SULFURYL FLUORIDE

First draft prepared by
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Explanation

Sulfuryl fluoride (O_2SF_2) is a gas used as a fumigant for the control of a range of insect pests. It has been used for structural fumigation since the early 1960s. In the USA it is approved for "food uses" (grain, dried fruit and tree nuts), while in the UK, Germany and Italy the structures being fumigated must be emptied of food items. Sulfuryl fluoride is thought to inhibit the glycolysis and fatty acid cycles via the release of fluoride ions, thereby depriving the insect of energy necessary for survival.

Sulfuryl fluoride has not been evaluated previously by the JMPR.

All the critical studies contained statements of compliance with good laboratory practice (GLP). Sulfuryl fluoride is sold under the trade names of "Vikane" and "Profume".

Evaluation for acceptable daily intake

Sulfuryl fluoride is a gas with a boiling point of minus 55 C (218 K), which makes the performance of studies of oral toxicity difficult. Therefore, all except three of the studies described in this monograph have involved exposure to atmospheres containing sulfuryl fluoride vapour. To convert from concentrations in air to a systemic dose in mg/kg bw per day, account was taken of the respiratory rate and respiratory volume of the animals[1] (Zielhuis & van der Kreek, 1979), the duration of exposure (hours per day and days per week) and the proportion (10%) of the inspired dose that was absorbed (based on the toxicokinetic study of Mendrala et al., 2002). In assessing the studies in which sulfuryl fluoride was administered by inhalation, the Meeting agreed that local effects on the respiratory tract were not relevant to the assessment of dietary exposures. However, comments on such local effects are included in this document for completeness. The Meeting agreed the finding of slight dental fluorosis in experimental animals was not an adverse effect. Increases in fluoride concentrations in tissues and blood are considered to be a marker of exposure and not an adverse effect per se.

Toxicity and toxicokinetic studies with sulfuryl fluoride have been performed over a period of about 40 years. Most studies contained documentation stating compliance with the principles of GLP and the GLP status of the individual studies is identified in the summary text. All studies are considered to have complied with the basic requirements of the applicable OECD (or equivalent national) test guidelines, unless identified in the summary text. Analysis of concentrations within the inhalation chambers showed that achieved concentrations were equivalent to nominal levels.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

No data were available.

[1] Twenty-four h respiratory volumes for test species are: rats, 0.96 m^3/kg bw; rabbits, 0.54 m^3/kg bw; mice, 1.8 m^3/kg bw; and dogs, 0.39 m^3/kg bw.

(b) Dermal route

No data were available.

(c) Inhalation route

Rats

In a GLP-compliant study, a number of experiments were conducted to determine the biokinetic behaviour and metabolism of sulfuryl fluoride ([^{35}S]sulfuryl fluoride: radiochemical purity, 100%; specific activity, 0.22–0.25 mCi/mmol) in male Fisher F344 rats. Animals were exposed to sulfuryl fluoride via nose-only inhalation, following the protocol outlined in Table 1.

The analysis of ^{35}S was by liquid scintillation counting (LSC). The concentration of fluoride ion in the urine of rats exposed to both concentrations of [^{35}S]sulfuryl fluoride was determined by ion selective electrode (ISE). Also, ISE determination of the fluoride ion concentration in the plasma, and the brain and kidney tissue homogenates, of rats exposed to both concentrations of non-radiolabelled sulfuryl fluoride was conducted. Selected samples of urine and faeces were pooled and underwent radiochemical profile analysis for possible metabolite identification.

Table 1. Toxicokinetic investigations in male rats exposed to sulfuryl fluoride by inhalation for 4 h

Sulfuryl fluoride	Target dose	No. of animals		Sampling
		Cannulae in jugular vein	Not cannulated	
[^{35}S]Sulfuryl fluoride	30 ppm (specific activity, 0.26 μCi/l)	4	4	Excreta collected in dry-ice cooled traps. Urine: 0 h (end of exposure) and at 6, 12, 24, 48, 72, 96, 120, 144 & 168 h after exposure. Faeces: 0 h and at 24, 48, 72, 96, 120, 144 & 168 h after exposure. Selected tissues collected 7 days after exposure and analysed for radioactivity. Venous blood samples collected via jugular cannulae at 0.25, 0.5, 1, 2, 3 & 4 h during exposure and at 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, 120, 144 & 168 h after exposure. When animals killed, blood obtained via cardiac puncture. Blood, urine, and faeces analysed for radioactivity, ^{35}S-labelled fluorosulfate, and sulfate and fluoride (urine and faeces only).
[^{35}S]Sulfuryl fluoride	300 ppm (specific activity 2.8 μCi/l)	4	4	As above
Unlabelled sulfuryl fluoride	30 ppm	0	18	Groups of three rats sacrificed at –2 h (during exposure), at 0 h (end of exposure), and at 2, 4, 8 & 2 h after exposure. When animals killed, blood obtained via cardiac puncture, and brain and kidney tissues collected and analysed for fluoride ion.
Unlabelled sulfuryl fluoride	300 ppm	0	18	As above
	0 ppm (control group)	0	8	Groups of two rats killed at –4, 0, 4 and 8 h. Blood obtained when animals killed, via cardiac puncture.

From Mendrala et al. (2002)

Achieved atmosphere concentrations were within 10% of nominal values. No animals died during the study. Sulfuryl fluoride was absorbed and excreted rapidly. Excretion was predominantly via the urine (> 80% in 24 h; Table 2). An initial urinary half-life for [^{35}S]sulfuryl fluoride-derived radioactivity was estimated as approximately 4 h at both exposure concentrations. This was followed by a second urinary elimination phase with a half-life of approximately 40 h. Low levels of radioactivity remained detectable in the urine for 7 days after exposure. No radioactivity was detected in charcoal traps exposed to exhaled air for 24 h after exposure at 300 ppm. It was considered likely that the 5% of the ^{35}S radiolabel recovered from tissues 7 days after exposure was due to non-specific incorporation of the ^{35}S radiolabel into amino acids and macromolecules. Some of the radioactivity found in faeces during exposure was thought to be due to contamination with urine resulting from the nose only restraint compromising the technique for separation of excreta.

As exposures were via inhalation of a test atmosphere the actual 'dose received' (total systemic dose) was estimated by addition of the total recovered radioactivity from urine, faeces and tissues. To provide a comparative estimate of total absorption a breathing rate of 0.96 m^3/kg per day for rats (Zielhuis & van der Kreek, 1979) has been assumed and a body weight of 250 g. This comparison indicates that the absorbed dose would be about 14% at 30 pm and about 11% at 300 ppm.

Inhaled sulfuryl fluoride is widely distributed. Highest concentrations at 168 h after exposure were in lungs, kidney, spleeen and nasal turbinates (Table 2). Comparison of findings for plasma and erythrocytes indicates an association of ^{35}S with erythrocytes. The AUCs for erythrocytes were about eightfold higher than for plasma, primarily due to an extended β-elimination phase (Mendrala et al., 2002).

(d) Biotransformation

(i) Chemical analysis

Blood extract samples showed only two radiolabelled components, tentatively identified as sulfate and fluorosulfate. No analysis for parent sulfuryl fluoride in the blood was performed because work previously done in this laboratory had shown rapid removal of sulfuryl fluoride from rat blood fortified with sulfuryl fluoride at high concentrations in vitro. The identification of fluorosulfate was confirmed by ^{19}F nuclear magnetic resonance (NMR) spectroscopy. The amount of fluorosulfate was approximately twice that of sulfate at all time-points after exposure, except at 15 min after the beginning of the exposure at 300 ppm, when the concentration of fluorosulfate was 6.5-fold higher, and 4 h after the end of this exposure, when only a small amount of fluorosulfate was detected. At all sample times, the concentrations of sulfate and fluorosulfate were approximately three- to fivefold higher after exposure at 300 ppm than after exposure at 30 ppm. Based on the limited amount of data available, the half-life for the elimination of fluorosulfate from whole blood was calculated to be 48 to 73 min, while the half-life for elimination of sulfate from whole blood was calculated to be 50 to 64 min.

