	6.3 ± 1.5
Carcass	1.5 ± 0.3	1.8 ± 0.1	1.2 ± 0.1	1.2 ± 0.3	2.6 ± 0.8	1.6 ± 0.2	1.4 ± 0.4	0.9 ± 0.4
GI tract	0.07 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.08 ± 0.02	0.27 ± 0.21	0.15 ± 0.02	0.09 ± 0.01	0.07 ± 0.02
Total	**97.3**	**96.6**	**93.6**	**94.7**	**95.9**	**91.3**	**95.4**	**91.3**

GI: gastrointestinal
Source: Quistad, Kovatchev & La Mar (2010, 2011a)

Kinetic parameters are presented in Table 2. Fluensulfone was rapidly absorbed at a dose level of 5 mg/kg bw, but the absorption phase was significantly longer at 500 mg/kg bw. Area under the plasma concentration–time curve (AUC) values increased proportionately (> 50-fold) with the administered dose (100-fold). The elimination from whole blood was longer than from plasma, indicating an affinity for blood cells. Investigative work showed that fluensulfone reacts with the free thiol moiety of globin to form a covalent linkage to the thiazole group and displacing butene sulfinic acid. The decline of ^{14}C-thiazole radiolabel from whole blood seems to follow the removal of derivatized haemoglobin, with a time required for the concentration to decline to half the peak concentration of 148–216 hours (6–9 days) for the low dose.

Table 2. Summary of kinetic parameters for rats (males and females combined) administered [^{14}C]fluensulfone

Kinetic parameter	Radiolabel	Dose rate	Whole blood	Plasma
C_{max} (µg eq/g)	Thiazole	Low	1.1–1.4	1.2–1.6
		High	75–92	39–49
	Butene	Low	0.97–0.99	1.2
		High	25–30	29–34
T_{max} (h)	Thiazole	Low	2–8	2
		High	48	1–8
	Butene	Low	4	4
		High	24	24
$t_{½}$ (h)	Thiazole	Low	146–208	14–15
		High	134–162	58–67
	Butene	Low	30–33	20
		High	30–32	24–26
$t_{1/10}$ (h)	Thiazole	Low	485–691	47–50
		High	445–537	193–221
	Butene	Low	100–110	66
		High	100–105	79–85
AUC (h·µg eq/g)	Thiazole	Low	237–306	40–45
		High	16 000 – 21 000	3 200–3 400
	Butene	Low	43–47	37–38
		High	2 000–2 100	1 900–2 000

AUC: area under the plasma concentration–time curve; C_{max}: maximum concentration; eq: equivalents; $t_{½}$: time for decline to half of C_{max}; $t_{1/10}$: time for decline to 1/10 of C_{max}; T_{max}: time to reach C_{max}
Source: Quistad, Kovatchev & La Mar (2010, 2011a)

The distribution of [^{14}C]fluensulfone (thiazole and butene labels) in rat tissues was investigated using a suspension in PEG 300, at single oral low (5 mg/kg bw) or high (500 mg/kg bw) doses administered to groups of three male and three female Wistar rats. The tissue distribution study was carried out with two radiolabels and three sacrifice intervals for each of the low and high doses. Collection intervals in each dose group were based on the previously determined kinetic parameters. The concentrations of fluensulfone-derived ^{14}C were measured in 32 tissues/organs and in the carcass.

Residues in males and females were generally similar. The thiazole label was slowly eliminated from red blood cells and organs associated with their formation/storage (bone, bone marrow, spleen). Highest initial concentrations were seen in liver, kidney and thyroid with the thiazole label and in liver, kidney and lung with the butene label. 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) (Supplementary Tables 1 and 2[1]) (Quistad & Kovatchev, 2011).

The disposition and metabolism of [^{14}C]fluensulfone were studied in male and female Wistar rats after 14 repeated daily oral doses of unlabelled fluensulfone, followed by a single oral dose of [^{14}C-thiazole]fluensulfone at 5 mg/kg bw, 1 day after the last unlabelled dose. Fluensulfone was administered as a suspension in PEG 300 by oral gavage. Rats were terminated 1 or 7 days after administration of the radiolabelled dose. The study determined the excretion pattern and distribution of radiolabel in selected tissues and identified the major metabolites of fluensulfone in urine and faeces.

The recovery of excreted radiolabel was 94–99% for individual rats. Radiolabel was found mainly in urine (71–83%), faeces (9–11%) and the cage wash (6–16%). One day after dosing with radiolabelled fluensulfone, an average of 0.6–0.8% of the administered radiolabel was in liver and 0.1% in kidney, with 1.7–2.1% in the carcass and 4.5–4.7% remaining in the gastrointestinal tract. By 7 days after treatment with radiolabelled fluensulfone, an average of 0.1–0.2% of the administered radiolabel remained in liver, and 0.03% in kidney. There were no significant sex differences in either elimination or tissue residues. The results are broadly in line with those from the equivalent single-dose study (Quistad, Kovatchev & La Mar, 2011b).

1.2 Biotransformation

Rats

Urine, faeces and cage wash samples from the study of Quistad, Kovatchev & La Mar (2011a,b, described above) were analysed for metabolites using radio–high-performance liquid chromatography. Fluensulfone was not a significant residue in either faeces or urine. The majority of the faecal radioactivity was not identified. It consisted of a number of peaks, each representing less than 2% of the administered dose; the only faecal metabolite representing more than 5% of the administered dose was thiazole sulfonic acid. In urine, there were no metabolites common to both radiolabels, which indicates that the initial step is cleavage at the sulfonyl bond. The initial reaction is probably with glutathione to release the trifluorobutene group and conjugation of the thiazole moiety (Table 3). The positioning of the radiolabel in the butene moiety did not permit the fate of the fluorine atoms to be determined. Similar results were seen after 14 daily doses of unlabelled fluensulfone and one dose of [thiazole-^{14}C]fluensulfone, indicating that there is no significant induction of the metabolism of fluensulfone.

A metabolic pathway is presented in Fig. 2.

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

2. Toxicological studies

2.1 Acute toxicity

Fluensulfone is of moderate acute toxicity following oral exposure and of low acute toxicity following dermal and inhalation exposure (Table 4). It is slightly irritating to the skin of rabbits (Simon, 2009c) and not irritating to rabbit eyes (Simon, 2009d), but it is a skin sensitizer in a maximization test in guinea-pigs (Simon, 2009e).

[1] Supplementary tables are available on the JMPR website.

Table 3. Comparison of urinary and cage wash metabolites in rats administered fluensulfone

Metabolite	% of administered dose							
	[thiazole-^{14}C]Fluensulfone				[butene-^{14}C]Fluensulfone			
	Low dose (5 mg/kg bw)		High dose (500 mg/kg bw)		Low dose (5 mg/kg bw)		High dose (500 mg/kg bw)	
	Males	Females	Males	Females	Males	Females	Males	Females
Thiazole mercapturate	40	45	49	63	–	–	–	–
Thiazole glucuronide MW 327-I	20	18	12	0.6	–	–	–	–
Thiazole glucuronide MW 327-II	7	11	10	21	–	–	–	–
Thiazole sulfonic acid	5	4	3	2	–	–	–	–
Other	3	4	2	3	–	–	–	–
Butene sulfinic acid	–	–	–	–	35	38	53	65
Butene sulfonic acid	–	–	–	–	4	6	4	5
Solvent front	–	–	–	–	8	8	4	3
Other	–	–	–	–	4	5	2	2

Source: Quistad, Kovatchev & La Mar (2011a)

2.2 Short-term studies of toxicity

Repeated-dose oral (dietary) toxicity studies with fluensulfone are available for rats, mice and dogs.

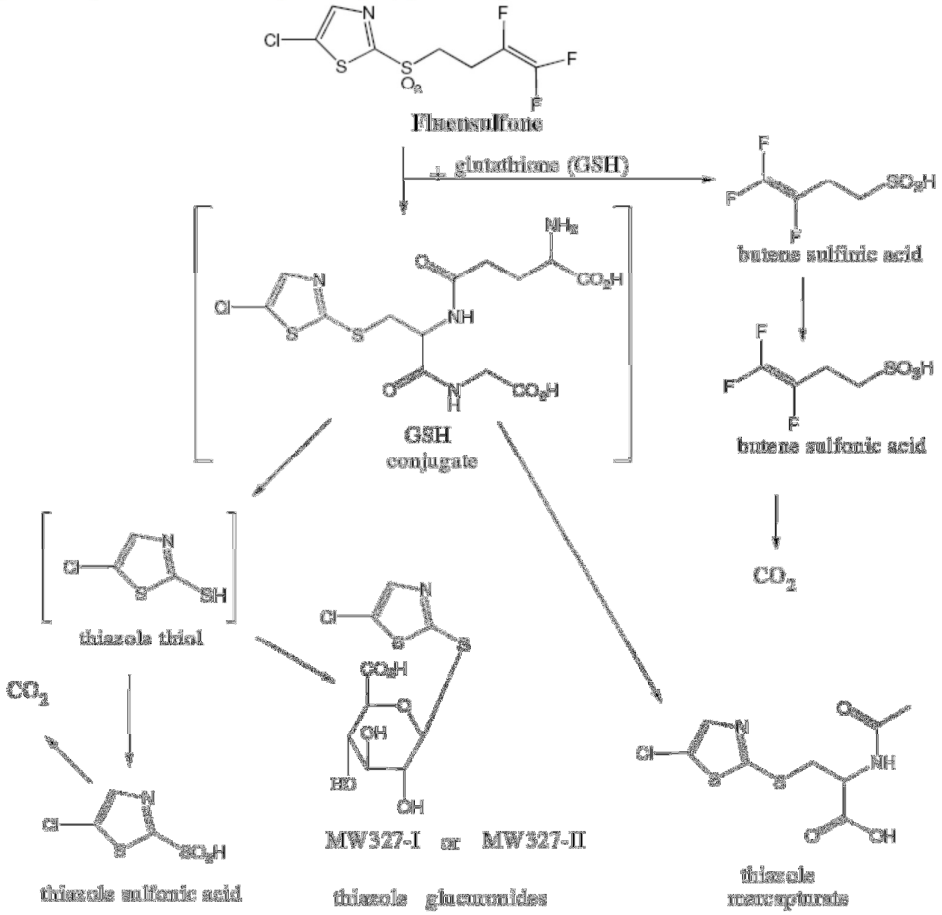
In all species, the liver was a target organ, although often the observed effects were limited to changes typical of those seen in animals responding to high levels of xenobiotics (e.g. hepatocyte hypertrophy concomitant with increased liver weight). Investigations on liver enzyme activities in all species revealed that fluensulfone administration does not result in significant induction of phase I cytochrome P450 (CYP) subfamilies, but that the (conjugating) phase II enzymes were induced to a limited extent.

The Meeting concluded that reductions in serum and hepatic alanine aminotransferase (ALAT) activities were not adverse (see below) and that effects related to the fluoride content of fluensulfone should be considered as part of total fluoride exposure. These effects have been considered as not adverse in the identification of no-observed-adverse-effect levels (NOAELs) for individual studies.

Mice

In a 4-week dietary study, groups of five male and five female Crl:CD1 mice received fluensulfone (purity 97%; batch NLL6692-7-5) in their diet at 0, 100, 500 or 2000 parts per million (ppm). Achieved doses were 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. Clinical observations were performed daily. Body weights and feed and water consumption were determined weekly. Clinical laboratory examinations were performed in week 4, and a gross necropsy (with organ weights and tissue sampling) was performed on all surviving animals at termination. An extensive range of organs and tissues was examined microscopically from control and top-dose animals plus a limited number of animals from the other groups.

Fig. 2. Proposed metabolic pathway of fluensulfone in rats



Source: Quistad, Kovatchev & La Mar (2011a)

Table 4. Summary of acute toxicity data for fluensulfone

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/L)	Purity; batch	Vehicle	Reference
Rat	Wistar	F	Oral	671 (OECD TG 423)	–	96.5%; 36372130-291-PF1	0.8% hydroxypropyl methyl cellulose	Haferkorn (2010)
Rat	HanRcc: Wistar	F	Oral	300–2 000 (OECD TG 423)	–	96.2%; 36372130-291-PF1	PEG 300	Simon (2009a)
Rat	HanRcc: Wistar	M/F	Dermal	> 2 000	–	96.2%; 36372130-291-PF1	PEG 300	Simon (2009b)
Rat	HanRcc: Wistar	M/F	Inhalation (4 h, nose only)	–	> 5.1 (MMAD < 2.5 µm)	96.2%; 36372130-291-PF1	None (aerosol)	Pothmann (2009)

F: female; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; M: male; MMAD: mass median aerodynamic diameter; OECD TG: Organisation for Economic Co-operation and Development Test Guideline; PEG: polyethylene glycol

No mortality and no clinical signs occurred. Feed intake was decreased by 25% or more at 500 ppm and above in both sexes. Water consumption was slightly increased at 2000 ppm in females. Body weight loss was observed at 2000 ppm in the 1st week of treatment in both sexes. At the end of treatment, the difference from the controls was −13% in males and −4% in females. Dose-related increases in red blood cell parameters were seen, achieving statistical significance at 2000 ppm in females (increases of erythrocyte and reticulocyte counts, concentration of haemoglobin, haematocrit values), indicative of a response to damage to mature erythrocytes. Increases in activities of plasma aspartate aminotransferase (ASAT) (2-fold) and plasma ALAT (2-fold) were recorded in both sexes at 2000 ppm and considered as correlates of the observed microscopic and organ weight changes in the liver.

The relative kidney weights were decreased slightly at 2000 ppm in males. Liver weights (relative) were increased (approximately 15%) at 500 ppm and above in females. Histopathology revealed treatment-related findings only in the liver at 2000 ppm in both sexes:

- a cytoplasmic alteration in the periportal region, characterized by a dense cytoplasmic structure, eosinophilia (females) or slight basophilia (males);
- degenerative changes of the periportal zone, which also encompassed single-cell necrosis and, in some of the females, mitotic figures; and
- a slight increase in the number and size of bile ducts in the portal regions (bile duct hyperplasia) in one male and three females.

The NOAEL of the study was considered to be 500 ppm for both sexes (equal to 101 mg/kg bw per day), based on body weight loss, changes in erythrocyte parameters (e.g. increased reticulocytes) and hepatotoxicity (altered cytoplasmic structure, single-cell necrosis, bile duct hyperplasia) at 2000 ppm (equal to 353 mg/kg bw per day) (Krötlinger & Rühl-Fehlert, 2003).

In a 90-day dietary toxicity study, groups of 12 male and 12 female Crl:CD1 (ICR)BR mice received diets containing 0, 60, 300 or 1500 ppm fluensulfone (purity > 97%; batch NLL 6692-7-5) for a period of 13 weeks. Achieved intakes were 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. Doses were selected on the basis of a 4-week range-finding study. Additional satellite groups of five mice of each sex were similarly treated at 0 or 1500 ppm for 4 weeks and then terminated for determinations of enzyme levels in liver tissue. Clinical observations were carried out daily, and body weight and feed and water consumption were measured weekly. Clinical laboratory investigations and a gross necropsy (with organ weights and tissue sampling) were performed on all surviving animals at their terminal sacrifice. Organs and tissues were examined microscopically. Biochemical investigations in liver tissue were done on satellite animals at day 28.

There were several mortalities during the study: 2, 2, 4 and 4 males and 3, 0, 0 and 5 females, respectively, for the control and treated groups, in order of ascending doses. A single female from the highest-dose group was the only animal in which clinical signs (piloerection, squatting position, poor general condition and emaciation) were recorded; all other mortalities were related to retro-orbital blood sampling on day 86 or 91.

At 1500 ppm, feed intake (both sexes) and water intake (males) were decreased. At 300 ppm, feed intake was decreased in females. Body weight gain was significantly retarded at 1500 ppm in both sexes during the 1st week of treatment, with a body weight loss recorded for females. At the end of treatment, mean body weights of animals in the high-dose group differed from the control values by approximately −10% for both sexes.

Haematological investigations showed evidence of increased red blood cell synthesis, with increases in erythrocyte counts (10%), haemoglobin (10%) and haematocrit (15%) values at 300 ppm and above in males, increased mean corpuscular volume and decreased mean corpuscular haemoglobin concentration (MCHC) in males at 1500 ppm, and 30% increases in reticulocyte counts in both sexes treated at 1500 ppm. Leukocyte counts were increased in males treated at and above 300

ppm. Clinical chemistry showed increased concentrations of total bilirubin at 300 ppm and above in both sexes and decreased urea concentrations in animals of both sexes treated at 1500 ppm. The activities of ASAT, ALAT and alkaline phosphatase were increased at 1500 ppm in both sexes, often attaining statistical significance.

No treatment-related macroscopic findings were noted at necropsy. At 1500 ppm, liver weights were increased in males (relative) and females (absolute and relative). Histopathology revealed a range of liver findings in both sexes treated at 1500 ppm, and hepatocellular hypertrophy was also observed in females receiving 300 ppm (Table 5). In the spleen, an increased incidence of animals with pigment deposition in the red pulp was recorded in both sexes at 1500 ppm.

Table 5. Incidence of microscopic findings in selected organs

Organ	Incidence of findings in males/females (n = 12)			
	0 ppm	60 ppm	300 ppm	1 500 ppm
Liver				
Hepatocyte hypertrophy	– / 1	– / 1	– / 6	12 / 10
- Grade 1	– / 1	– / 1	– / 6	– / –
- Grade 2	– / –	– / –	– / –	3 / –
- Grade 3	– / –	– / –	– / –	9 / 10
Bile duct hyperplasia (grades 1–3)	– / –	– / –	– / –	11 / 10
Oval cell proliferation (grades 1–3)	– / –	– / –	– / –	7 / 2
Degeneration (grades 1–4)	– / –	– / –	– / –	10 / 10
Pigment deposits (grades 1–2)	– / –	– / –	– / –	7 / 9
Spleen				
Pigment deposits (grades 1–3)	5 / 5	5 / 6	6 / 8	12 / 11

Source: Krötlinger (2003)

Determinations in liver tissues in satellite animals sacrificed after 28 days of treatment showed no evidence for toxicologically significant effects on the concentration of cytochrome P450 at 1500 ppm. In male mice, only the monooxygenase 7-ethoxycoumarin deethylase was marginally increased. The phase II enzymes epoxide hydrolase (EH) and glutathione *S*-transferase (GST) were marginally increased, whereas uridine diphosphate (UDP)–glucuronyltransferase (UDPGT) was not affected. In female mice, the monooxygenases were not significantly affected, whereas all phase II enzymes (EH, GST, UDPGT) were significantly induced at 1500 ppm.

The NOAEL of the study was 60 ppm (equal to 11 mg/kg bw per day), based on haematological effects and hepatocyte hypertrophy at 300 ppm (equal to 51 mg/kg bw per day) (Krötlinger, 2003).

Rats

Groups of five male and five female Wistar rats received fluensulfone (purity > 98%; batch NLL6692-3) in their diet at 0, 125, 500 or 2000 ppm for approximately 4 weeks. Achieved doses were 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. Animals were inspected once a day for morbidity and mortality. In the 4th exposure week, sensory reactivity to stimuli of different types (auditory, visual, touch, nociceptive) and grip strength were assessed. Urine and blood samples were taken prior to sacrifice. In addition to the standard parameters, immunoglobulins (Ig) A, G and M, triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) concentrations were also determined. At terminal sacrifice, selected organs were weighed, and an extensive range of tissues from top-dose and control animals was examined histopathologically; lung, liver and kidney were examined in other groups. Liver

specimens were taken to investigate liver enzymes and substrates. Other liver specimens were excised and frozen to analyse tissue for cell proliferation potential. Blood (by cardiac puncture), left femur and half of the spleen were taken and immediately placed in chilled test tubes containing sterile physiological saline for immunotoxicity investigations.

There were no mortalities, clinical signs or effects in open-field investigations or on sensory reactivity. Mean feed consumption was slightly decreased in animals receiving 2000 ppm during the first few days of treatment, whereas water consumption was slightly increased in males of this group, but not in females. Body weight gain was retarded (~30%) in male and female rats receiving 2000 ppm and, to a lesser extent, in males receiving 500 ppm (Table 6). No treatment-related changes were noted in haematology. Clinical chemistry revealed dose-related decreases in the activity of ALAT in all treatment groups of both sexes (Table 7). In 2000 ppm males, cholesterol and total protein were slightly, but statistically significantly, increased, and triglyceride concentration was decreased (Table 7). Urine analysis did not indicate test compound–related effects.

Table 6. Body weight and selected relative organ weights in rats fed diets containing fluensulfone for 28 days

Organ	Relative organ weight (mg/100 g)			
	0 ppm	125 ppm	500 ppm	2 000 ppm
Male rats				
Terminal body weight (g)	*294*	*291*	*267*	*263*
Liver	4 552	4 887	4 856	5 205**
Kidneys	685	722	765	827**
Spleen	201	191	194	210
Female rats				
Terminal body weight (g)	*203*	*194*	*200*	*181***
Liver	4 211	4 186	4 470	4 467
Kidneys	704	692	701	756
Spleen	223	212	225	257*

*: $P < 0.05$; **: $P < 0.01$
Source: Temerowski & Rühl-Fehlert (2002)

Table 7. Selected clinical chemistry parameters in rats fed diets containing fluensulfone for 28 days

Parameter	0 ppm	125 ppm	500 ppm	2 000 ppm
Male rats				
ALAT (U/L)	91.6	63.0**	35.2**	11.8**
Cholesterol (nmol/L)	1.55	1.64	1.82	2.19*
Triglycerides (nmol/L)	1.73	1.84	1.30	0.82**
Total protein (g/L)	60.9	62.9	60.8	64.6*
Female rats				
ALAT (U/L)	74.2	54.6**	31.9**	11.0**
Cholesterol (nmol/L)	2.06	1.93	1.89	2.34
Triglycerides (nmol/L)	1.10	1.02	1.02	1.19
Total protein (g/L)	64.7	64.7	64.8	62.6

ALAT: alanine aminotransferase; U: units; *: $P < 0.05$; **: $P < 0.01$
Source: Temerowski & Rühl-Fehlert (2002)

Analysis of organ weight data showed statistically significant increases in mean relative liver (15%) and kidney weights (20%) in male rats treated at 2000 ppm and in relative spleen weight (10%) in females of the same group. Gross pathology revealed surface changes in the kidneys of males dosed at 500 ppm (1/5 animals) and 2000 ppm (5/5 animals). Histopathology revealed degeneration and hyaline inclusions in the proximal tubules. At 2000 ppm, focal tubular necrosis was observed in one male rat (Table 8). Histopathology of the liver, spleen and female kidneys revealed no toxicological effects. Immunotoxicity examinations showed no notable effects on the splenic and bone marrow cell counts, in the composition of the splenic subpopulations or in antibody titres (although splenic samples from top-dose females were lost).

Table 8. Histopathological findings in kidneys of male rats fed fluensulfone for 28 days

Lesion	Incidence of finding (n = 5)			
	0 ppm	125 ppm	500 ppm	2 000 ppm
Basophilic tubules	5	5	5	4
- Grade 1	4	5	3	1
- Grade 2	1	–	2	3
Hyaline droplets	–	–	1	5
- Grade 2	–	–	1	4
- Grade 3	–	–	–	1
Degeneration of proximal tubules	–	–	2	5
- Grade 1	–	–	2	–
- Grade 2	–	–	–	3
- Grade 3	–	–	–	2
Mononuclear infiltration	1	1	–	2
- Grade 1	1	1	–	2
Intratubular protein	–	–	2	1
- Grade 1	–	–	2	–
- Grade 2	–	–	–	1
Tubular necrosis	–	–	–	1
- Grade 1	–	–	–	1

Source: Temerowski & Rühl-Fehlert (2002)

Monooxygenases were markedly decreased in females at 500 ppm and above and, to a lesser extent, in males at 2000 ppm. Phase II enzymes (GST, UDPGT and EH) were induced in males at 500 ppm and above and in females at 2000 ppm (by 40–170%).

The NOAEL of the study 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) (Temerowski & Rühl-Fehlert, 2002).

In a 90-day dietary toxicity study, groups of 10 male and 10 female Wistar rats received diets containing 0, 60, 120, 500 or 2000 ppm fluensulfone (purity 97%; batch NLL 6692-7-5) for a period of 3 months. Doses were chosen on the basis of a 28-day range-finding study. Additional recovery groups were similarly treated at 0 or 2000 ppm and then maintained without treatment for a recovery period of 28 days. Additional groups of five males were treated for 1 week at 0 or 2000 ppm for investigation of α_{2u}-globulin accumulation in kidneys. Mean daily intakes were 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 of the main groups, respectively. Clinical observations were carried out daily, and body weight and feed and water

consumption were measured weekly. A detailed clinical observation and an open-field observation were performed weekly. A functional observational battery was performed in week 12 on both main and recovery groups and in week 16 on recovery groups. Motor activity assessments were carried out in week 12 on main groups and in weeks 13 and 16 on recovery groups. At the end of both the treatment and recovery periods, haematology, clinical chemistry and urine analysis examinations were performed. Animals were necropsied, organ weights were determined, and organs and tissues were subjected to a histopathological examination. The liver, teeth and left femur were retained at both necropsies from all animals; the total fluoride concentrations in teeth and left femur ashes were determined, and enzyme activities and cytochrome P450 content were determined in liver tissue homogenates.

No mortality occurred during the study. Clinical observations of animals revealed no treatment-related findings, and detailed behavioural investigations (functional observational battery, motor activity) gave no evidence of any neurotoxicity. No abnormalities were detected in ophthalmological investigations or urine analysis. Body weight gains were reduced (10%) in males and females treated at 2000 ppm and slightly reduced in males receiving 500 ppm (7%). During the recovery period, animals of the 2000 ppm group gained slightly more body weight than the respective control groups. Feed consumption of females was decreased at 2000 ppm during the treatment period and was not affected by the treatment in the other groups. Water intake was increased in males at 500 ppm (10%) and in males (23%) and females (15%) at 2000 ppm. During the recovery period, mean daily water intake remained similarly increased, compared with the control groups.