Two radioactive peaks tentatively identified as sulfate and fluorosulfate were detected in the urine. Analysis for parent sulfuryl fluoride in urine was not conducted because sulfuryl fluoride has been shown to be rapidly hydrolysed in aqueous solutions. During the exposure, the amount of fluorosulfate eliminated in the urine was 3- to 3.5-fold higher than the amount of sulfate (Table 3). After the exposures, the amount of sulfate recovered in the urine was greater than that of fluorosulfate. By 12 h after exposure, the amount of sulfate recovered in the urine was five- to sevenfold greater than that of fluorosulfate. The total amount (μmol) of sulfate plus fluorosulfate recovered in the urine as determined by high performance liquid chromatography/radioactivity monitor (HPLC/RAM) compares well with the amount (μmol eq) sulfuryl fluoride in the urine plus rinse, measured in the portion of this study that used radiolabelled sulfuryl fluoride. Conversion of urinary concentrations of sulfate and fluorosulfate to rate estimates to correct for unequal collection intervals allowed calculation of half-lives for the elimination of sulfate and fluorosulfate in urine. Sulfate was eliminated in the urine with half-life

Table 2. Excretion, kinetic and tissue data from male rats given ^{35}S-labelled sulfuryl fluoride by inhalation

Sample		Excretion after inhalation (µg eq sulfuryl fluoride)	
		Lowest dose (30 ppm)	Highest dose (300 ppm)
Urine (+ rinse)	–4–0 h[a]	273	2766
	0–6 h	167	936
	6–12 h	54	429
	12–24 h	43	199
	24–48 h	20	124
	48–120 h	18	130
	120–144 h	2.9	21
	144–168 h	2.0	14
	–4–168 h[b]	581	4618
Faeces	–4–0 h[a]	9.6	325
	0–24 h	32	222
	24–48 h	22	158
	48–120 h	8.9	67
	120–144 h	0	0.6
	144–168 h	0	4.4
	–4–168 h[b]	73	777
Expired air		—	—
Cage wash		—	—
Tissues (day 7)		35	298
Total recovery		688	5692
Tissue concentrations at 168 h			
Lung		0.78	6.3
Spleen		0.39	3.1
Kidney		0.37	2.8
Respiratory turbinates		0.31	3.5
Olfactory turbinates		0.29	3.2
Brain		0.23	1.9
Liver		0.13	0.9
Fat		0.03	0.5
Plasma			
T_{max} (h)		0.5	0.5
C_{max} (µg.eq/g)		4.7	38
AUC (–4–∞) (µg.h/ml)		97	756
$T_{½}$ α (h)		2.6	2.4
$T_{½}$ β (h)		82	56
Erythrocytes			
T_{max} (h)		0.5	1.0

C_{max} (μg.eq/g)	4.1	48
AUC (–4–∞) (μg.h/ml)	863	5492
$T_{½}$ α (h)	"similar to plasma''[c]	"similar to plasma" [c]
$T_{½}$ β (h)	222	139

From Mendrala et al. (2002)

AUC, area under the curve of concentration–time

[a] Period of exposure

[b] Period of exposure plus duration of study

[c] Terminology used in study report

of 2.2 and 3.8 h at 30 and 300 ppm, respectively. Fluorosulfate was eliminated slightly faster, with a half-life estimate of 1.2 and 2.4 h at 30 and 300 ppm, respectively.

(ii) Fluoride analysis

Urine: Elevated concentrations of fluoride ion were detected in the urine during and after the exposures to sulfuryl fluoride. Non-exposed control rats had concentrations of fluoride ion of approximately 2.5 μg/g urine. By the end of the 4 h exposure at 30 ppm, the urinary concentration of fluoride ion reached a maximum of 9.3 μg/g urine. This concentration was maintained during the 6 h after exposure. By 12 h after exposure, the concentration had decreased to 2.7 μg/g urine, that is, close to background levels. In a similar fashion, by the end of the 4 h exposure at 300 ppm, the urinary concentrations of fluoride ion reached a maximum of 76 μg/g urine, about eightfold higher than that obtained after exposure at 30 ppm. By 6 h after exposure, however, concentrations diminished to 32 μg/g urine, and by 24 h after exposure to 5.0 μg/g urine. The overall shapes of the three curves for urine HPLC and ion-sensitive electrode (ISE) data during and after exposure to sulfuryl fluoride were similar; this would suggest that that the kinetics of formation and elimination of the three metabolites (urinary sulfate, fluorosulfate and fluoride) were interrelated, as would be expected.

ISE analysis of plasma and tissues was conducted with rats exposed to unlabelled sulfuryl fluoride and with control rats exposed to clean air (Table 4). Plasma concentrations of fluoride ion

Table 3. Radiolabelled metabolites found in the urine of rats exposed by nose-only inhalation to ^{35}S-labelled sulfuryl fluoride for 4 h

Metabolite fraction	Metabolite recovered in urine (μmol)				Fluoride ion (μg fluoride ion/g urine)	
	Lowest dose (30 ppm)		Highest dose (300 ppm)		Lowest dose (30 ppm)	Highest dose (300 ppm)
	S	FS	S	FS	F^-	F^-
Not exposed	—	—	—	—	2.23	2.55
Exposure period –4–0 h	0.58	1.68	6.35	22.36	9.33	76.25
Post-exposure: 0–6 h	0.64	0.46	5.38	3.96	9.21	31.90
6–12 h	0.37	0.06	2.59	0.59	2.71	6.34
12–24 h	—	—	1.43	0.22	—	5.04
Total	1.59	2.2	15.76	27.14	1.119	6.29

From Mendrala et al. (2002)

F^-, fluoride ion; FS, fluorosulfate; S, sulfate.

Table 4. Concentrations of fluoride ion in the plasma, brain and kidneys of rats exposed by nose-only inhalation to unlabelled sulfuryl fluoride for 4 h

Scheduled time of death	Fluoride ion (µg fluoride ion/g tissue)								
	Plasma			Brain			Kidney		
	Control	30 ppm	300 ppm	Control	30 ppm	300 ppm	Control	30 ppm	300 ppm
Before exposure (–4 h)	0.63	—	—	0.46	—	—	2.3	—	—
During exposure (–2 h)	—	0.87	2.5	—	1.1	1.5	—	4.8	5.4
End of exposure (0 h)	0.46	0.76*	2.5*	0.60	0.79	2.3	2.3	5.4*	5.6*
After exposure (2 h)	—	0.56	0.86	—	0.78	1.3	—	5.4	4.9
4 h	0.44	0.53*	0.71*	0.46	0.80	0.98	2.5	5.7*	5.0*
8 h	1.2[a]	0.38*	0.55*	0.76	—	—	1.9	—	—
20 h	0.56	0.47	0.52	—	—	—	—	—	—

From Mendrala et al. (2002)

* Significant difference from values for controls at the indicated scheduled time of death; alpha = 0.05.

[a] No clear reason for high background reading

in control animals ranged from 0.430 to 1.338 µg/g plasma throughout the 24 h cycle. An apparent slight elevation of plasma fluoride above control concentrations was noted during the exposures at 30 ppm and 300 ppm, but rapidly returned to control concentrations by about 2 h after the exposure period. Plasma concentrations of fluoride at the end of exposure at 30 and 300 ppm, respectively, were 1.6- and 5.4-fold higher than those for the controls. The maximum plasma concentration of fluoride measured at the termination of the 4 h exposure to sulfuryl fluoride at 30 ppm was similar to that measured after exposure to sulfuryl fluoride at 30 ppm in the 13-week study using administration by inhalation (Nitschke et al., 1987a), although considerable variation was reported. However, the plasma concentration at 300 ppm reported in this study was almost twice that reported in the 13-week study with sulfuryl fluoride administered by inhalation (Nitschke at al, 1987a) at the same dose. It was considered that based on the rapid clearance of fluoride from the plasma (background levels about 2 h after the end of exposure), a delay in sacrificing the animals after exposure in the 13-week study may have resulted in measurements of fluoride that were below peak concentrations. Again, the three curves of concentration measured during and after exposures to sulfuryl fluoride were found to be similar for the three metabolites (urinary sulfate, fluorosulfate and fluoride).

Fluoride concentrations in kidney tissue during and after exposure at 30 and 300 ppm were roughly 2- to 2.5-fold higher at all collection times than in control rats. Control rats had mean fluoride concentrations of 2.2 µg/g (0.12 µmol/g) kidney tissue, while rats at 30 and 300 ppm had concentrations of about 5 µg/g (0.26 µmol/g) kidney tissue. These levels were measured by the second hour of exposure and were maintained for 4 h after exposure.

A slight 1.5-fold elevation in fluoride concentrations in brain tissue relative to control rats was observed during and after exposure to sulfuryl fluoride at 30 ppm. Concentrations of fluoride ion in brain tissue from rats in the control group were about 0.6 µg/g (0.03 µmol/g) tissue at all measured times, increasing to 0.8 µg/g (0.04 µmol/g) brain after exposure to sulfuryl fluoride at 30 ppm. At the end of the exposure at 300 ppm, a range of 1.3 to 3.7 µg/g (0.05 to 0.12 µmol/g) brain (mean, 2.3 µg/g) was measured, two- to fivefold higher than in the controls. These concentrations of fluoride returned to control levels at about 4 h after exposure.

On the basis of the identification of fluorosulfate and sulfate in the blood and urine, the likely metabolic pathway for sulfuryl fluoride is initial hydrolysis to fluorosulfate, with release of fluoride, followed by further hydrolysis to sulfate and release of the remaining fluoride.

2. Toxicological studies

2.1 Acute toxicity

(a) General toxicity

Because sulfuryl fluoride is a gas with a boiling point of –55 °C (218 K), the studies of acute toxicity after oral and dermal administration therefore had to be performed in a non-standard manner. In the studies of inhalation toxicity, there appeared to be a non-linear effect of concentration, with 95% mortality after a 0.2 h exposure at 15 000 ppm (3000 ppm.h), but 50% mortality after exposure at 1000 ppm for 6 h (6000 ppm.h) (Anonymous, 1959b). Signs reported during exposures by inhalation were sedation, dark nasal and ocular exudate and convulsions, with blueish tails being the main sign at lower concentrations. Centrilobular hepatocyte degeneration and renal tubule degeneration were seen in rats exposed to lethal concentrations (Table 5).

(b) Ocular irritation, dermal irritation and dermal sensitization

Performing studies of irritancy and sensitization with a gas is not straightforward. Whole-body exposures to sulfuryl fluoride at relatively high concentrations (e.g. 9600 ppm for 4 h) did not provide any evidence of irritation to skin, eyes or respiratory tract. Studies of repeated whole-body exposure provided no evidence of skin sensitization or respiratory sensitization. There have been no reports of significant irritancy or sensitization from users of Vikane over a 40-year period.

Table 5. Acute toxicity of sulfuryl fluoride

Species	Strain	Sex	Route and vehicle	LD_{50}(mg/kg bw) LC_{50} (mg/l air)	Purity (%)	Reference
Rat	NS	M & F	Oral, in corn oil	About 100	NA	Anonymous (1959a)
Guinea-pig	NS	M & F	Oral, in corn oil	About 80	NA	Anonymous (1959a)
Rat	Fisher F344	M & F	Dermal[a] (4 h)	> 9600 ppm in air	99.7	Bradley et al (1990)
Rat	Wistar	M & F	Inhalation (4 h, whole-body?)	4.7 mg/l (1000 ppm)	"Vikane"	Anonymous (1959b)
Rat	Wistar	M & F	Inhalation (4 h, whole-body)	5.8 mg/l (M, 1122 ppm) 5.2 mg/l (F, 991 ppm)	99.7	Miller et al. (1980)
Mouse	$B6C3F_1$	M & F	Inhalation (4 h, whole-body)	1.7–2.8 mg/l (400–600 ppm)	99.6	Nitschke & Lomax (1989)
Mouse	$B6C3F_1$	M & F	Inhalation (4 h, whole body)	2.8 mg/l (M, 660 ppm) 2.7 mg/l (F, 642 ppm)	99.6	Nitschke & Quast (1990)

F, female; M, male; NA, not available—solution prepared by bubbling "Vikane" into the corn oil; NS, not stated.

[a]By exposure to gas; head in fresh air

2.2 Short-term studies of toxicity

Oral toxicity was investigated in a 66-day dietary study in rats. Repeat-dose dermal toxicity has not been investigated specifically, but would have been included in the many studies that investigated whole-body exposure. Studies of inhalation have been performed in rats, dogs, mice and rabbits, for periods spanning 2 weeks to 1 year.

(a) Oral administration

Mice

No data were available.

Rats

In a 66-day study, groups of 10 male and 10 female Wistar rats (aged at least 50 days) were maintained for 66 days on diets fumigated with 0, 2, 10, 100 and 200 lb per 1000 cu ft of Vikane (purity not specified). The study did not comply with GLP (being performed before GLP requirements were established) and did not satisfy OECD guideline 407 (1981). Fluoride concentrations in the control and treated diets were determined (Table 6). The rats were weighed twice weekly for 28 days and then weekly. They were observed "frequently" for gross changes in appearance and behaviour. The teeth of all rats were checked for any visual evidence of fluorosis. Food consumption was recorded for the first month. Samples of urine were obtained before termination from all males for fluoride analysis; terminal haematology values were obtained from five female rats at 0, 2 and 10 lbs and from two males from each dietary concentration. At necropsy, the lungs, heart, liver, kidneys, spleen, and testes were weighed. Portions of these organs as well as the pancreas and adrenals were examined histologically. Samples of blood, urine (male), kidney, lung, liver and bones from the rats killed at 30 days were collected and analysed for fluoride.

No mortalities occurred and no clinical signs of toxicity were reported. Reductions in body-weight gain were statistically significant in males at 53 ppm (net concentration of fluoride), and above. In females there was a clear reduction in body-weight gain at the highest "dose" (Table 7).

No information on food and water consumption was given and no ophthalmic observations were undertaken. No clinical chemistry was undertaken. No effects on haematocrit, haemoglobin, total or differential counts of leukocytes were reported. Full urine analysis was not undertaken, only fluoride concentrations were assessed. A clear dose-related increase in urinary fluoride was seen in treated males; females were not assessed (Table 8).

Table 6. Concentration of fluoride in diets fumigated with Vikane

Group	Rate of fumigation with Vikane (lbs/1000 cu ft)	Average concentration of fluorides (ppm) [a]	
		Total	Net[b]
1	Control diet (no fumigation)	36	—
2	2	55	19
3	10	89	53
4	100	386	350
5	200	740	704

From Anonymous (1959c)

[a] The identity of the "fluoride" in the diets was not given.

[b] Level minus value for control

Teeth were noted to have fluorosis banding, altered pigmentation and staining at 53 ppm and above. Altered liver and testes weights appeared secondary to lower body weights (Table 7).

Fluoride content in the urine and bone increased in a dose-related manner. In tissue other than bone, fluoride content did not correlate well with dietary concentration, especially the spurious result in kidneys from the groups at 53 ppm (Table 8).

Histopathology findings were reported as "glomerular" involvement of the kidneys, but no doses were specified.

On the basis of the limited level of detail provided in the study report, it was not possible to derive a no-observed-adverse-effect concentration (NOAEC) with any confidence. However, a tentative value of 19 ppm (net amount of fluoride above the control diet value of 34 ppm in the fumigated diet; equivalent to a total fluoride intake of 2.5 mg/kg bw per day) can be proposed on the basis of overt fluorosis of teeth in both sexes and reduced body-weight gain in males at 53 ppm (equivalent to a total fluoride intake of 4.5 mg/kg bw per day) (Anonymous, 1959c).

Dogs

No data were available.

(b) Dermal exposure

No data were available.

(c) Exposure by inhalation

Mice

(i) 2-Week pilot study

In a pilot study of subacute toxicity after inhalation, groups of 10 male and 10 female CD-1 mice were given sulfuryl fluoride (purity, 99.6%; batch No. 752; lot No. 880329) at a concentration of 0, 30, 100 or 300 ppm during 6 h/day for 5 days/week for 2 weeks (nine whole-body exposures). These concentrations corresponded to intakes of sulfuryl fluoride of 0, 4.1, 13, and 39 mg/kg bw per day. The study complied with GLP and with OECD test guideline 412 (1981), 84/449/EEC, Method B8 (1984); California SB950.