At 2000 ppm, decreases in haemoglobin concentrations and MCHC and increased reticulocytes were recorded, most of which were reversible during the recovery period. Significant reductions in serum ALAT activities were seen in both sexes treated at 500 or 2000 ppm, with values returning to normal during the recovery phase. Clinical chemistry tests gave evidence for effects on liver function (significantly increased triglycerides) at 500 ppm and above during treatment, with subsequent recovery. At the end of the treatment period, concentrations of urea were significantly increased at 2000 ppm in males.

At 2000 ppm, relative weights of livers were increased 9% in females and 17% in males; relative kidney weights were significantly increased in both sexes (males: 21%, females: 14%), but not at the end of the recovery period. In this dose group, changes of the surface of the kidneys were observed in five males at necropsy at the end of the treatment period. Histopathological investigations revealed hepatocyte hypertrophy in both sexes at 2000 ppm and hyaline droplet nephropathy accumulation in males at 500 and 2000 ppm, associated with increased α_{2u}-globulin. Hyaline droplet accumulation was reversible within the recovery period, whereas other tubular changes observed at 500 ppm and above after the treatment period were still discernible in 2000 ppm males after the recovery period. In females, a slightly increased grade of Schmorl-positive pigment was seen in renal tubules at 2000 ppm. This change was reversible within the recovery period.

In the forestomach, a diffuse basal cell hyperplasia was observed in both sexes at 500 ppm (40% of rats) and 2000 ppm (80% of males and all females). At the end of the recovery period, this change partly regressed, but was still detectable in a few animals (30% of males and 20% of females) at 2000 ppm (500 ppm animals did not form part of the recovery phase).

At necropsy at the end of the treatment period, discoloration of teeth was observed in 6/10 males at 2000 ppm. Treatment with the test substance increased the content of fluoride in ashes from bones and teeth of treated male and female rats in a dose-related manner (Supplementary Table 3). Statistically significant increases in fluoride concentrations were observed at 120 ppm and above. At the end of the recovery period, fluoride concentrations, although still significantly increased in the top-dose groups, were of a smaller magnitude than at the end of dosing, indicating a possible reversibility of this effect. Histopathological examinations revealed no abnormalities in teeth and investigated bones. There were no investigations of bone density, bending strength or compression resistance.

The NOAEL was 120 ppm (equal to 8 mg/kg bw per day for males and 12 mg/kg bw per day for females), based on forestomach hyperplasia, increased triglycerides and increased water

consumption in females and and decreased body weight/body weight gains in males at 500 ppm (equal to 35 mg/kg bw per day for males and 53 mg/kg bw per day for females) (Schladt & Rühl-Fehlert, 2003).

[The authors of the study report identified the NOAEL as 60 ppm (equal to 4 mg/kg bw per day), based on the increased content of fluoride in ashes from bones and teeth of rats treated at 120 ppm (equal to 8 mg/kg bw per day) and above.]

Dogs

In a 4-week dietary study, groups of two male and two female Beagle dogs received fluensulfone (purity 96.7%; batch 36372130-291-PF1) in their diet at 0, 50, 200 or 900 ppm for 30 days. Mean intakes were 0, 1.9, 7 and 31 mg/kg bw per day for males and 0, 2, 8 and 30 mg/kg bw per day for females, respectively. All males and females were sacrificed on day 30 and subjected to standard investigations. In addition, thyroid hormone levels in blood, ALAT activity in liver tissue and metabolic enzyme activity in liver were also determined. Animals were examined regularly for clinical signs, body weight and feed consumption. Ophthalmoscopic and veterinary examinations plus blood and urine sampling were performed pretest and during the last week of treatment. At necropsy, a number of organs were weighed, and histological examination was performed on 14 organs and tissues as well as on all gross lesions from all animals.

No mortality occurred and no clinical signs were noted during the study. A slight reduction in mean feed consumption and corresponding body weight stasis in males and body weight loss in females were noted at 900 ppm during the treatment period when compared with the pretreatment values or control group. No treatment-related changes were recorded in ophthalmological examination or in haematological or urine analysis parameters. Decreased ALAT activity in the blood was observed in all treated groups, and decreased protein and albumin levels and albumin to globulin ratio were noted in the females at 900 ppm. TSH values were increased 4- to 5-fold in male and female dogs at 900 ppm when compared with the control values. However, no differences in T_3 or T_4 levels were observed in the treated groups when compared with the control values.

Marked increased absolute liver (> 20%), relative liver (30%) and relative thyroid (> 50%) weights were recorded in the males at 900 ppm; organ weights in females were unaffected. There was no associated macroscopic or microscopic histopathology. Fluensulfone caused a reduction to 55–65% of control values in liver ALAT activity in both sexes at 900 ppm. The results of liver enzyme determinations showed some increases (up to 4-fold) in some total cytochrome P450 and specific phase I and phase II hepatic enzyme activities.

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 organ weights (liver and thyroid) at 900 ppm (equal to 30 mg/kg bw per day) (Braun, 2009a).

In a 90-day dietary study, groups of four male and four female Beagle dogs received fluensulfone (purity 96.7%; batch 36372130-291-PF1) in their diets at 0, 5, 50 or 500 ppm for 13 consecutive weeks. Doses were set on the basis of a range-finding study. Achieved intakes were 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. Similar-sized recovery groups for the control and the high dose were treated for 13 consecutive weeks and sacrificed after an additional 28-day period without treatment. Throughout the study, all animals were observed daily for viability and clinical signs. In addition, body weight and feed consumption were recorded. Blood and urine samples were collected pretest, in week 6 and prior to sacrifice for routine examinations and thyroid hormone levels. Ophthalmoscopy examinations were performed pretest and prior to sacrifice. At scheduled necropsy, a part of the liver was taken from all animals for liver enzyme activity determinations. Additionally, blood samples were drawn from the jugular vein. Organ weights were recorded, and samples from an extensive range of organs were subjected to histopathological examination.

There were no mortalities, clinical signs or changes observed in ophthalmological or veterinary examinations or urine analysis. There were no effects on feed consumption, but female animals receiving 500 ppm gained less weight than the controls. Increases in reticulocyte counts and MCHC were seen at 500 ppm in weeks 6 and 13. Clinical chemistry investigation revealed statistically significant decreases in ALAT after 6 weeks in males and females at 50 and 500 ppm; however, values were within historical control ranges for the performing laboratory, and the decreases were not evident at 13 weeks. Decreased total protein and albumin levels and albumin to globulin ratios were recorded after 6 and 13 weeks at 500 ppm. A tendency to higher TSH levels was seen in male and female dogs treated at 500 ppm, but the results were driven by high values in one dog of each sex; no notable differences in T_3 and T_4 levels were observed

Increased liver weights were seen in males and females at 500 ppm, but were not apparent at the end of the recovery period (Table 9). Slight diffuse hepatocellular hypertrophy was seen in one main study female at 500 ppm. There was some evidence of enzyme induction in the livers of 500 ppm dogs, but the increases in activity were approximately 2-fold. Hepatic ALAT activity was reduced at 500 ppm, to 40% and 70% of control values in males and females, respectively.

Table 9. Body and liver weights in dogs (n = 4) exposed to fluensulfone

	Control[a]	5 ppm	50 ppm	500 ppm[a]
Males				
Body weight (kg)	10.6 *(12.0)*	–	–	10.0 *(10.8)*
Liver weight				
- Absolute (g)	286.0 ± 63.8 *(337.3 ± 80.8)*	295.0 ± 68.3	280.6 ± 22.5	315.0 ± 50.8 *(309.8 ± 24.7)*
- Relative to body weight (%)	2.7 ± 0.3 *(2.8 ± 0.6)*	2.6 ± 0.7	2.6 ± 0.2	3.2 ± 0.2 *(2.9 ± 0.2)*
Females				
Body weight (kg)	10.5 *(10.0)*	9.8	9.3	8.7 *(9.0)*
Liver weight				
- Absolute (g)	318.2 ± 47.3 *(267.8 ± 9.3)*	304.7 ± 30.5	299.1 ± 63.8	343.6 ± 19.1 *(254.3 ± 21.5)*
- Relative to body weight (%)	3.1 ± 0.4 *(2.7 ± 0.4)*	3.2 ± 0.4	3.2 ± 0.3	4.0 ± 0.3** *(2.9 ± 0.1)*

**: $P < 0.01$

[a] Recovery group shown in parentheses.

Source: Braun (2009b)

The NOAEL was 50 ppm (equal to 1.7 mg/kg bw per day), based on alterations in reticulocytes and marked increases in relative and absolute liver weights at 500 ppm (equal to 17 mg/kg bw per day) (Braun, 2009b).

In a 1-year oral toxicity study, fluensulfone (purity 96.7%; batch 36372130-291-PF1) was administered in the diet at 0, 5, 50, 100 or 500 ppm to purebred Beagle dogs (four of each sex per group) for a period of 52 weeks, with selected animals retained for an 8-week recovery period. Achieved intakes were 0, 0.1, 1.5, 3 and 16 mg/kg bw per day, respectively. Throughout the study, all animals were observed daily for viability and clinical signs. Body weight was recorded at selected intervals, and feed consumption was recorded daily. Ophthalmoscopic and veterinary examinations

were performed pretest, during weeks 13, 26 and 52 and during week 8 of recovery. Blood and urine samples were collected for clinical laboratory investigations pretest, during weeks 6, 13, 26 and 52 and during week 8 of recovery. Additional blood samples were collected for liver parameter determinations during weeks 1, 2, 4, 8, 10, 17 and 39 and during week 4 of recovery as well as for hormone analyses pretest and during week 52 and week 8 of recovery. Following completion of the treatment or recovery period, a detailed necropsy was performed on all animals, and selected organs were weighed. The collected tissues were placed in fixative, processed and examined microscopically. Additional samples of liver and bone were collected and frozen for specific analyses.

Body weight gain was reduced at 500 ppm (Table 10). Alterations in red blood cell parameters were seen at 500 ppm (Tables 11 and 12). Marked decreases in plasma ALAT activity were observed at 50, 100 and 500 ppm. Increased relative liver and kidney weights were seen at 100 ppm and above (Table 13), with brown pigment deposition and diffuse hypertrophy observed in the liver of the majority of dogs at 500 ppm. There were no treatment-related histopathological findings in the kidneys or increases in plasma urea levels. Increases in hepatic activities of EH and GST were seen at 500 ppm, together with decreased ALAT activity at 50 ppm and above. A dose-related increase in fluoride content was evident at doses of 50, 100 and 500 ppm in the ashes from bone and teeth, attaining statistical significance only at the high dose level (Supplementary Table 4). No signs of dental fluorosis were observed during the study, and no abnormality in bone structure was observed at microscopic examination. With the exception of the pigment deposition in the sinusoidal cells of the liver and fluoride levels, all the findings showed extensive resolution during the recovery period.

Table 10. Body weight and body weight gain in dogs receiving fluensulfone for 12 months

Dietary concentration (ppm)	Males			Females		
	Mean body weight (kg)		Overall body weight gain (%)	Mean body weight (kg)		Overall body weight gain (%)
	Day 1	Day 358		Day 1	Day 358	
0	8.5	13.4	57.6	7.9	12.0	51.4
5	9.1	12.3	35.5	7.7	12.6	63.8
50	8.2	12.1	47.6	7.6	11.5	50.5
100	8.4	12.1	43.1	7.4	10.9	46.5
500	8.6	11.4	32.9*	7.7	10.1	32.0

*: $P < 0.05$
Source: Braun (2011b)

The NOAEL was 100 ppm (equal to 3 mg/kg bw per day), based on altered red cell parameters, increased pigment deposition in the liver and increased liver weights at 500 ppm (equal to 16 mg/kg bw per day) (Braun, 2011b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female CD-1 mice were treated for 78 weeks with 0, 30, 200 or 1200 ppm fluensulfone (purity 96.7%; batch 36372130-291-PF1) in their feed. Achieved mean intakes were 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. Additionally, six males and six females were used for liver enzyme determination after 13 weeks of treatment. Viability/mortality, clinical signs, feed consumption and body weights were recorded periodically throughout the study. Blood samples from all main group mice were taken after 52 and 78 weeks for determination of haematology and at 78 weeks for determination of limited clinical biochemistry parameters. After 13 weeks, satellite group mice were killed; livers were removed and homogenized, and ex vivo activity of metabolic liver enzymes was determined. All animals sacrificed after 78 weeks of treatment with fluensulfone as well as all animals

Table 11. Selected haematology parameters in male dogs receiving fluensulfone for 12 months

Dietary concentration (ppm)	Hb (mmol/L)	MCH (fmol)	MCHC (mmol/L)	HDW (mmol/L)	Reti abs ($\times 10^9$/L)	Reti rel	Plat ($\times 10^9$/L)
Pretest							
0	8.2	1.37	20.77	1.01	57	0.010	419
5	8.1	1.36	21.03	1.05	59	0.009	324
50	8.0	1.37	21.21	1.08	52	0.009	391
100	7.8	1.33	20.80	1.05	63	0.011	443
500	8.3	1.34	20.94	1.04	65	0.011	384
Week 6							
0	8.7	1.37	21.00	1.08	57	0.009	373
5	8.8	1.38	21.29	1.13	61	0.009	299
50	8.5	1.35	21.03	1.11	51	0.008	339
100	8.9	1.35	21.08	1.13	61	0.010	403
500	8.4	1.33	20.18**	1.44**	108**	0.020*	396
Week 52							
0	10.8	1.45	21.76	1.08	60	0.008	342
5	10.4	1.45	21.82	1.14	65	0.009	278
50	10.5	1.43	21.67	1.13	79	0.012	398
100	10.0	1.40	21.55	1.13	62	0.010	451
500	9.3**	1.34**	20.47**	1.53**	108*	0.015*	457
Week 8 of recovery							
0	12.4	1.41	21.00	1.01	58	0.007	257
500	10.7	1.39	21.42	1.13	72	0.010	318

Hb: haemoglobin; HDW: haemoglobin distribution width; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; Plat: platelets; Reti abs: absolute reticulocyte count; Reti rel: relative reticulocyte count (ratio of reticulocytes to total erythrocytes); *: $P < 0.05$; **: $P < 0.01$
Source: Braun (2011b)

Table 12. Selected haematology parameters in female dogs receiving fluensulfone for 12 months

Dietary concentration (ppm)	Hb (mmol/L)	MCH (fmol)	MCHC (mmol/L)	HDW (mmol/L)	Reti abs ($\times 10^9$/L)	Reti rel	Plat ($\times 10^9$/L)
Pretest							
0	8.5	1.36	20.81	0.98	64	0.009	382
5	8.5	1.35	20.97	1.03	69	0.011	418
50	8.2	1.39	21.22	1.11*	76	0.011	377
100	9.1	1.39	21.20	1.14**	98	0.016	391
500	8.8	1.38	20.81	1.04	90	0.014	439
Week 6							
0	9.5	1.37	21.15	1.10	74	0.011	347
5	9.5	1.36	21.12	1.11	72	0.011	352
50	8.6	1.39	21.11	1.11	49	0.008	361
100	9.9	1.40	21.37	1.12	92	0.014	343
500	8.4**	1.36	20.10**	1.40**	108	0.017	454

Table 12 (continued)

Dietary concentration (ppm)	Hb (mmol/L)	MCH (fmol)	MCHC (mmol/L)	HDW (mmol/L)	Reti abs ($\times 10^9$/L)	Reti rel	Plat ($\times 10^9$/L)
Week 52							
0	10.6	1.44	21.72	1.07	57	0.007	387
5	10.5	1.43	21.85	1.14	72	0.009	417
50	9.9	1.46	21.80	1.13	63	0.009	406
100	10.5	1.45	21.73	1.13	78	0.011	398
500	9.8	1.40	20.66**	1.41**	110**	0.015*	557*
Week 8 of recovery							
0	10.9	1.41	21.86	1.09	74	0.008	374
500	10.5	1.41	21.51	1.10	70	0.009	375

Hb: haemoglobin; HDW: haemoglobin distribution width; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; Plat: platelets; Reti abs: absolute reticulocyte count; Reti rel: relative reticulocyte count (ratio of reticulocytes to total erythrocytes); *: $P < 0.05$; **: $P < 0.01$
Source: Braun (2011b)

Table 13. Kidney and liver weights in dogs (n = 4) exposed to fluensulfone for 1 year

	Control[a]	5 ppm	50 ppm	100 ppm	500 ppm[a]
Males					
Kidney weight					
- Absolute (g)	60 *(56)*	53	53	63	64 *(56)*
- Relative to body weight (%)	0.45 *(0.42)*	0.44	0.45	0.53	0.68* *(0.47)*
Liver weight					
- Absolute (g)	380 *(326)*	322	315	372	351 *(330)*
- Relative to body weight (%)	2.8 *(2.5)*	2.7	2.7	3.1	3.2 *(2.8)*
Females					
Kidney weight					
- Absolute (g)	49 *(46)*	47	46	50	50 *(46)*
- Relative to body weight (%)	0.40 *(0.43)*	0.40	0.41	0.48	0.52 *(0.45)*
Liver weight					
- Absolute (g)	317 *(326)*	314	278	316	366 *(296)*
- Relative to body weight (%)	2.5 *(3.0)*	2.5	2.5	3.1	3.7* *(2.9)*

*: $P < 0.05$
[a] Recovery animals shown in parentheses.
Source: Braun (2011b)

found dead or killed in extremis during the study were necropsied, and tissues and organs were collected. Organ weights were recorded. A defined part of the liver was used for enzyme activity determinations, and the remaining portion of the liver as well as all other organs were fixed for histopathological evaluation. A wide range of tissues from control and top-dose animals was examined histopathologically, with lungs and gross lesions examined from the mid- and low-dose groups.

Survival to termination in week 78 was greater than 70% in all groups. There were no effects on survival, clinical signs, organ weights, palpable nodules and masses or clinical chemistry parameters. Absolute feed consumption was reduced throughout the study in both sexes at 1200 ppm: by 17% in males and 24% in females. Absolute mean body weight and body weight gain were statistically significantly decreased by the treatment in a dose-dependent manner in males at 200 and 1200 ppm and in females at 1200 ppm. The mean body weights at the end of the treatment period were reduced by 6% and 17% in the males of the mid- and high-dose groups and by 12% in the females of the high-dose group.

After 52 weeks, decreases were seen in absolute red blood cell count (< 10%) in mice of both sexes at 1200 ppm and decreased absolute white blood cell count in females only (no specific cell type affected). None of these changes was still present at terminal sacrifice after 78 weeks.

After 13 weeks of treatment, fluensulfone caused a slight decrease of hepatic ALAT activity in females, but no change in ALAT activity was recorded after 78 weeks of treatment. In addition, after 13 weeks, a weak increase in microsomal cytochrome P450 content, accompanied by a slight increase in the activities of Cyp4a1 and Cyp1a1 in females only, and a slight to moderate induction of phase II enzymes (UDPGT, GST and EH), mainly in females, were recorded (Supplementary Table 5).

Absolute weights of a number of organs were reduced in a manner consistent with the lower body weights. Increases in mean organ weights were associated with a small number of animals with very high organ weights. The only pathological changes of any note were in the lungs. An increased incidence of nodules in the lung of females, but not males, was seen at 200 and 1200 ppm (Table 14). The incidence and severity of bronchiolization of the lungs (described as a change from flattened epithelium to cuboidal epithelium) was increased at 200 and 1200 ppm (Table 14). An increased number of alveolar/bronchiolar adenomas was observed in females treated at 200 and 1200 ppm. The number of alveolar/bronchiolar carcinomas in females treated at 1200 ppm did not attain statistical significance with a Fisher's exact test, whereas it was positive with the Peto trend test (Table 14). In control and 30 ppm females, the first lung lesion was seen at terminal sacrifice; at 200 ppm, three mice with lung tumours died during the study (first seen at week 67); at 1200 ppm, the first lung tumour was seen in an animal that died at week 50, with five animals dying during the study having lung tumours. The incidence of adenomas was clearly outside the historical control range (Table 14).

The NOAEL for carcinogenicity was 30 ppm (equal to 6.4 mg/kg bw per day), based on increased incidences of alveolar/bronchiolar adenomas and carcinomas in females at 200 ppm (equal to 39 mg/kg bw per day). The NOAEL for non-neoplastic effects was 30 ppm (equal to 4.2 mg/kg bw per day), based on decreased body weights and lung lesions (bronchiolization) at 200 ppm (equal to 28 mg/kg bw per day) (Kaiser, 2011a).

Mechanism of action of lung tumours in mice and supporting data

The notifier presented a relevance assessment of the lung tumours in mice based on the International Programme on Chemical Safety (IPCS) framework (Strupp et al., 2012). This was based on a number of elements:

- Published literature indicates that species-specific lung tumours can be produced in the mouse by a number of chemicals. The underlying cause is a high metabolic activity of the mouse lung, due to relatively high abundance of Clara cells in the mouse compared with humans and the mouse-specific cytochrome P450 isoform 2f2 in the Clara cells. The compounds are activated to reactive intermediates, leading to local cytotoxicity that promotes sustained cell

Table 14. Pathology findings in lungs of mice receiving fluensulfone for 78 weeks

	Males				Females				Historical control range for females
	0 ppm	30 ppm	200 ppm	1 200 ppm	0 ppm	30 ppm	200 ppm	1 200 ppm	
Survival week 78	36	42	46	41	43	37	36	36	–
No. examined	50	50	50	50	50	50	50	50	265
Nodules	11	8	8	12	1	6	11	13	–
Bronchiolar/alveolar hyperplasia	3	3	1	2	0	3	3	2	–
Lymphoid hyperplasia	17	26	22	16	26	22	26	33	–
Bronchiolization	1	0	24**	31**	5	7	43**	48**	–
- Mean grade	1.0	0	1.3	1.6	1.0	1.0	1.8	2.6	–
Alveolar/bronchiolar adenoma	7	9	5	12	2	4	14**	9*	0–6% Mean 3.5%
Alveolar/bronchiolar carcinoma	8	3	3	4	2	1	1	4[#]	0–10% Mean 3.5%
Adenoma and carcinoma combined	15	12	8	16	4	5	15**	13*	–

[#]: $P < 0.05$ (Peto trend test); */**: $P < 0.05$ and $P < 0.01$, respectively (Fisher's exact test, one-sided and pairwise)
Source: Kaiser (2011a)

proliferation, leading finally to tumour formation. Rats have lower metabolic activity in the lung compared with mice (below the threshold needed to cause lung tumours upon lifetime exposure), and activity in humans is orders of magnitude lower than in rats. The carcinogenic risk in the human lung is very low for this mode of action.

- Fluensulfone has shown an increased incidence of lung tumours in female mice at doses above a threshold of 30 ppm. Lung tumours are not seen in rats.
- Fluensulfone is not genotoxic.
- Fluensulfone was found to be extensively metabolized by mouse lung microsomes, whereas no metabolic activity is seen in human lung microsomes.
- Mouse-specific Cyp2f2 is a major contributor in fluensulfone's metabolism, and Cyp2e1 is not involved.
- Administration of fluensulfone to mice led to an early increase in Clara cell proliferation.

The Meeting considered that the proposed carcinogenicity relevance assessment was not extensive enough to conclusively identify the mode of action, having a number of deficiencies:

- It is based substantially on generic data on compounds such as isoniazid, which produced lung tumours in both sexes.
- It does not address the issue of why the non-neoplastic lesions are produced equally in male and female mice, but there is an increase in tumours only in female mice.
- The metabolic data show no differences between male and female mice. In males, there are no tumours.
- Alternative modes of action have not been addressed. There are no specific genotoxicity data on lung tissue/cells.
- The temporal association is unclear. There is an initial Clara cell proliferation at 2 weeks, but no histopathological findings at or before 13 weeks, and then bronchiolization and tumours at 50 weeks and beyond.

The Meeting concluded that the relevance to humans of the mouse lung tumours produced by fluensulfone cannot be entirely excluded, but the weight of evidence is that the mode of action is non-genotoxic and threshold based.

The full IPCS framework assessment is available as a supplementary document on the JMPR website.

Rats

In a combined chronic toxicity and carcinogenicity study in rats, four groups of HanRcc Wistar rats (50 males and 50 females in each group) were treated for 104 weeks with 0, 30, 200 or 1200 ppm fluensulfone (purity 96.7%; batch 36372130-291-PF1) in their diets. Achieved intakes were 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. Satellite groups of 20 males and 20 females were similarly treated for 52 weeks and used for clinical chemistry, haematology and urine analysis investigations. Viability/mortality, clinical signs, feed consumption and body weights were recorded periodically throughout the study. Water consumption was recorded once during week 78. Ophthalmoscopic examinations were performed during acclimatization, in week 52 (satellite animals) and week 104. A functional observational battery was performed in week 52 (satellite groups). Blood and urine samples from all satellite animals were drawn in weeks 13 and 26 and after 52 weeks for determination of haematology, coagulation, clinical biochemistry and urine parameters. Blood samples from all main group animals were drawn after 78 and 104 weeks for determination of haematology parameters. All animals were necropsied, and tissues and organs were collected. Organ weights were recorded. In five satellite animals per group, a part of the liver was used for enzyme activity determinations; the remaining portion of the liver as well as all other organs fixed in neutral phosphate-buffered 4% formaldehyde solution underwent histopathological evaluation. Histological examinations were performed on organs and tissues from all control and high-dose animals and the lungs and oesophagus of animals from the mid- and low-dose groups. After 52 and 104 weeks, the right femur and the incisors of the maxilla of 10 animals of each sex per group were taken for fluoride determination.

There were two deaths (control females) in the satellite group, and survival to termination at 104 weeks was greater than 65% in all groups. Treatment with fluensulfone at dietary concentrations up to 1200 ppm for up to 104 weeks had no effects on survival, clinical signs, functional observational battery/motor activity results or ophthalmoscopic findings. There were no increases in palpable nodules or masses or in any type of neoplastic finding. There were no treatment-related histopathological changes in the liver, kidney, femur, joints or teeth.