All animals were observed daily for overt signs of toxicity or changes in demeanour. Behaviour pattern and nervous system activity were assessed by specific observations for lethargy

Table 7. Final body weights, liver and testes weights in rats fed diets treated with "Vikane"

Net concentration of fluoride (ppm)	Males						Females			
	No. of rats	Body weight (g)	Liver		Testes		No. of rats	Body weight (g)	Liver	
			g	g/100 g	g	g/100 g			g	g/100 g
0	8	306	8.72	2.86	3.02	0.99	9	173	5.40	3.12
19	9	285	8.43	2.96	2.86	1.01	10	185	5.66	3.06
53	10	275*	8.36	3.03*	2.78	1.01	10	171	540	3.15
350	9	247**	7.31	2.96	2.80	1.09*	9	169	5.28	3.12
704	10	207**	6.23	3.00*	2.72	1.33*	10	145**	4.52	3.12

From Anonymous (1959c)

*p = 0.01 to 0.05 (statistical test not specified)

**p = < 0.01 (statistical test not specified)

Table 8. Urinary and tissue content of fluoride (mean, ppm) in rats given diets treated with Vikane for 30 days

Net concentration of fluoride (ppm)	Sample											
	Males						Females					
	Blood	Urine	Kidney	Lung	Liver	Bone	Blood	Urine	Kidney	Lung	Liver	Bone
0	0.0	9.9	0.9	13.5	0.0	260	4.4	—	1.3	20.3	1.6	276
19	2.7	11.9	2.4	5.0	0.0	408	3.0	—	1.3	13.0	2.0	339
53	0.4	13.3	10.3	1.0	0.3	413	0.0	—	9.6	20.4	2.0	339
350	0.4	85.6	2.3	17.6	0.0	1615	0.6	—	0.0	17.2	0.0	1875
704	6.2	174.4	2.3	6.0	8.4	1920	2.9	—	5.7	25.3	0.3	3445

From Anonymous (1959c)

tremors, convulsions, salivation, lachrymation, diarrhoea, or other signs of altered central nervous system functions. All animals were weighed on test days 1, 3, 5, 8, and 11. Blood samples were collected before sacrifice on the day following the last exposure to the test material. Weights of the brain, heart, liver, kidneys and testes (males) were recorded. All animals were examined for gross pathological alterations. A complete set of tissues was collected from each animal and preserved in neutral phosphate-buffered 10% formalin. The lungs were infused with buffered formalin to their approximate normal inspiratory volume and the nasal cavity was flushed with formalin via the pharyngeal duct to ensure rapid fixation. The necropsy included in situ examination of the eyes by a glass-slide technique with fluorescent illumination. Histopathological examination of brain, liver, kidney and respiratory tract (nasal tissue, trachea, larynx, and lungs) was conducted on all animals.

At 300 ppm, all males and four out of five females died during the second week of the study. The decedents were thin and many had roughened haircoat and exhibited body tremors (males only). All animals exposed to sulfuryl fluoride at < 300 ppm showed no overt signs of toxicity. The body weights of mice exposed at 300 ppm were significantly lower than those of the controls (> 25%).

Food and water consumption as not assessed. Ophthalmic observations were not conducted after exposure.

Erythrocyte counts in male mice and haemoglobin concentrations in male and female mice exposed at 100 ppm were statistically significantly elevated; however, these values were within the range for the historical control data from this laboratory and are not considered to be adverse.

Urine analysis was not conducted. All clinical chemistry parameters measured in mice exposed to sulfuryl fluoride were comparable to tvalues for the controls.

Several gross pathological changes were observed in male and female mice at 300 ppm that died before scheduled necropsy. Thes included decreased amount of ingesta in the digestive tract, decreased fat and erosions of the stomach. There were no effects at 100 or 30 ppm.

Owing to the decreased body weight in the sole surviving female mouse exposed at 300 ppm, several absolute and/or relative organ weight values differed from those of the controls. These differences included an increase in relative brain, heart and kidney weights and a decrease in absolute liver weights. At 100 or 30 ppm, no effects on organ weights were noted.

Effects were observed in the cerebrum and medulla of the brain of mice exposed at 100 or 300 ppm. Multifocal vacuoles, which were very slight in degree, were observed in the cerebrum of four male and two female mice at 100 ppm. More severe vacuoles graded as moderate were observed in the cerebrum of the majority of mice exposed at 300 ppm. In addition, four male mice and one female mouse at 300 ppm had vacuoles in the medulla that were very slight or moderate in severity. There were no histopathological changes in the medulla of mice exposed to sulfuryl

Table 9. Histopathological findings in mice exposed to sulfuryl fluoride by inhalation for 2 weeks

Organ	Finding	Severity	Concentration (ppm)							
			0	30	100	300	0	30	100	300
			Males				Females			
No of mice examined (deaths)			5	5	5	(5)	5	5	5	1 (4)
Brain	Vacuolation, cerebrum, bilateral, multifocal	Very slight	0	0	4	(1)	0	0	2	0
		Moderate	0	0	0	(4)	0	0	0	(3)
	Vacuolation, medulla, bilateral, multifocal	Very slight	0	0	0	(2)	0	0	0	(1)
		Moderate	0	0	0	(2)	0	0	0	0
Kidney	Atrophy, tubule, unilateral, focal,	Very slight	1	0	0	0	1	2	0	0
	Atrophy, tubule, unilateral, multifocal	Very slight	0	0	0	0	0	0	1	0
	Atrophy, tubule, bilateral, focal	Slight	0	0	0	0	1	0	1	0

From Nitschke & Quast (2002)

fluoride at 100 ppm and no lesions were noted in the cerebrum or cerebellum of mice at 30 ppm (Table 9).

Nine out of 10 mice exposed to sulfuryl fluoride at 300 ppm had hepatocellular and lachrymal gland atrophy, which was considered to be secondary to inanition/the moribund condition of these animals; there were no histopathological effects at 100 ppm or below. All other microscopic changes in mice were considered to be incidental findings that were unrelated to exposure to sulfuryl fluoride; there were no compound-related effects on the kidney.

In this study in CD-1 mice, the NOAEC for males and females was 30 ppm (approximately equivalent to 4.1 mg/kg bw per day) on the basis of vacuolation in the cerebrum at 100 ppm (approximately equivalent to 13 mg/kg bw per day) (Nitschke & Quast (2002).

Groups of 10 male and 10 female CD-1 mice were exposed by inhalation (whole-body) to sulfuryl fluoride (purity, 99.6%; lot No. WP 880329 752 MAR/88) at a concentration of 0, 10, 30 or 100 ppm for 6 h/day, 5 days/week for 13 weeks. These concentrations corresponded to intakes of 0, 1.4, 4.1 and 13 mg/kg bw per day. Additional groups of four males and four females were used for nervous system investigations. The study complied with GLP and US EPA guideline 82-4, OECD 412, 84/449/EEC Method B8.

Clinical observations were made daily, a detailed clinical examination was made weekly, a functional observational battery (FOB) was conducted on all mice after 4, 8 and 12 weeks of exposure and body weights were measured each week. Haematology (haematocrit, haemoglobin, erythrocytes, leukocyte/differential, platelets) was carried out for 10 mice of each sex per group before necropsy, and clinical chemistry (alainine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase, AP; urea nitrogen, UN; creatinine, total protein, albumin, globulin, glucose, cholesterol, triglycerides, total bilirubin, calcium, phosphorus and fluoride) on 10 mice of each sex per group at necropsy. Gross pathology examinations were carried out for 10 mice of each sex per dose. Histopathological examinations were made on a wide range of tissues from the control group and the group at 100 ppm, and histological examinations were also made of nasal tissues, trachea, lungs, brain, thyroid, heart, liver, kidneys, salivary glands and testes from mice in the groups at 10 and 30 ppm.

The remaining animals, four of each sex per dose, were used to measure serum fluoride concentrations. In addition, tissues of these four animals of each sex per dose were perfused with glutaraldehyde/formaldehyde fixative and neural tissues (cerebellum, cerebrum, medulla oblongata, thalamus/hypothalamus, spinal cord, dorsal root ganglia, trigeminal ganglia, peripheral nerves, including sciatic, tibial and sural nerves, as well as the head, liver, kidneys and lung) were examined histopathologically.

Three mice died during the study. All deaths were unrelated to exposure to sulfuryl fluoride (one control died due to inanition attributable to tongue abscess, and the other two mice died from accidental handling trauma).

There were no exposure-related cageside, clinical or functional observational effects in any of the exposure groups.

FOB results were similar in treated and control groups. The auditory brainstem response in about half of the CD-1 mice from several different batches of mice purchased by the conducting laboratory had been shown to be poor, therefore the startle response, typically measured in FOBs, was not measured in this study.

In general, mean body weights of male and female mice exposed at 100 ppm were statistically significantly decreased relative to control values from day 25 to the end of the study. By the end of the study, the mean body weights for male and female mice at 100 ppm were 89 and 91% of control values, respectively.

Food and water consumption was not assessed. Ophthalmic observations were not assessed after treatment. Urine analysis was not conducted. There was no evidence of an exposure-related effect on haematology parameters.

There were statistically significant increases (dose related) in serum fluoride concentrations in females at exposures $\geq$ 30 ppm, and males at 100 ppm. There were slight but statistically significant increases in AP activity and triglyceride concentrations, and decreases in inorganic phosphate in males at 100 ppm (Table 10).

There were no exposure-related gross pathological changes noted with sulfuryl fluoride at concentrations of up to 100 ppm.