Feed consumption in males treated with 1200 ppm was slightly lower during the first 4 weeks of treatment, but no further effects on feed consumption were recorded thereafter. Absolute mean body weight was decreased (< 10%) by the treatment in a dose-dependent manner in males treated at 200 and 1200 ppm.

A number of changes in haematology and biochemistry parameters were noted, mainly in animals treated with 1200 ppm. Two-fold increases in liver marker enzyme activities were seen in top-dose animals, but are not considered adverse in isolation. Dose-related, statistically significant increases in sodium levels were seen throughout the study in males and in females at week 52 (Tables 15 and 16). The sodium levels were all within the concurrent control ranges, and there were no fluctuations in sodium levels in the 90-day rat study with fluensulfone (all groups had mean values of 142 mmol/L); therefore, the fluctuations in sodium levels were considered not to be an adverse effect of treatment. Urinary ketone levels were increased in top-dose animals.

At necropsy after 52 weeks, no gross lesions that could be attributed to treatment with fluensulfone were observed. Absolute and relative weights of livers, kidneys and adrenals were increased in males treated at 1200 ppm. Kidney weight relative to body weight was also increased in females at 1200 ppm (Supplementary Table 6). Microscopically, hyperkeratosis of the oesophagus of minimal severity was observed in animals of both sexes treated with 1200 ppm, but no abnormality was noted in lungs. Phase II enzymes were significantly induced at 1200 ppm.

Table 15. Clinical chemistry parameters for male rats receiving fluensulfone in satellite group

(a) Week 13

Dietary concentration (ppm)	Chol (mmol/L)	Trigl (mmol/L)	Phos-lip (mmol/L)	ASAT (U/L)	ALAT (U/L)	LDH (U/L)	Na (mmol/L)
0	2.15	0.33	2.06	82.1	36.8	173.9	151.8
30	2.06	0.33	2.06	82.4	36.3	107.0**	148.9
200	2.29	0.36	2.18	79.8	38.1	114.8**	148.5
1 200	2.86**	0.47*	2.70**	87.0	34.8	165.6	149.9
Dietary concentration (ppm)	K (mmol/L)	Cl (mmol/L)	Ca (mmol/L)	P (mmol/L)	Tot prot (g/L)	Alb (g/L)	Glob (g/L)
0	4.01	108.4	2.77	2.01	74.44	45.45	28.99
30	3.91	107.2	2.76	2.01	72.43	44.27	28.17
200	3.90	106.6	2.74	2.03	71.34	43.88*	27.46
1 200	4.05	105.5*	2.83	2.18**	75.45	46.30	29.04

(b) Week 26

Dietary concentration (ppm)	Chol (mmol/L)	Trigl (mmol/L)	Phos-lip (mmol/L)	ASAT (U/L)	ALAT (U/L)	LDH (U/L)	Na (mmol/L)
0	2.47	0.39	1.83	78.8	32.8	166.2	145.5
30	2.49	0.43	1.88	80.1	33.9	162.6	145.6
200	2.60	0.46	1.93	89.5	34.8	192.6	147.0**
1 200	3.50**	0.66**	2.57**	89.9	36.0	269.4**	149.2**
Dietary concentration (ppm)	K (mmol/L)	Cl (mmol/L)	Ca (mmol/L)	P (mmol/L)	Tot prot (g/L)	Alb (g/L)	Glob (g/L)
0	3.81	102.7	2.75	1.76	73.87	42.84	31.03
30	3.79	102.9	2.76	1.82	74.27	42.81	31.46
200	3.88	103.5	2.74	1.74	74.57	43.37	31.20
1 200	4.11**	103.0	2.89**	1.98**	80.53**	46.02**	34.52**

(c) Week 52

Dietary concentration (ppm)	Chol (mmol/L)	Trigl (mmol/L)	Phos-lip (mmol/L)	ASAT (U/L)	ALAT (U/L)	LDH (U/L)	Na (mmol/L)
0	2.50	0.66	1.83	70.9	30.7	109.7	146.2
30	2.76	0.82	2.05	77.6	34.5	126.8	147.2*
200	2.82	0.72	2.05	79.7	31.5	123.3	149.2**
1 200	3.83**	1.05**	2.63**	91.8**	31.8	181.3**	152.0**
Dietary concentration (ppm)	K (mmol/L)	Cl (mmol/L)	Ca (mmol/L)	P (mmol/L)	Tot prot (g/L)	Alb (g/L)	Glob (g/L)
0	3.83	102.6	2.74	1.41	73.96	42.59	31.37
30	3.88	104.0**	2.77	1.47	75.88*	42.40	33.48**
200	3.93	106.1**	2.80**	1.55*	76.64**	43.48	33.16*
1 200	4.27**	105.3**	2.94**	1.57**	82.91**	46.00**	36.91**

ALAT: alanine aminotransferase; Alb: albumin; ASAT: aspartate aminotransferase; Chol: cholesterol; Glob: globulin; LDH: lactate dehydrogenase; Phos-lip: phospholipids; Tot prot: total protein; Trig: triglycerides; U: unit; *: $P < 0.05$; **: $P < 0.01$

Source: Kaiser (2011b)

Table 16. Clinical chemistry parameters for female rats receiving fluensulfone in satellite group

(a) Week 13

Dietary concentration (ppm)	Chol (mmol/L)	Trigl (mmol/L)	Phos-lip (mmol/L)	ASAT (U/L)	ALAT (U/L)	LDH (U/L)	Na (mmol/L)
0	1.88	0.24	2.19	90.6	34.4	125.0	148.8
30	1.64	0.24	2.09	79.0*	29.2	115.9	144.9**
200	2.02	0.27	2.32	84.3	27.4*	121.0	146.7**
1 200	2.16	0.26	2.51*	79.6	28.1*	157.8*	148.9
Dietary concentration (ppm)	K (mmol/L)	Cl (mmol/L)	Ca (mmol/L)	P (mmol/L)	Tot prot (g/L)	Alb (g/L)	Glob (g/L)
0	3.48	108.0	2.77	1.41	77.51	51.50	25.95
30	3.26	105.5**	2.66**	1.46	77.28	52.18	24.98
200	3.34	106.2**	2.68**	1.61*	76.55	50.05	26.50
1 200	3.52	107.1	2.70	1.57	77.51	49.95	27.56*

(b) Week 26

Dietary concentration (ppm)	Chol (mmol/L)	Trigl (mmol/L)	Phos-lip (mmol/L)	ASAT (U/L)	ALAT (U/L)	LDH (U/L)	Na (mmol/L)
0	2.40	0.32	2.29	94.0	30.7	193.6	148.1
30	2.02	0.33	2.00	90.9	27.6	189.9	143.1**
200	2.58	0.35	2.41	85.6	28.1	143.4	144.9**
1 200	2.53	0.32	2.35	75.7*	25.3*	179.6	146.4**
Dietary concentration (ppm)	K (mmol/L)	Cl (mmol/L)	Ca (mmol/L)	P (mmol/L)	Tot prot (g/L)	Alb (g/L)	Glob (g/L)
0	3.19	104.8	2.82	1.47	81.37	52.31	29.06
30	3.29	102.3**	2.76	1.45	78.29*	51.52	26.77**
200	3.29	103.2**	2.78	1.44	78.39*	50.64	27.75
1 200	3.43*	103.9	2.79	1.36	78.46*	49.87*	28.59

(c) Week 52

Dietary concentration (ppm)	Chol (mmol/L)	Trigl (mmol/L)	Phos-lip (mmol/L)	ASAT (U/L)	ALAT (U/L)	LDH (U/L)	Na (mmol/L)
0	2.25	0.30	1.96	116.2	40.4	131.3	144.7
30	2.11	0.33	1.97	101.5	38.5	126.6	145.0
200	2.63	0.35	2.21	105.3	32.9	160.6	147.2**
1 200	2.67	0.40**	2.47**	89.3*	28.6**	149.9	151.5**
Dietary concentration (ppm)	K (mmol/L)	Cl (mmol/L)	Ca (mmol/L)	P (mmol/L)	Tot prot (g/L)	Alb (g/L)	Glob (g/L)
0	3.44	102.6	2.73	1.32	78.12	48.89	29.23
30	3.48	102.8	2.80*	1.35	79.17	50.49	28.68
200	3.53	104.8**	2.80*	1.40	79.38	49.51	30.26
1 200	3.51	106.5**	2.87**	1.40	80.39	50.85	29.54

ALAT: alanine aminotransferase; Alb: albumin; ASAT: aspartate aminotransferase; Chol: cholesterol; Glob: globulin; LDH: lactate dehydrogenase; Phos-lip: phospholipids; Tot prot: total protein; Trig: triglycerides; U: unit; *: $P < 0.05$; **: $P < 0.01$

Source: Kaiser (2011b)

At necropsy after 104 weeks, an increased number of macroscopic foci in the lungs was recorded in the treated animals, compared with controls, attaining statistical significance only in males at 1200 ppm (Table 17). Enlarged livers were recorded in four females (compared with one in the control group) treated at 1200 ppm. Microscopically, an increased incidence of hyperkeratosis in the oesophagus was recorded in animals of both sexes treated at 200 and 1200 ppm. The incidence of chronic interstitial inflammation in the lungs was increased in males (Table 17) and females treated at 1200 ppm and in females treated at 200 ppm. This lesion was characterized by focal/multifocal changes consisting of interstitial or intra-alveolar inflammatory cells associated with hypertrophied reactive type II pneumocytes, associated with the presence of foamy intra-alveolar macrophages, which was also increased in mean severity grade. Although the incidences were not all statistically significant, the pattern of effects was considered outside the normal range by the study report authors.

Table 17. Incidence of lungs and oesophagus lesions in male rats receiving fluensulfone

Finding	0 ppm		30 ppm		200 ppm		1 200 ppm	
Lung foci (n = 50)	3	4	5	8	5	9	15**	11
Lung interstitial inflammation (n = 50)	2	3	4	1	2	7	13*	9
Oesophageal hyperkeratosis (week 52; n = 20)	0	0	0	0	0	0	12**	6*
Oesophageal hyperkeratosis (week 104; n = 50)	3	2	2	3	7	8*	21**	20**

*: $P < 0.05$; **: $P < 0.01$
Source: Kaiser (2011b)

Fluoride measurements revealed a marked increase in the fluoride content in the ashes from bones and teeth at 200 and 1200 ppm and in bones from 30 ppm rats at week 104 (Supplementary Table 7). Changes in bone and tooth fluoride levels were not associated with signs of dental fluorosis (discoloration of the teeth) or skeletal fluorosis (mobility problems, changes in external appearance of bones, changes in bone histopathology).

The NOAEL for chronic treatment was 30 ppm (equal to 1.4 mg/kg bw per day), based on hyperkeratitis of the oesophagus, chronic interstitial inflammation in the lungs and reduced body weight gain at 200 ppm (equal to 9.6 mg/kg bw per day). Fluensulfone showed no carcinogenic potential in rats at dose levels up to 1200 ppm (equal to 58 mg/kg bw per day), the highest dose tested (Kaiser, 2011b).

2.4 Genotoxicity

Fluensulfone has been tested for reverse mutation in bacterial and mammalian cells, for clastogenicity in vitro and for the induction of micronuclei in mouse bone marrow erythrocytes (Table 18). A weakly positive result was seen with *Salmonella typhimurium* TA100 in an early study, but not in two subsequent studies. Positive results were seen in the chromosomal aberration test at markedly cytotoxic concentrations. The micronucleus test was negative. It is concluded that fluensulfone is not genotoxic in vivo.

2.5 Reproductive and developmental toxicity

The reproductive toxicity of fluensulfone was evaluated in a two-generation reproduction study in rats. Developmental toxicity was assessed in rats and rabbits.

(a) Two-generation reproductive toxicity study in the rat

In a two-generation study, groups of 24 parent male and female rats (HanRcc Wistar) received fluensulfone (purity > 96%; batch 36372130-291-PF1) in their diets at a concentration of 0, 30, 250 or 1800 ppm during a 10-week pre-pairing period, throughout the mating period, during

Table 18. Results of studies of genotoxicity with fluensulfone

Test substance (vehicle)	End-point	Test object	Concentration	Purity; batch	Result	Reference
In vitro						
Fluensulfone (DMSO)	Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537	16–5 000 µg/plate, ±S9 mix Repeat 1 000–6 000 µg/plate with TA100	98.8%; NLL6692-5	Weak positive TA100 ±S9 Negative in other strains	Herbold (2002a,b)
Fluensulfone (DMSO)	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 uvrA	3.16–5 000 µg/plate (±S9 mix)	96.8%; 411-33-1	Negative	Donath (2011)
Fluensulfone (DMSO)	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 uvrA	3–5 000 µg/plate (±S9 mix)	96.2%; 36372130-291-PF1	Negative	Sokolowski (2008)
Fluensulfone (DMSO)	Reverse mutation	Chinese hamster lung V79 cells – *HPRT* locus	24–72 µg/mL (−S9 mix) 20–80 µg/mL (+S9 mix)	98.4%; NLL6692-13.1	Negative	Herbold (2003a)
Fluensulfone (DMSO)	Chromosomal aberration	Human lymphocytes (4 and 24 h exposures)	62.5–1 000 µg/mL (−S9 mix) 125–1 000 µg/mL (+S9 mix)	96.5%; 36372130-291-PF1	Positive at cytotoxic concentrations ±S9 mix	Flügge (2010)
In vivo						
Fluensulfone (DMSO)	Bone marrow micronuclei	NMRI mice (M; $n = 5$)	75, 150 and 300[a] mg/kg bw, twice by intraperitoneal route	98.4%; NLL6692-13.1	Negative	Herbold (2003b)

DMSO: dimethyl sulfoxide; HPRT: hypoxanthine–guanine phosphoribosyltransferase; M: male; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Increased normochromatic erythrocye:polychromatic erythrocyte ratio.

pregnancy and up to weaning of the F_1 generation (on day 21 postpartum). Treatment of the F_1 generation (24 males and 24 females per group) continued during development to adulthood (at least 91 days), mating, pregnancy and lactation of the F_2 generation until weaning of the F_2 pups. Mean

achieved intakes were 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 at 0, 30, 250 and 1800 ppm, respectively.

Animals of both generations were observed regularly for clinical signs and viability/mortality. Body weight and feed consumption were recorded routinely, except during the pairing period. F_1 and F_2 pups were culled on day 4 postpartum to yield as nearly as possible four males and four females per litter. Pups were weighed on days 0 or 1, 4, 7, 14 and 21 postpartum; sex ratio was assessed on days 0 or 1, 4 and 21. The onset of ear and eye opening, lower incisor eruption and hair growth, and, for the F_1 animals selected for breeding of the F_2 generation, the age and weight at which vaginal opening or preputial separation occurred were recorded. At termination, all animals were necropsied, organ weights were determined, and reproductive organs as well as target organs were fixed and examined in control and high-dose animals. Liver, kidney and thyroid gland were also examined in the 30 and 250 ppm groups. Incisors and femur bones were collected from all F_1 adult animals; fluoride content was determined in right femur and half of the incisors from 10 F_1 parent animals of each sex per group and from 10 F_2 pups (aged 21 days) of each sex per group. Sperm analyses were performed in males of the control and high-dose groups of both generations. Quantitative evaluation of primordial follicles and antral follicles from 10 sections per ovary in the first 10 females of the P and F_1 generations of the control and high-dose groups was also performed.

The clinical signs noted during the study were not remarkable. At 1800 ppm, treatment with the test item resulted in a statistically significant reduction of feed consumption in both P and F_1 generations during the pre-pairing period. In the F_1 generation, this reduction was prolonged into the after pairing period in males and through the gestation period and on days 7–14 of lactation in females. Body weight was statistically significantly reduced in both generations, whereas body weight gain was decreased in the P generation only during the pre-pairing period. Kidney and liver weights were statistically significantly increased in both generations.

At 250 ppm, a reduction in feed consumption in F_1 parent males was recorded during the first half of the pre-pairing period. As feed consumption recovered and there was no corresponding decrease in body weight at this dose level, this was not considered to be adverse.

No effects on any reproductive parameters were recorded; slight increases in postnatal loss to day 4 at 1800 ppm did not have an adverse effect on litter size (Table 19). Analysis of sperm motility, sperm morphology and sperm head count did not reveal any effects. Developmental indices (onset of ear and eye opening, lower incisor eruption and hair growth) and sexual maturation (vaginal opening and preputial separation) did not indicate any test item–related effect, in terms of date or body weight.

In both F_1 and F_2 pups, pup weight was statistically significantly reduced (10%) during the last week of lactation at 1800 ppm. Secondarily, spleen weight was also reduced. During the histopathological examinations, glycogen deposits were reduced in the liver of F_2 male and female pups, possibly associated with the lower body weight.

At 1800 ppm, centrilobular hypertrophy of the liver was noted at minimal to slight severity in both P and F_1 parental animals. This finding correlated with the increase in liver weight. The incidence and severity of renal tubular hyaline droplets were increased in males of P and F_1 generation parental animals. The incidence and/or severity of tubular basophilia, mononuclear cell infiltration and tubular casts were also increased in males of P and F_1 generation parental males. These lesions were considered to be secondary lesions resulting from the hyaline droplets described above. Follicular hypertrophy of the thyroid gland was recorded at minimal to slight severity in four males of the P generation and six F_1 generation parental males.

In the F_1 generation parental animals and the F_2 generation pups, there were significant increases in the fluoride content of bones and teeth (Supplementary Table 8). There were no effects on incisor length or the coloration of the teeth reported in either the F_1 adults or the F_2 pups.

The NOAEL for systemic toxicity in parental animals of both generations was 250 ppm (equal to 16 mg/kg bw per day), based on the effects on body weight development throughout the study in males plus liver and kidney weight increases at 1800 ppm (equal to 122 mg/kg bw per day).

Table 19. Breeding data for the P generation of rats receiving fluensulfone

Parameter	Mean ± SD[a]			
	0 ppm	30 ppm	250 ppm	1 800 ppm
No. of litters	23	24	22	24
Duration of gestation (days)	21.6 ± 0.59	21.4 ± 0.58	21.6 ± 0.49	21.5 ± 0.51
No. of implantations	13.6 ± 2.13	14.1 ± 1.72	13.5 ± 2.30	14.2 ± 1.58
Postimplantation loss (% of implantations)	2.0 ± 2.40	1.2 ± 1.53	0.9 ± 0.97	1.6 ± 1.41
Living pups at first litter check	11.7 ± 3.41	12.9 ± 2.07	12.6 ± 2.48	12.6 ± 1.79
Postnatal loss days 0–4 postpartum				
- No. of litters affected	1	2	3	6
- Total no.	1	2	4	8*
- Mean ± SD	0.0 ± 0.21	0.1 ± 0.28	0.2 ± 0.50	0.3 ± 0.64
Living pups day 4 postpartum	7.6 ± 1.70	8.0 ± 0.00	8.0 ± 0.00	8.0 ± 0.00
Living pups day 21 postpartum	7.6 ± 1.70	8.0 ± 0.20	8.0 ± 0.0	8.0 ± 0.20

SD: standard deviation; *: $P < 0.05$
[a] Except where noted otherwise.
Source: Ceccatelli (2011)

The NOAEL for offspring toxicity was 250 ppm (equal to 16 mg/kg bw per day in males), based on reduced pup weight noted at 1800 ppm (equal to 122 mg/kg bw per day).

The NOAEL for reproductive effects was 1800 ppm (equal to 122 mg/kg bw per day), the highest dose tested (Ceccatelli, 2011).

(b) Developmental toxicity

Rats

In a developmental toxicity study, groups of 30 mated Wistar rats received fluensulfone (purity 96–97%; batch NLL6692-7-5) in 2% Cremophor, 0.5% carboxymethyl cellulose and deionized water at 0, 7.7, 49 or 292 mg/kg bw per day on days 6–19 of gestation. All dams were sacrificed on gestation day 20, at which time gross external and internal necropsies were performed. All fetuses were evaluated for external anomalies. Approximately half of all fetuses from each litter were examined for visceral effects at necropsy and also evaluated for cranial effects using Wilson's technique. The remaining fetuses underwent an evaluation for general skeletal (including cartilage) development.

Three dams from the mid-dose group and four from the top-dose group were found dead. Maternal compound-related findings observed in dams treated at 292 mg/kg bw per day included red vaginal discharges ($n = 3$) and body weight loss coupled with decreased feed consumption (Table 20) at the start of dosing that persisted to the end of the study. The initial body weight deficits (20 g/rat on days 6–10) were consistent with the reduction in feed consumption (6 g/rat per day). Increased absolute and relative (10%) liver and kidney weights were seen at the top dose level.

There was a decrease in the number of fetuses per litter at the low and top doses but not the middle dose (Table 21). This was due in part to reduced implantation rates, which would be unrelated to dosing. Four non-viable fetuses were seen in one top-dose litter. No compound-related effects on fetal external, visceral or skeletal malformations were observed. There were no increases in external or visceral variations. There was evidence of slightly increased ossification, particularly of the skull, with significantly lower incidences of enlarged fontanelles/sutures in the mid- and high-dose groups (Table 21). These latter findings are not considered adverse.

Table 20. Body weight and feed consumption in rats administered fluensulfone during gestation

Day of pregnancy	Mean ± standard error			
	Control	7.7 mg/kg bw per day	49 mg/kg bw per day	292 mg/kg bw per day
Body weight (g)				
0	208.2 ± 2.33	207.7 ± 2.88	211.0 ± 2.35	206.1 ± 2.43
6	228.8 ± 2.59	224.8 ± 3.42	228.7 ± 2.38	228.3 ± 2.90
7	230.4 ± 2.80	227.1 ± 3.68	228.7 ± 2.49	223.5 ± 3.28
10	241.0 ± 2.85	235.9 ± 4.30	240.4 ± 2.66	220.2 ± 3.38**
15	258.5 ± 4.09	255.8 ± 3.53	262.8 ± 2.71	237.1 ± 4.39**
20	310.0 ± 5.06	299.9 ± 5.20	314.1 ± 4.24	284.2 ± 5.17**
Feed consumption (g/kg bw per day)				
4–6	84.9 ± 1.70	79.2 ± 1.65	83.2 ± 2.68	86.1 ± 2.23
6–8	83.8 ± 3.03	82.4 ± 2.36	78.4 ± 2.63	52.6 ± 3.83**
8–10	85.8 ± 3.01	78.0 ± 2.97	82.0 ± 2.64	52.5 ± 3.10**
10–12	86.6 ± 2.82	78.7 ± 2.73	82.3 ± 2.14	60.8 ± 3.87**
12–14	87.3 ± 2.54	83.6 ± 2.91	82.9 ± 2.21	97.4 ± 9.80
18–20	71.9 ± 2.32	76.0 ± 2.39	74.7 ± 2.46	75.1 ± 8.71

**: $P < 0.01$
Source: Young (2003)

Table 21. Reproductive data in rats administered fluensulfone during gestation

Parameter	0 mg/kg bw per day	7.7 mg/kg bw per day	49 mg/kg bw per day	292 mg/kg bw per day
No. of pregnant rats	24	27	26	24
No. found dead	0	3	0	4
No. of litters	24	24	26	20
Litter size				
- Mean ± SE	10.7 ± 0.63	9.2 ± 0.76	11.4 ± 0.44	9.9 ± 0.50
- Range	1–15	0–14	5–15	5–14
No. of viable fetuses	257	220	296	193
- Mean ± SE per dam	10.7 ± 0.63	9.2 ± 0.76	11.4 ± 0.44	9.6 ± 0.56**
No. of corpora lutea (mean)	13.3	12.9	13.4	13.4
No. of implantations	11.3	10.8	12.2	10.4
Postimplantation loss (% of implantations)	0.6	1.6	0.8	0.7
No. of non-viable fetuses	0	0	0	4
Median % male fetuses	57	50	59	50
Mean weight of fetuses (g) (mean ± SE)	3.6 ± 0.05	3.6 ± 0.05	3.6 ± 0.04	3.4 ± 0.07
Malformations				
Fetal incidence	3	0	1	1
Litter incidence	3	0	1	1
Variations				
Fetal incidence (%)	150	127	167	121

Parameter	0 mg/kg bw per day	7.7 mg/kg bw per day	49 mg/kg bw per day	292 mg/kg bw per day
	(58.4%)	(57.7%)	(56.4%)	(62.7%)
Litter incidence	23	22	26	20
Enlarged anterior fontanelle	41	23	19**	11**
Enlarged sagital suture	39	22	18**	6**
Enlarged posterior fontanelle	42	32	18**	7**
Enlarged squamosal suture	13	8	4*	1*
Left-sided umbilical artery	15	12	11	20

SE: standard error; *: $P < 0.05$; **: $P < 0.01$
Source: Young (2003)

The NOAEL for maternal toxicity was 49 mg/kg bw per day, based on reduced body weight and clinical signs at 292 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 49 mg/kg bw per day, based on the four non-viable fetuses in a litter at 292 mg/kg bw per day. Fluensulfone was not teratogenic to rats (Young, 2003).

Rabbits

In a developmental toxicity study, groups of 20 mated Himalayan rabbits were administered fluensulfone (purity 96.7%; batch 36372130-291-PF1) in 0.5% aqueous carboxymethyl cellulose at 0, 2.5, 10 or 40 mg/kg bw per day at a dosing volume of 5 mL/kg bw on days 6–27 of gestation. All does were sacrificed on gestation day 28, at which time gross external and internal necropsies were performed. Fetuses were removed from the uterus, sexed, weighed individually and examined for gross external and visceral abnormalities. The heads of 50% of the fetuses were serially sectioned and examined. The remaining fetuses were used for skeletal examinations (alizarin red S and alcian blue staining).