Several absolute organ-weight values in males and females exposed at 100 ppm were reduced. These differences included a statistically significant decrease in absolute brain (Table 11), heart and liver weights for male and female mice, and absolute kidney weights in male mice. These absolute organ-weight changes were not accompanied by significant histopathological changes and the relative organ weights for these animals were comparable to those of the controls. The decrease in absolute organ weights was considered to be a secondary reflection of the body-weight decrease.

Table 10. Clinical chemical results in mice exposed to sulfuryl fluoride for 13 weeks

Dose (ppm)	Males				Females		
	PO_4	Tri (mg/dl)	AP (mU/ml)	F^- (ppm)	PO_4 (mg/dl)	AP (mU/ml)	F^- (ppm)
0	7.1	91	43	0.107	4.8	45	0.090
10	6.8	83	42	0.112	4.9	52	0.088
30	6.7	89	47	0.156	4.6	49	0.132*
100	5.8	143*	57*	0.259*	4.4	58	0.233*

From Nitschke & Quast (1993)

AP, alkaline phosphatase; F, fluoride; PO_4, phosphate; Tri, triglyceride.

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

Table 11. Findings (means or No. of animals) in groups of mice (n = 10) exposed to sulfuryl fluoride for 13 weeks

Finding		Concentration (ppm)			
		0	10	30	100
Body weight at 13 weeks (g)	M	39.6	38.8	38.9	35.3*
	F	30.3	30.7	31.2	27.6*
Absolute brain weight (g)	M	0.50	0.49	0.48	0.47*
	F	0.51	0.50	0.50	0.48*
Releative brain weight (g)	M	1.3	1.3	1.3	1.4
	F	1.7	1.6	1.6	1.8
Thyroid follicular cell hyperplasia (slight)	M	0	0	0	9*
	F	0	0	0	6*
Cerebrum—vacuolation of caudate putamen (bilateral)	M	0	0	0	9*
	F	0	0	0	8*
Cerebrum—vacuolation of external capsule (bilateral)	M	0	0	0	9*
	F	0	0	0	10*
Thalamus/hypothalamus—external capsule vacuolation (bilateral)	M	0	0	0	9*
	F	0	0	0	10*

From Nitschke & Quast (1993)

M, male; F, female.

* $p < 0.05$

Histopathology showed a number of microscopic observations in mice from the various groups, primarily regarding the three mice that died during the study, and the one mouse with a liver abscess. These observations were not considered to be exposure-related. Treatment-related effects were observed in the brain and thyroid gland of mice exposed at 100 ppm (Table 11). There were no adverse histopathology findings at 30 ppm.

Tissues were examined from the nervous systems of the four mice that were perfused (whole-body) for neuropathology assessment. To maximize the detection of possible changes in the tissue fixed by perfusion, in contrast to the tissue fixed by immersion, a total of nine sections of brain were prepared from each mouse from regions 3, 4 and 7[2]. Despite the additional evaluation of extra brain sections, the microvacuoles in the various affected regions appeared to be less prevalent in the perfusion-fixed than in the immersion-fixed tissue.

The NOAEC was 30 ppm (approximately equivalent to 4.1 mg/kg bw per day) on the basis of reduced body-weight gain (about 10%) and vacuolation in the brain at 100 ppm (approximately equivalent to 13 mg/kg bw per day) (Nitschke & Quast, (1993).

Rats

In this range-finding study for a 13-week study, groups of five male and five female Fischer 344 rats were exposed by nose-only inhalation to Vikane gas fumigant (purity, 99.8%; lot No. TWP 830919-408) at a concentration of 0, 100, 300 or 600 ppm during 6 h/day for 5 days per

[2] Section 3: corpus callosum, caudate putamen, globus pallidus, optic chiasm, cortex (frontal & parietal), lateral ventricles.

Section 4: hippocampus, thalamus, hypothalamus, cortex (parietal & temporal), third ventricle.

Section 7: cerebellum, inferior colliculus, the fifth cranial nerve (the trigeminal nerve) nerve, pyramidal tract, nucleus trapezoid body.

week for 2 weeks (nine exposures). These concentrations corresponded to intakes of 0, 7.2, 22 and 44 mg/kg bw per day. The study complied with GLP, but not with any guideline.

Animals were observed daily and body weights were recorded several times throughout the study. Before the ninth exposure, blood samples were collected from rats for haematology and urine samples were collected for urine analysis. At terminal necropsy, serum samples were prepared for clinical chemistry and organs (brain, heart, liver, kidneys, thymus and testes) were weighed. A complete necropsy was performed on all animals and extensive histopathology was completed on the controls and on animals at 600 and 300 ppm. Examination of tissues from animals in the group at 100 ppm was limited. Special stains were used on the brain, lung, kidney, heart and stomach from rats receiving the highest dose in order to further investigate effects.

All males and four out of five females at 600 ppm died or were found in a moribund condition between the second and ninth exposures. No other rats died in this study.

Clinical signs of toxicity were not specified in the report.

In the group at 600 ppm, body weights were significantly lower than those of the controls for most of the study.

Measurement of food and water consumption was not undertaken.

Ophthalmic observations were not undertaken. Eyes were examined at necropsy and during histopathology.

Leukocyte count was elevated in both sexes, and erythrocyte parameters were elevated in females at the highest dose (Table 12).

Urine analysis revealed no changes in survivors.

There were some statistically identified alterations in the surviving female rat at the highest dose (urea nitrogen, ALT, glucose, albumin and globulin).

Decreased thymus size was observed in rats at the highest dose. There were some slight increases (< 10%) in male rat kidney weight at 300 ppm and a statistically significant increase in relative kidney weight in female rats at the two higher doses (Table 13). The heart weights of rats were generally variable. The relative heart weight was elevated for females at 300 and 600 ppm though the absolute weight was significantly reduced for the surviving female at 600 ppm.

Decedents had severe microscopic kidney lesions that included papillary necrosis as well as degeneration and regeneration of collecting tubules and proximal tubules. Numerous microscopic pathological observations in these rats were considered secondary to the renal effects or terminal changes. The one female rat that survived until termination was in poor condition and had a variety of changes secondary to kidney lesions. Five out of 10 rats exposed at 300 ppm had minimal renal changes. There were no renal effects at 100 ppm. The only lesion seen in the brain was slight oedema in one female that died.

Table 12. Haematology results for F344 rats exposed to sulfuryl fluoride for 2 weeks

Conc. (ppm)	Males					Females				
	No.	Erythrocytes ($\times 10^6$/mm^3)	Hb (g/dl)	EVF (l/l)	Leukocytes ($\times 10^3$/mm^3)	No.	Erythrocytes ($\times 10^6$/mm^3)	Hb (g/dl	EVF (l/l)	Leukocytes ($\times 10^3$/mm^3)
0	5	8.14	17.3	0.482	6.7	5	7.78	17.0	0.480	5.3
100	5	8.20	17.4	0.486	7.4	5	7.87	17.0	0.484	6.6*
300	5	7.96	16.9	0.476	8.8*	5	7.80	17.0	0.480	7.9*
600	0	—	—	—	—	1	8.76*	19.2*	0.530*	11.6*

From Eisenbrandt et al. (1985)

*Statistically identified difference from control mean by Dunnett's test, alpha = 0.05.

Conc., concentration; EVF, erythrocyte volume fraction; Hb, haemoglobin

Table 13. Organ-weight changes in F344 rats exposed to sulfuryl fluoride for 2 weeks

Concentration (ppm)	No.	Terminal body weight (g)	Heart		Kidney	
			(g)	(g/100g)	(g)	(g/100g)
Males						
0	5	173.7	0.617	0.355	1.452	0.834
100	5	167.7	0.637	0.380*	1.443	0.861
300	5	172.3	0.614	0.356	1.545	0.896
600	0	—	—	—	—	—
Females						
0	5	112.2	0.459	0.409	1.071	0.955
100	5	109.4	0.467	0.427	1.123	1.028
300	5	107.6	0.471	0.438*	1.155	1.075*
600	1	67.6*	0.313*	0.463*	0.961	1.422*

From Eisenbrandt et al. (1985)

*Statistically identified difference from control mean by Dunnett's test, alpha = 0.05.

The NOAEC for F344 rats was 100 ppm (approximately equivalent to 7.2 mg/kg bw per day) on the basis of very slight or slight renal hyperplasia and mineralization in rats at the next dose 300 ppm (approximately equivalent to 22 mg/kg bw per day). Changes in leukocyte counts in females at 100 ppm were not considered to be toxicologically significant as they were not reproduced in the 13 week study (see below) (Eisenbrandt et al., 1985).

Groups of 10 male and 10 female Fischer 344 rats were given sulfuryl fluoride (purity, 99.8%; lot No. TWP 830919-408) at a concentration of 0, 30, 100 or 300 ppm for 6 h/day, 5 days per week for 13 weeks by whole-body inhalation. Intake of the test article was 0, 2.2, 7.2 and 22 mg/kg bw per day. The study complied with GLP and US EPA guideline 82-4 (19).

Investigations included daily clinical observations; weekly body weights; haematology and clinical chemistry at terminal necropsy, and urine analysis after 11 weeks of exposure. At termination, organ weights (brain, heart, liver, kidneys, thymus, testes) and gross pathology were assessed for all animals. A complete histopathological examination of a wide range of tissues was performed on control rats and rats at the highest dose; histopathological examination was limited to brains, kidneys, nasal tissues, trachea and lungs of rats in the groups at 30 and 100 ppm. Special staining was performed on brain sections from three rats of each sex from the group receiving the highest dose, and electron microscopy of rat brain sections was attempted, but failed owing to numerous artifacts (tissues had been previously fixed for light microscopy).

There were no mortalities. No adverse clinical signs of toxicity were observed. Body weights of animals at 300 ppm were statistically significantly decreased from control values after 45 days in males and 24 days in females. Overall body-weight gains at 30, 100 and 300 ppm were 103%, 104% and 63% and 88%, 92% and 54% of controls in males and females, respectively. Food and water consumption was not assessed. No treatment-related effects on the eyes were noted in the report.

The erythrocyte counts for males at 300 ppm were statistically significantly lower than control values; the erythrocyte counts of females at 300 ppm were slightly lower than control values (Table 14). Platelet counts for males at 300 ppm were statistically significantly higher than control values. Since the erythrocyte-count alteration was $< 10\%$ and the platelet counts were

within the range of historical control values, these differences were not considered to be toxicologically significant. The white blood cell differential counts of rats exposed at concentrations of up to 300 ppm were not notably different from those of the controls.

At 300 ppm, statistically significant effects on various parameters were seen (Table 14). Serum fluoride concentrations were increased slightly at 300 ppm (Table 14). The specific gravity of urine from rats at 300 ppm was decreased in females, and was statistically significantly decreased in males.

At necropsy, gross examination of rats at 100 or 300 ppm revealed mottled upper and lower incisors. Pale foci were observed on the lung surface of most rats at 300 ppm.

Many of the absolute and relative organ weights of rats at 300 ppm were statistically significantly different from the respective control values. These changes were considered to be a secondary reflection of the decreased body weights. The absolute brain weight of female rats at 100 ppm was statistically significantly decreased from control values (about 4%); although the relative brain weight was comparable to that of controls, brain weight is normally well conserved in cases of reduced body weight and as the brain is a target organ for sulfuryl fluoride, this finding is considered to be potentially treatment-related.

The results of histopathology examination are summarized in Table 15. Subacute inflammation was detected histopathologically in the nasal tissues of all males and females at 300 ppm. All females and most males had minimal inflammation in the nasal tissue. A few males had more severe inflammation in the respiratory and olfactory mucosa with mucopurulent exudate in the nasal passages. The more extensive inflammation was accompanied by degeneration and reactive changes in the mucosa.

Slight, subpleural histiocytosis was observed in the lungs of rats at 300 ppm.

Microscopic vacuolation was observed in the brains of all rats at 300 ppm. The minimal vacuolation was in the area of the caudate-putamen nuclei and was more prominent in the white fibre tracts of the internal capsule than in the adjacent neuropil. Special stains of the brain with LFB-PAS or Sevier Munger stain did not reveal any additional effects.

Table 14. Haematology and clinical chemistry results from a 13-week study of toxicity in rats exposed to sulfuryl fluoride by inhalation

Parameter	Concentration (ppm)							
	0	30	100	300	0	30	100	300
	Males				Females			
Erythrocytes (10^6/mm^3)	9.07	9.05	9.08	8.64*	8.63	8.73	8.74	8.28
Platelets (10^3/mm^3)	502	516	548	565*	582	565	599	619
Alkaline phosphatase (mU/ml)	65	67	72	85*	48	51	53	72*
Blood urea nitrogen (mg/dl)					16	16	17	21*
Globulin (g/dl)	2.5	2.5	2.7	2.9*				
Total protein (g/dl)					6.0	5.9	5.9	5.6*
Albumin (g/dl)					3.7	3.6	3.6	3.4*
Calcium (mg/dl)					11.0	10.8	10.8	10.7*
Fluoride (µg/ml)	1.0	0.7	0.9	1.2	0.6	.07	0.6	1.4

From Nitschke, Dittenber & Eisenbrandt (1987)

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

Table 15. Main gross and histopathological findings in a 13-week study of toxicity in rats exposed to sulfuryl fluoride by inhalation

Organ	Finding	Severity	Concentration (ppm)							
			Males				Females			
			0	30	100	300	0	30	100	300
No. of rats examined			10	10	10	10	10	10	10	10
Brain	Absolute weight (g)		1.9	1.9	1.9	1.8	1.82	1.78	1.75*	1.72*
	Relative weight (g/100g)		0.67	0.67	0.66	0.77*	1.11	1.12	1.11	1.22*
	Vacuolation, cerebrum, bilateral, focal	Slight	0	0	0	10*	0	0	0	10*
Kidney	Aggregate(s) of reticuloendothelial cells:		0	0	0	0	1	1	0	1
	Atrophy, individual nephron	Very slight	9	7	8	3*	4	0	1	2
	Hyperplasia, collecting ducts	Very slight	0	0	0	0	0	0	0	9*
	Mineralization, tubule(s), multifocal	Slight	6	2	3	3	4	0	1	2
	Decreased protein droplets, cortex	Very slight	0	0	0	10*	0	0	0	0
Lungs (alveoli)	Histiocytosis, subpleural, focal	Very slight	0	1	0	0	2	1	2	0
	Histiocytosis, subpleural, multifocal	Slight	0	0	0	10*	0	0	0	10*
Nasal turbinates	Inflammation—subacute, mucosa, diffuse	Very slight	0	0	0	4	0	1	0	9*
		≥ Slight	0	0	0	6	0	0	0	1
	Inflammation—subacute to chonic, respiratory mucosa, multifocal	Very slight	0	0	0	0	2	2	2	0
		Slight	0	2	1	0	2	1	4	0

From Nitschke et al. (1987)

* $p < 0.05$

A slight decrease in protein droplet formation was observed in the renal cortical tubules of males at 300 ppm and was likely to be secondary to the decreased body weight. Most females at 300 ppm had very slight hyperplasia of the renal collecting ducts, which was most apparent in the outer portion of the inner zone of the medulla.

There was no histopathological correlate to the mottled teeth that were observed at necropsy.

The NOAEC in F344 rats was 30 ppm (approximately equivalent to 2.2 mg/kg bw per day) on the basis of overt dental fluorosis (mottled teeth) and decreased brain weight in females at exposure levels ≥ 100 ppm (approximately equivalent to 7.2 mg/kg bw per day) (Nitschke et al., 1987a).

Rabbits

Groups of three male and three female New Zealand White rabbits were given sulfuryl fluoride (purity, 99.8%; Vikane gas fumigant, lot No. TWP 830919-408) at a concentration of 0, 100, 300 or 600 ppm during 6 h/day for 5 days per week for 2 weeks by inhalation. The intakes

were 0, 4.1, 13 and 25 mg/kg bw per day. The study complied with GLP but was not conducted according to any guideline, being a range-finding study for a subsequent 13-week study.

Animals were observed daily and body weights were recorded several times throughout the study. Selected organs (brain, heart, liver, kidneys, and testes) were weighed and blood samples taken at the terminal necropsy. A complete necropsy was performed on all animals and extensive histopathology was completed on animals in the control group and in groups at the highest dose. Otherwise, examination of tissues from animals at the intermediate dose was confined to target organs and several other tissues. Measurement of food and water consumption was not undertaken. Ophthalmic observations were not undertaken, although eyes were examined at necropsy and histopathology. Urine analysis was not performed.

One female rabbit at 600 ppm had convulsions after the fifth exposure, resulting in a fractured tibia. Another rabbit at 600 ppm was found to have a fractured vertebra after the sixth exposure, although convulsions were not observed. Both these animals were euthanized. Male and female rabbits that survived exposure at 600 ppm were slightly hyperactive. Other rabbits appeared normal and there were no significant gross pathological findings.