All does survived until the scheduled necropsy, and no clinical signs related to treatment were observed. Mean body weight gain was slightly reduced in does treated at 40 mg/kg bw per day between days 6 and 9, attaining statistical significance on days 7 and 8. This effect was accompanied by a reduction in feed intake noted since the beginning of treatment, more evident between days 18 and 21 and attaining statistical significance between days 21 and 24 (−22.2% compared with the control group). Over the initial dosing period, rabbits in the top-dose group had a slightly lower feed consumption than controls, but the deficit in feed consumption (3 g/rabbit per day) does not match the body weight deficit of 42 g/rabbit over 3 days. Over the period from days 9 to 15, the top-dose rabbits had a body weight gain higher than that of any other groups; although the feed consumption was higher than that of the controls, it was below that of the low- and mid-dose groups. Overall body weight gain (days 6–28) in the top-dose group was approximately 10% less than that of the controls, but was in line with those of the low- and mid-dose groups (Table 22).

There were no adverse effects on measured litter or fetal parameters (Supplementary Table 9). A lower fetal weight in the top-dose group was secondary to the higher number of fetuses per litter and is not considered adverse.

The NOAEL for embryo and fetal toxicity was 40 mg/kg bw per day, the highest dose tested. The maternal NOAEL was 10 mg/kg bw per day, based on body weight loss. Fluensulfone was not teratogenic to rabbits (Ceccatelli, 2009).

2.6 Special studies

(a) Neurotoxicity

Acute neurotoxicity in the rat

In a preliminary acute neurotoxicity study, groups of three male and three female RccHan Wistar rats were administered single oral doses of fluensulfone at 0, 100, 500, 1000 or 2000 mg/kg

Table 22. Body weight, body weight gain and feed consumption in pregnant rabbits dosed with fluensulfone from days 6 to 27

	0 mg/kg bw per day	2.5 mg/kg bw per day	10 mg/kg bw per day	40 mg/kg bw per day
Group size[a]	20	19	19	17
Body weight (g)				
- Day 6	2 551	2 546	2 576	2 561
- Day 7	2 552	2 550	2 581	2 535
- Day 8	2 555	2 556	2 583	2 530
- Day 15	2 641	2 640	2 663	2 642
- Day 28	2 773	2 753	2 782	2 761
Body weight gain (g)				
- Days 6–9	13	24	10	–29
- Days 9–12	19	10	23	34
- Days 12–15	58	60	54	76
- Days 21–24	33	37	34	28
- Days 6–28	222	207	206	200
Mean feed consumption (g/rabbit per day)				
- Days 6–9	95	113	103	92
- Days 9–12	100	108	106	99
- Days 12–15	94	103	100	99
- Days 21–24	108	103	105	84*
- Days 6–28	98	101	105	93

*: $P < 0.05$

[a] Animals with viable litters only.

Source: Ceccatelli (2009)

bw (purity 98.7%; batch 36372130-291-PF1) in PEG 300 and were observed for the following 8 days. At the end of the observation period, all surviving animals were sacrificed and assessed by gross pathology. All animals of the high-dose group (2000 mg/kg bw) died spontaneously within 24 hours after dose administration after they had developed signs of overt toxicity from 0.5 hour. All animals of the remaining dose groups survived to terminal sacrifice without showing any signs of toxicity. No macroscopic findings were recorded in any animals of any dose groups at necropsy. A peak systemic effect was observed on the day of dosing in animals of both sexes treated at 2000 mg/kg bw approximately 5 hours after administration (Sommer, 2009).

Groups of 10 male and 10 female RccHan Wistar rats were administered single oral doses of fluensulfone at 0, 100, 400 or 1200 mg/kg bw (purity 98.7%; batch 36372130-291-PF1) in PEG 300 and observed for 14 days. General cage-side observations were made in all animals prior to treatment start and once daily thereafter throughout the study. In addition, a functional observational battery evaluation was performed on all animals prior to treatment start and on days 1 (approximately 1–4 hours after dose administration), 7 and 14. Motor activity was assessed over a period of 30 minutes after each functional observational battery evaluation. Feed consumption and body weights were measured regularly. At the end of the scheduled observation period, five rats of each sex per group were perfusion fixed in situ, and selected nervous system tissues were dissected, processed and examined neurohistopathologically.

One female dosed at 1200 mg/kg bw died within 24 hours after dose administration from unknown causes. On day 8, the mean body weight gain was lower in males and females treated at 1200 mg/kg bw. Feed consumption was dose dependently reduced in males and females at 400 and 1200 mg/kg bw on days 1–8, with statistical significance attained in females at 1200 mg/kg bw only.

Administration of a single oral gavage dose of fluensulfone to Wistar rats at 100, 400 or 1200 mg/kg bw produced reversible effects on motor activity and functional observational battery parameters. At 100 mg/kg bw, a rapidly reversible effect on motor activity was observed in females only, and an increased incidence of abnormal righting reflex was seen in males. Changes in functional observational battery parameters (Tables 23 and 24) were observed at all dose levels on day 1, but not subsequently. Detailed weekly clinical observations revealed no treatment-related effects on days 1, 8 and 15. No test item–related effects on absolute or relative brain weights were evident in males or females. There were no findings in the nervous system during macroscopic and microscopic pathology examinations.

Table 23. Incidences of remarkable observations noted during functional observational battery assessments at 1–4 hours post-dosing in rats (n = 10) administered fluensulfone

Day 1 finding	0 mg/kg bw		100 mg/kg bw		400 mg/kg bw		1 200 mg/kg bw	
	M	F	M	F	M	F	M	F
Soft faeces	0	0	0	0	0	1	2	4
Skin cold to touch	0	0	0	0	0	0	2	0
Reduced activity	0	0	3	1	6	6	8	7
Decreased rearing	0	0	2	1	4	8	6	8
Piloerection	0	0	3	0	2	2	8	6
Righting response reduced	0	0	4	1	7	7	7	8
Hunched posture	0	0	0	0	0	0	2	0
Salivation	0	0	0	0	1	0	0	1

F: females; M: males
Source: Sommer (2010)

Table 24. Summary of locomotor activity at 1–4 hours post-dosing (day 1) in rats administered fluensulfone

Parameter	Dose (mg/kg bw)	Males		Females	
		Activity counts	% difference from control	Activity counts	% difference from control
Total distance	0	2 487.9 ± 513	–	2 615.2 ± 812	–
	100	2 565.4 ± 853	+3	1 939.4* ± 614	−26
	400	1 418.6* ± 529	−43	1 504.8** ± 441	−42
	1 200	1 016.0** ± 679	−59	1 088.5** ± 525	−58
Centre time	0	124 ± 111	–	124 ± 79	–
	100	107 ± 51	−14	68* ± 39	−45
	400	67 ± 45	−46	51** ± 27	−59
	1 200	27** ± 29	−78	29** ± 26	−77
Rearing activity	0	48 ± 27	–	48 ± 20	–
	100	43 ± 43	−10	27** ± 21	−44
	400	20** ± 20	−58	17** ± 8	−65
	1 200	11** ± 13	−77	9** ± 7	−81

*: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Sommer (2010)

The lowest-observed-adverse-effect level (LOAEL) for general toxicity and neurotoxicity was 100 mg/kg bw, the lowest dose tested, in males and females, based on clinical signs and reduced activity in the functional observational battery and decreased motor activity. There was no neuropathological finding at any dose level (Sommer, 2010).

Using the United States Environmental Protection Agency's (USEPA) benchmark dose software (BMDS version 2.2), the following $BMDL_{SD}$ values ($BMDL_{SD}$ is the 95% lower confidence limit of the dose corresponding to a change equivalent to 1 standard deviation in the response for unexposed animals) were derived using the lowest value from the models with a good fit for dichotomous data or Hill model for continuous data (Table 25).

Table 25. BMD_{10}/BMD_{SD} and $BMDL_{10}/BMDL_{SD}$ values for end-points from the acute neurotoxicity study with fluensulfone

End-point	$BMDL_{10}/BMDL_{SD}$ (mg/kg bw)	BMD_{10}/BMD_{SD} (mg/kg bw)	Models
Reduced righting response: males	31	47	Gamma, Weibull, quantal linear (10% change)
Locomotor distance: males	54 / 147	335 / 371	Hill (extra 10%) / Hill (1 SD)
Locomotor distance: females	7 / 33	20 / 94	Hill (extra 10%) / Hill (1 SD)
Centre time: males	10 / 124	78 / 470	Hill (extra 10%) / Hill (1 SD)
Centre time: females	0.5 / 6	9 / 74	Hill (extra 10%) / Hill (1 SD)
Rearing activity: males	9 / Did not run	87 / 368	Hill (extra 10%) / Hill (1 SD)
Rearing activity: females	3 / 19	12 / 59	Hill (extra 10%) / Hill (1 SD)

BMD_{10}: benchmark dose for a 10% response; BMD_{SD}: benchmark dose for a 1 standard deviation response; $BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; BMD_{SD}: lower 95% confidence limit on the benchmark dose for a 1 standard deviation response; SD: standard deviation

The large variability in results in the control groups (standard deviation [SD] often approximating to the value of the mean) indicates that using a 10% change is unrealistic, and the default of the software to use 1 SD is considered to be more appropriate for the continuous data sets. The lowest $BMDL_{SD}$ is 6 mg/kg bw for centre time in females, but this is a value with a low confidence level, given the 12-fold gap from the $BMDL_{SD}$ to the BMD_{SD}. Other $BMDL_{SD}$ values for females, the more sensitive sex, from the low end of the distribution are 19 and 33, and it is concluded that these support a point of departure (POD) of 25 mg/kg bw.

[The notifier performed a simple linear regression, which indicated that the NOAEL would be in the region of 25 mg/kg bw.]

Subchronic neurotoxicity in the rat

In a subchronic dietary neurotoxicity study, groups of 12 male and 12 female Wistar rats received fluensulfone (purity 95.7%; batch 36372130-291-PF1) in their diet at a concentration of 0 (control), 100, 500 or 2500 ppm for 90 days. Achieved intakes were 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. General cage-side observations, body weights and feed consumption values were determined routinely. Functional observational batteries were performed on all animals prior to first administration and in weeks 2, 5, 9 and 13. Locomotor activities were assessed after each functional observational battery evaluation. Ophthalmoscopic examinations were performed on all animals once prior to first administration and in animals of groups 1 and 4 in week 13. At the end of the treatment period, five rats of each sex per group were perfusion fixed in situ. Selected nervous system tissues were dissected, processed and examined microscopically.

All animals scheduled for neurohistological examination survived the scheduled study period. One mid-dose female died on day 87, unrelated to treatment. Treatment-related effects were confined to lower feed consumption, body weight and body weight gain in males at 2500 ppm. Terminal body weight in top-dose males was 15% lower than in controls. Detailed clinical observations and functional observational battery evaluations of clinical symptoms revealed no treatment-related effects. Locomotor activity (total distance and rearing counts) was significantly lower in males at 2500 ppm during week 5, but not at week 9 or 13, and this is considered to be a chance finding. There were no notable effects on neuropathological examination.

The NOAEL for neurotoxicity was 2500 ppm (equal to 153 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 500 ppm (equal to 31 mg/kg bw per day), based on lower body weight in males at 2500 ppm (equal to 153 mg/kg bw per day) (Sommer, 2011).

(b) Immunotoxicity in mice

Although immunotoxicity end-points were already included in the 28-day dietary study in rats and mice, fluensulfone was also tested in mice, according to the applicable USEPA guideline, to evaluate its immunotoxic potential.

In a study evaluating potential immunotoxicity, groups of 10 female CD-1 mice received fluensulfone (purity 96.7%; batch 36372130-291-PF1) in the diet at a concentration of 0, 100, 500 or 2500 ppm. After 1 week of treatment, one mouse in the high-dose group died, and signs of overt toxicity (decreases in body weight, feed and water consumption, clinical signs in a few animals) were noted in the other animals of this group. Following 3 days on untreated diet, the dietary concentration was therefore reduced to 1500 ppm for this group, and treatment was continued for an additional 21 days. The treatment period for all groups was extended to 32 days in order to complete the planned 28-day treatment period for all treated and negative control animals on study. Achieved mean intakes were 0, 17, 86 and 204 mg/kg bw per day (note that the study report erroneously presents intakes on a daily basis when they are weekly). Cyclophosphamide was used as a positive control and was administered intraperitoneally at 25 mg/kg bw per day during days 27–31 to a group of 10 female mice. On day 27, a single intravenous dose of 0.2 mL of 1×10^8 sheep red blood cells (sRBC) in phosphate-buffered saline was administered to all surviving animals. Five days after immunization (day 32), all mice were weighed and euthanized, serum samples were collected from each mouse for anti-sRBC IgM titres, and the spleen and thymus were weighed.

Apart from the effects observed when the highest-dose animals were receiving 2500 ppm in their diet, treatment did not affect body weight and feed or water consumption in mice at 100 and 500 ppm, and these parameters returned to, or were similar to, vehicle control values in the highest-dose mice when treated at 1500 ppm. No effects on absolute or relative spleen and thymus weights were noted. The mean anti-sRBC IgM values for the 100, 500 and 2500/1500 ppm groups were reduced in a dose-related manner, but with a small magnitude (Table 26). The magnitude of the changes and the extremely wide variation seen in these assays are not indicative of fluensulfone having significant immunotoxic potential.