At 600 ppm, body-weight gains were significantly lower than those of the controls for most of the study. Terminal body weights of some rabbits at 300 or 600 ppm were slightly decreased (Table 16). Some of these rabbits also had decreased liver weights at termination; the increased liver weight at 100 ppm is considered to be a chance finding. The heart weight of the surviving female at 600 ppm appeared to be increased (Table 16).

There were no notable effects on haematology parameters. There were no notable effects on clinical chemistry parameters, apart from slightly reduced albumin in some rabbits at 300 and 600 ppm.

Histopathology revealed treatment-related, focal malacia (necrosis) in the cerebrum of all rabbits at 600 ppm, as well as in one male and one female at 300 ppm. Notably, the same part of the cerebrum was vacuolated in all rabbits at 300 or 600 ppm. Most rabbits exposed at 300 or 600 ppm had moderate inflammation of the nasal tissues. Some of these rabbits also had acute inflammation of the trachea and one female had inflammation of the bronchi and bronchioles. A variable haematopoietic response was associated with the inflammation in the respiratory system

Table 16. Organ and body weights of rabbits exposed to sulfuryl fluoride by inhalation for 2 weeks

Concentration (ppm)	No.	Terminal body weight (g)	Brain		Heart		Liver	
			(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
Males								
0	3	3250	9.7	0.30	6.7	0.21	115	3.5
100	3	3212	9.3	0.29	7.0	0.22	118	3.7
300	3	3062	9.9	0.32	5.8	0.19	92	3.0
600	3	2918*	9.0*	0.31	5.9	0.20	84	2.9
Females								
0	3	3503	9.3	0.27	6.6	0.19	97	2.8
100	3	3594	9.9	0.28	6.7	0.19	137*	3.8*
300	3	3384	9.7	0.29	6.4	0.19	98	2.6
600	1	3316	10.3	0.31	8.5*	0.26*	89	2.7

From Eisenbrandt et al. (1985)

*Statistically identified difference from control mean by Dunnett's test, alpha = 0.05.

of some rabbits. Alterations included lymphoid hyperplasia in the mediastinal lymph nodes and spleen. There were no histopathological changes at 100 ppm.

The NOAEC for rabbits was 100 ppm (approximately equivalent to 4.1 mg/kg bw per day) on the basis of vacuolation in the cerebrum at 300 ppm (approximately equivalent to 13 mg/kg bw per day). A local effect of inflammation of the respiratory tract was also present at 300 ppm (Eisenbrandt et al., 1985).

Groups of seven male and seven female New Zealand White rabbits were given sulfuryl fluoride (purity, 99.8%; Vikane gas fumigant, Lot No. TWP 830919-408) at a concentration of 0, 30, 100, 600/300 ppm by whole-body inhalation during 6 h/day for 5 days per week for 13 weeks. Intakes were 0, 1.4, 4.1 and 13 mg/kg bw per day. The study complied with GLP and was conducted according to US EPA guideline 82-4.

Animals were observed daily and body weights were recorded several times throughout the study. Selected organs (brain, heart, liver, kidneys, and testes) were weighed and blood samples taken at the terminal necropsy. A complete necropsy was performed on all animals and extensive histopathology was completed on controls and groups at the highest dose. In the groups receiving the lowest dose or the intermediate dose, histopathological examinations were limited to gross lesions, brain, kidney, liver and respiratory tract. Special staining was peformed on brain sections. Measurement of food and water consumption was not undertaken. Ophthalmic observations were not undertaken, although eyes were examined at necropsy and histopathology. Urine analysis was not performed.

Chamber concentrations were confirmed analytically as being acceptable. The highest concentration was reduced to 300 ppm after nine exposures as one female rabbit exposed at 600 ppm died after the eighth exposure and convulsions were seen in one male and one female after nine exposures. After reduction of the highest concentration, no further clinical signs were noted.

Body-weight gain was reduced by > 10% in groups exposed at 100 ppm and above. Leukocyte count was increased at the highest concentration, significantly in males and by about 20% in females, with no clear changes in differential counts. There were no significant effects on haematology at 100 or 30 ppm (Table 17).

A number of clinical chemistry changes were noted, but the only finding exhibiting a dose–response relationship was an increase in blood urea nitrogen in females from the group at the highest dose Serum fluoride concentrations were increased in both sexes at all concentrations (Table 17).

There were no gross pathological findings in animals surviving to the end of the study. The animal that died had a fractured vertebra.

Absolute and relative liver weights were reduced at 600/300 ppm and 100 ppm in both sexes, achieving statistical significance in females at 600/300 ppm.

Histopathology revealed treatment-related vacuolation, gliosis and malacia of rabbits at 600/300 ppm. One female exposed at 100 ppm had moderate cerebral vacuolation; although this was more severe than in any of the animals at 300 ppm, it cannot be discounted, given the absence of this finding in animals in the control group or at 30 ppm. Irritation of the nasal tissues was present in most rabbits at 600/300 ppm and in one male at 100 ppm

The NOAEC was 30 ppm (approximately equivalent to 1.4 mg/kg bw per day) on the basis of vacuolation in the cerebrum at 100 ppm (approximately equivalent to 4.1 mg/kg bw per day) (Nitschke et al., 1987b).

Dogs

Groups of one male and one female beagle dogs were exposed to sulfuyl fluoride (purity, 99.6%; lot No. 880329 752 MAR/88) at a concentration of 0, 30, 100 or 300 ppm (0, 0.13, 0.42 or 1.25 mg/l, respectively) by whole-body inhalation for 6 h/day for 5 days per week for 2 weeks

Table 17. Clinical chemistry and histopathological findings in rabbits exposed to sulfuryl fluoride by inhalation for 13 weeks

Observations			Concentration (ppm)							
			Males				Females			
			0	30	100	300	0	30	100	300
Blood urea nitrogen (mg/dl)			20	19	18	19	20	21	21	24*
Fluoride (µg/ml)			0.07	0.17*	0.4*	0.6*	0.6	0.7*	0.8*	1.0*
Leukocytes (10^3/mm^3)			7.6	7.8	7.5	9.9*	7.2	7.6	7.9	8.6
Organ/finding/severity:										
No. of rabbits examined			7	7	7	7	7	7	7	7
Brain	Cerebrum, focal gliosis	Slight	0	0	0	0	0	0	0	2
	Cerebrum, focal malacia	Severe	0	0	0	3	0	0	0	1
	Cerebrum, focal vacuolation	Very slight	0	0	0	3	0	0	0	3
		Slight	0	0	0	0	0	0	0	2
		Moderate	0	0	0	0	0	0	1	0

From Nitschke, Zimmer & Eisenbrandt, (1987)

*$p < 0.05$

(nine exposures). Intakes were 0, 0.9, 2.9 and 5.8 mg/kg bw per day. The study complied with GLP but not with any guidelines (US EPA 82-4, supplemental).

Whole-body exposures were conducted under dynamic airflow conditions. Animals were observed daily and weighed regularly. Animals were necropsied on the day after the last exposure.Serum samples were obtained from each animal at least twice before the initial exposure to sulfuryl fluoride and again at necropsy for haematology and clinical chemistry determinations. Urine analysis was conducted on samples from the urinary bladders at necropsy. An ophthalmology examination was conducted before the study and after the last exposure. Major organs were weighed and a limited number of tissues examined histopathologically. Food and water consumption was not assessed.

No deaths occurred. At 300 ppm, intermittent episodes of tremors and tetany were observed in both dogs beginning with the fifth exposure. On day 9, during the seventh exposure, the tremors and tetany were sufficiently severe that the exposure was terminated after approximately 5.5 h. Within 30 min after terminating the exposure, both dogs appeared to be normal. Similar clinical effects were noted during subsequent exposures and were rapidly reversible even during the exposure period. There were no exposure-related effects noted in dogs at 30 or 100 ppm.

Owing to the body-weight range in the dogs used, each dog was used as its own control, with body weights obtained several times before the first exposure. The body weight on day 11 of the female dog at 300 ppm was decreased by approximately 500 g from values before exposure. Body weights for the male dog at 300 ppm, or males or females at 30 or 100 ppm were comparable to values before exposure.

No treatment-related effects after ophthalmic examination were noted.

Haematology values for all dogs were comparable to those before exposure.

Urine analysis revealed values for male and female dogs at 300 ppm that were comparable to control values. The specific gravity for the male at 100 ppm was lower than that for the control or the dog at 300 ppm. This was not considered to be exposure-related, since there was no apparent dose–response relationship.