Table 26. Anti-sRBC IgM titres in an immunotoxicity study in mice

	Mean ± standard error of duplicate samples				
	0 ppm	100 ppm	500 ppm	2 500/1 500 ppm	Positive control
Anti-sRBC IgM (U/mL)	1 526.9 ± 371.64	1 008.2 ± 214.27	1 036.9 ± 169.65	917.6 ± 154.52	167.4 ± 57.55**

IgM: immunoglobulin M; sRBC: sheep red blood cells; U: units; **: $P < 0.01$
Source: Struve (2011)

The NOAEL for systemic toxicity was 500 ppm (equal to 86 mg/kg bw per day), based on deaths and clinical signs at 2500 ppm. The NOAEL for immunotoxicity was 2500/1500 ppm (equal to a mean intake of 204 mg/kg bw per day), the highest dose tested (Struve, 2011).

(c) Investigation of possible modes of action leading to decreases in ALAT activity

A series of studies was performed to investigate possible modes of action leading to decreases in ALAT activity in serum and liver homogenates of animals exposed to fluensulfone. In a study with fluensulfone-treated dog plasma samples, the addition of excess pyridoxal-5′-phosphate had no significant effect on the measured activity of ALAT (Sagelsdorff, 2009), and measurements of pyridoxal-5′-phosphate in a 2-week dog study with fluensulfone (500 ppm) were unaltered, although ALAT activity was reduced by approximately 85% (Braun, 2011a). The addition of fluensulfone to 100 × g supernatants of dog liver homogenates did not produce any inhibition of measured ALAT activity. Although it would be expected that these homogenates would have some metabolic capability and levels of fluensulfone decreased during the assays, there were no investigations to confirm that the range of fluensulfone metabolites seen in vivo was produced (Sagelsdorff, 2010). There were no marked changes in ALAT messenger ribonucleic acid (mRNA) or protein expression in the livers of dogs exposed to fluensulfone (500 ppm) for 2 weeks (Braun, 2011a). Although these studies failed to identify the underlying cause, the evidence is indicating that direct inhibition of ALAT by fluensulfone itself, altered mRNA expression and an action involving pyridoxal-5′-phosphate do not appear to be involved. A paper by Fuentes-Almagro et al. (2012) showed that reactive molecules similar to the butene metabolites of fluensulfone could directly inhibit related enzymes.

ALAT enzyme depression subsequent to dietary administration of fluensulfone in laboratory animals was not considered to represent an adverse effect in humans (Gordon, 2011). The Meeting concluded that as the inhibition of ALAT was reversible and as alternative metabolic pathways are available, the reductions in hepatic ALAT activity seen in animals exposed to fluensulfone were not adverse.

(d) Lung tumour mechanistic studies

Comparative metabolic activity of human and mouse lung microsomes against fluensulfone

Lung microsomes were prepared from CD-1 (ICR) mice (12 of each sex) and from commercial material from 10 human non-smokers. Microsomes were analysed for CYP2E1 and Cyp2f2 enzyme activities against a standard substrate (chloroxazone) and the ability to metabolize fluensulfone (2 µmol/L). The results showed that the mouse microsomes were 300- to 500-fold more active than human microsomes against chloroxazone (Table 27). Mouse microsomes exhibited significant capability to metabolize fluensulfone, whereas human microsomes had negligible, if any, activity (Table 27). The microsomes from male mice had a specific activity similar to those from females. The use of a specific Cyp2f2 inhibitor (5-phenyl-1-pentyne) showed that the mouse-specific Cyp2f2 played a significant role in the metabolism of fluensulfone.

Table 27. Relative metabolic capability of mouse and human lung microsomes

	Male mouse microsomes	Female mouse microsomes	Human microsomes
Chloroxazone activity (pmol/mg per minute)	86.2	68.4	0.22
Fluensulfone remaining at 120 min (%); no inhibitor	2.1	4.6	102
Fluensulfone remaining at 120 min (%); + CYP2E1 inhibitor	6.2	8.7	94
Fluensulfone remaining at 120 min (%); + Cyp2f2 inhibitor	27	23	96

Source: Jaeger (2011)

Mouse lung cell proliferation assay

Groups of 10 female CD-1 mice received fluensulfone (purity 96.2%; batch 36372130-291-PF1) at 0 or 1200 ppm for 3 or 7 days or isoniazid (positive control) at 1305 ppm in the diet for 3 or 7 days. At 14 and 2 hours prior to termination, animals received an intraperitoneal injection of 5-bromo-2′-deoxyuridine (BrdU). After termination, lung (bronchiolar epithelial) cells were assessed for cell proliferation (BrdU-positive cells). With both isoniazid and fluensulfone, there was an approximate 5-fold increase in the numbers of BrdU-positive cells after 3 days of exposure, but after 7 days, the results were similar in control and test groups. This study demonstrates that exposure to fluensulfone induces an initial transient proliferation in bronchiolar epithelial cells, similar to that produced by isoniazid (Kaiser, 2011c).

(e) Toxicity studies on metabolites

Thiazole sulfonic acid (metabolite M-3625)

Thiazole sulfonic acid is of low acute oral toxicity (Table 28). It was not genotoxic in an Ames test and was negative for chromosomal aberrations in mammalian cells and in an in vivo micronucleus assay in rats (Table 29). Thiazole sulfonic acid is of lower acute oral toxicity than fluensulfone and is not genotoxic.

Table 28. Summary of acute toxicity data for fluensulfone metabolites

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/L)	Metabolite; purity; batch	Vehicle	Reference
Rat	HanRcc: Wistar	F	Oral	> 2 000 (OECD TG 423)	–	Thiazole sulfonic acid (M-3625) 93.6% 231PAL071	Water	Arcelin (2010a)
Rat	HanRcc: Wistar	F	Oral	300–2 000 (OECD TG 423)	–	Methyl sulfone (M-3626) 98.8% 231PAL052	Corn oil	Mallaum (2010)
Rat	HanRcc: Wistar	F	Oral	> 2 000 (OECD TG 423)	–	Butene sulfonic acid (M-3625) 99.5% 215PAL44	Water	Arcelin (2010b)

F: female; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; OECD TG: Organisation for Economic Co-operation and Development Test Guideline

Methyl sulfone derivative (metabolite M-3626)

The methyl sulfone derivative is of moderate acute oral toxicity, with an LD_{50} of ≥ 300 mg/kg bw (Table 28). It was weakly mutagenic in an Ames test for test strain *Salmonella typhimurium* TA100 at the highest tested concentration in the absence of metabolic activation, was equivocal for gene mutation in mammalian cells in the presence and absence of metabolic activation and was negative in an in vivo micronucleus assay in rats and for unscheduled deoxyribonucleic acid (DNA) synthesis in rats (Table 29). The methyl sulfone derivative is of similar acute oral toxicity to fluensulfone and is not genotoxic in vivo.

Table 29. Results of studies of genotoxicity with fluensulfone metabolites

Test substance (vehicle)	End-point	Test object	Concentration	Purity; batch	Result	Reference
In vitro						
Thiazole sulfonic acid (M-3625) (water)	Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* (WP2uvrA)	3–5 000 µg/plate, ±S9 mix	93.6% 231PAL071	Negative	Sokolowski (2010a)
Thiazole sulfonic acid (M-3625) (water)	Chromosomal aberration	Chinese hamster V79 cells	592, 1 185 or 2 370 µg/mL, ±S9 mix	93.6% 231PAL071	Negative	Hall (2010a)
Methyl sulfone (M-3626) (DMSO)	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* (WP2uvrA)	3–5 000 µg/plate, ±S9 mix	98.8% 231PAL052	Weakly positive −S9 Negative +S9	Sokolowski (2010b)
Methyl sulfone (M-3626) (DMSO)	Reverse mutation (HPRT)	Chinese hamster V79 cells	3.8–175 µg/mL −S9 mix 37.5–1 000 µg/mL +S9 mix	98.8% 231PAL052	Equivocal ±S9	Hall (2011)
Butene sulfonic acid (M-3627) (water)	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* (WP2uvrA)	3–5 000 µg/plate, ±S9 mix	99.5% 215PAL44	Negative	Sokolowski (2010c)
Butene sulfonic acid (M-3627) (water)	Chromosomal aberration	Chinese hamster V79 cells	533, 1 066 or 2 131 µg/mL, ±S9 mix	99.5% 215PAL44	Negative	Hall (2010b)
In vivo						
Thiazole sulfonic acid (M-3625) (water)	Bone marrow micronuclei	Wistar rats (M)	0, 500, 1 000 or 2 000 mg/kg bw (gavage) single	93.6% 231PAL071	Negative	Merker (2011a)
Methyl sulfone (M-3626) (corn oil)	Bone marrow micronuclei	Wistar rats (M)	0, 125, 250, 500 mg/kg bw (gavage) single	98.8% 231PAL052	Negative	Merker (2011b)
Methyl sulfone (M-3626) (corn oil)	Liver UDS	Wistar rats (M)	0, 250, 500 mg/kg bw (gavage) single	98.8% 231PAL052	Negative	Merker (2011c)
Butene sulfonic acid (M-3627) (water)	Bone marrow micronuclei	Wistar rats (M)	0, 500, 1 000 or 2 000 mg/kg bw (gavage) single	99.5% 215PAL44	Negative	Merker (2010)

DMSO: dimethyl sulfoxide; HPRT: hypoxanthine–guanine phosphoribosyltransferase; M: male; S9: 9000 × *g* supernatant fraction from rat liver homogenate; UDS: unscheduled DNA synthesis

Butene sulfonic acid (metabolite M-3627)

The butene sulfonic acid is of low acute oral toxicity (Table 28). It was not genotoxic in an Ames test and was negative for chromosomal aberrations in mammalian cells and in an in vivo micronucleus assay in rats (Table 29). The butene sulfonic acid metabolite is of lower acute oral toxicity than fluensulfone and is not genotoxic.

3. Observations in humans

Medical data on fluensulfone are limited, but no reports of adverse effects were identified during routine monitoring of production plant workers and among personnel involved in the experimental biological testing or field trials. There is no evidence or data available to support any findings in relation to poisoning with fluensulfone.

3.1 Medical data

(a) Report on medical surveillance on manufacturing plant personnel

A declaration of health record of industrial workers has been provided by Makhteshim Chemical Works, Beer Sheva, 84100 Israel. More than 20 workers were estimated to have been involved with the development of the synthesis and manufacturing of fluensulfone and formulations. Additional workers were exposed during the regulatory and field biological testing in Israel and elsewhere. Makhteshim, up to 25 December 2011, did not receive any reports of illness or adverse health effects attributed to exposures associated with the handling, testing or manufacturing of fluensulfone and formulations (Exponent, 2012).

Comments

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 that 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 discoloration 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 ALAT 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 ALAT activity and that there was no significant interaction with the cofactor pyridoxal-5′-phosphate. mRNA levels appeared slightly induced, whereas protein expression appeared stable. No effect on the general health of the dogs accompanied the reduction of hepatic ALAT 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 ALAT activity was reversible, and alternative metabolic pathways are available. The Meeting concluded that a reduction in hepatic ALAT 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 hyperplasia, 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, respectively), 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 between weeks 13 and 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 Cyp2f2 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 embryo 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 at 40 mg/kg bw per day, 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 in the range 19–33 mg/kg bw for females, and the Meeting identified 25 mg/kg bw as a reference point or 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, and the NOAEL for general toxicity was 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-sRBC 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 ppm. Fluensulfone did not affect splenic or bone marrow cell counts or IgA, IgG or IgM titres in rats exposed to 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 unscheduled DNA synthesis, 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 acceptable daily intake (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 acute reference dose (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.

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	1 200 ppm, equal to 69 mg/kg bw per day[e]	–
	Multigeneration reproductive toxicity study[a]	Reproductive toxicity	1 800 ppm, equal to 122 mg/kg bw per day[e]	–
		Parental toxicity	250 ppm, equal to 16 mg/kg bw per day	1 800 ppm, equal to 122 mg/kg bw per day
		Offspring toxicity	250 ppm, equal to 16 mg/kg bw per day	1 800 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
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

$BMDL_{SD}$: 95% lower confidence limit on the benchmark dose for a 1 standard deviation response; F: female; LOAEL: lowest-observed-adverse-effect level; M: male; NOAEL: no-observed-adverse-effect level; POD: point of departure

[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	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.1 mg/L
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Not irritating
Dermal sensitization	Sensitizing (guinea-pig 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 sulfonic acid (M-3625): Oral $LD_{50} > 2\,000$ 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 $LD_{50} > 2\,000$ mg/kg bw; not genotoxic
Medical data	
	No adverse effects reported

$BMDL_{SD}$: lower 95% confidence limit on the benchmark dose for a 1 standard deviation response; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; NOAEC: no-observed-adverse-effect concentration; NOAEL: no-observed-adverse-effect level; POD: point of departure: T_{max}: time to reach the peak concentration in plasma

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

ADI: acceptable daily intake; ARfD: acute reference dose

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