Serum fluoride concentrations for dogs at 100 or 300 ppm were two- to fourfold higher than control values. Serum fluoride concentrations for dogs at 30 ppm and all other clinical chemistry values were comparable with those for controls and/or values before exposure.

No treatment-related effects were observed after gross pathology examination (including teeth). There were no treatment-related effects on organ weights.

Minimal microscopic inflammatory changes were observed in the nasal turbinates of the male and female dog and trachea of the female dog at 300 ppm. Although numerous microscopic sections were examined from the cerebral cortex, brain stem, cerebellum and medulla oblongata, there were no changes detected in dogs at 300 ppm. There were no exposure-related effects noted in dogs at 30 or 100 ppm.

The NOAEC (within the limits of the study design) was 100 ppm (approximately equivalent to 2.9 mg/kg bw per day) on the basis of tremors and tetany beginning with the fifth exposure at 300 ppm (approximately equivalent to 8.7 mg/kg bw per day). Histopathological examinations revealed no changes in the central nervous system. At 300 ppm, the upper respiratory tract showed histopathological findings typical of inflammatory changes (Nitschke & Quast, 1991).

Groups of four male and four female beagle dogs were exposed to sulfuryl fluoride (purity, minimum 96.3%; Lots WP880329 752 and WP901011 907) by whole-body inhalation at a concentration of 0, 30, 100 or 200 ppm for 6 h/day, 5 days per week for 13 weeks. Intakes were 0, 0.9, 2.9 and 5.8 mg/kg bw per day. The study complied with GLP and conformed to the following guidelines: California SB-950, US EPA 82-1 and 82-4R, OECD 409 and 413, EEC Method No. L 133/3 pages 12 and 20.

Parameters measured included daily clinical observations, weekly measurement of body weights, haematology and clinical chemistry on three occasions (before exposure, about week 6, and in week 12), ophthalmology before the start of the study and within 1 week of scheduled necropsy, urine analysis at necropsy; gross pathology of all animals; organ weights (brain, heart, lung, liver, kidneys, thyroid with parathyroid, pituitary, adrenals, ovaries and testes); microscopic pathology of a wide range of tissues on all animals; additional immunohistochemical staining of brain sections for glial fibrillary acidic protein (GFAP). Food and water consumption was not assessed.

No deaths occurred.One male exhibited lateral recumbency, tetany, tremors, salivation and incoordination 75 min after exposure to sulfuryl fluoride at 200 ppm on day 19. One hour later, during the weekly clinical examination, the activity of this animal was decreased relative to controls, but was otherwise normal.

By the end of the study, the mean body weight values of male and female dogs at 200 ppm fluoride were 88% and 96%, respectively, of the control values. When body-weight values for male and female dogs within a group were combined (to increase statistical power, thought to be reasonable since there were no statistical time–sex–dose interactions) the differences between controls and dogs at 200 ppm were statistically significant. No exposure-related changes in organ weights were noted.

No ophthalmic effects were reported. There were no significant changes in haematology parameters. Urine analysis showed similar results in test and control groups.

AST activity and albumin concentrations in male and female dogs at 200 ppm were slightly but significantly lower than control values. Given the general variation in these parameters between groups and over time, the toxicological significance of these findings is equivocal.

There were no visible exposure-related findings related to gross pathology.

In the midbrain region of one male and one female dog at 200 ppm there was a single, small bilaterally symmetrical focal microscopic change noted in the putamen. This focal change was characterized microscopically by vacuolation, gliosis (microglial gitter cells), perivascular cuffing, hypertrophy of endothelial cells and individual cells showed nuclear pyknosis and

karyorrhexis. It was noted that the size of the lesion was extremely small and barely recognizable microscopically. The focal reaction was slightly more prominent in the affected male dog than in the female. Examination of these brain sections with GFAP immunohistochemistry did not reveal any reaction. This was consistent with the absence of any gliosis noted in sections stained with haematoxylin and eosin, with the exception of the microglial gitter cell reaction.

Given the results of the 1 year study (below) fluorosis would have been expected in this study.

The NOAEC was 100 ppm (approximately equivalent to 2.9 mg/kg bw per day) on the basis of slightly reduced body weights in males and females and histopathological changes in the brain at 200 ppm (approximately equivalent to 5.8 mg/kg bw per day). The brain findings comprised very slight, small, bilateral, focal vacuolation and gliosis in the putamen region of two of eight dogs at 200 ppm (Nietsche et al., 1992).

Groups of four male and four female beagle dogs were exposed by whole-body inhalation to sulfuryl fluoride (purity, 95.1–98.8%; lot Nos WP 910826-929, WP 920131- 940 and WP 920619-953) at a concentration of 0, 20, 80 or 200 ppm for 6 h/day, 5 days per week for 1 year. Owing to excessive morbidity/mortality by approximately 9 months, the group at 200 ppm was removed from the study and necropsied. Intakes were 0, 0.6, 2.3 and 5.8 mg/kg bw per day. The study complied with GLP and was conducted in accordance with the following guidelines: US EPA 83-1, OECD 452, 87/302/EEC: chronic toxicity test.

Animals were observed daily and weighed before the initial exposure, at weekly intervals for the first 13 weeks and monthly thereafter until approximately 9 months. After 9 months the body weights were taken biweekly to monitor the progress of the chronic toxicity. Whole blood and serum samples were obtained from each animal twice before initiation of the study, at 3, 6 and 9 months, and once during the last 2 weeks of exposure for haematology and clinical chemistry. Urine analysis was performed at 6 months and at 12 months. An ophthalmological examination took place before the start of the study and again shortly before the scheduled necropsy at 1 year. One half of the lower jaw with teeth and one femur from each dog were collected and frozen at necropsy for possible fluoride analysis however, fluoride analyses were not conducted on these samples. Animals were necropsied on the day after the last exposure, when 51 different tissues were collected in formalin or Bouin's fixative. Major organs (brain, heart, lung, liver, kidneys, thyroid with parathyroid, pituitary, adrenals, ovaries, and testes) were weighed from all animals except those assigned to the group at 200 ppm, which was terminated early owing to excessive pulmonary toxicity (not weighed owing to lack of concurrent controls). A complete set of tissues collected at necropsy from all animals was examined by light microscopy. Food and water consumption was not assessed.

Owing to excessive morbidity and mortality (two males and three females), the group at 200 ppm was taken off test at 9 months (day 282) and necropsied. A male dog at 200 ppm died on day 267, and another became moribund and was necropsied on day 271. Three female dogs from the group at 200 ppm were terminated in a moribund condition on days 278 and 281. There were no other deaths.

At approximately 9 months into the study, several male and female dogs at 200 ppm exhibited clinical signs of toxicity. The observations included, but were not limited to, laboured breathing, shallow rapid respiration, and pale or blue mucous membranes. One of the two remaining males from the group at 200 ppm exhibited minor respiratory changes before removal from the study on day 282. This dog had lost 782 g of body weight during the previous month. The heaviest male dog in the group at 200 ppm was clinically normal, but was also necropsied on day 282. Although the remaining female in this group did not show clinical signs of altered respiration, she had lost 647 g of body weight during the previous month.

The body temperature of some dogs was occasionally elevated; however, this was not consistently observed in the same dog on repeated examinations, nor within the group. Upon auscultation of the thoracic cavity, the heart rate and sounds were normal. In addition, the

intensity of the femoral pulse appeared to be normal. Both of these findings indicated that the altered respiratory function was not caused by cardiac arrhythmia. There were no indications of neurotoxicity.

Although microbiological cultures were taken from the lungs of several dogs, only one showed a *Haemophilus* bacterial infection. This bacterium was likely to be an opportunist infecting this debilitated dog and not a primary infectious agent.

No effects on ophthalmogy were reported.

There were no exposure-related effects on haematology parameters in dogs at 20 or 80 ppm throughout the study, or in dogs at 200 ppm during the first 6 months. A number of changes were noted subsequently, specifically a decrease in erythroid parameters and an increase in leukocytes with a neutrophilia, in dog of each sex at 200 ppm that became ill.

There were no biologically significant findings from urine analysis.

There was a statistically significant decrease in AP activity in the males at 20 ppm; however, the difference may have been caused by a control dog having an elevated value in both samples before study. There were a number of minor changes in clinical chemistry observed in some of the dogs at 200 ppm that were ill and were removed from study; these were interpreted as secondary to the generally poor overall condition of the dogs rather than indicating any primary target organ effect.

There were no significant exposure-related effects on gross pathology observed in tissues other than the lungs of animals at 200 ppm. An occasional dog in the control group and at 80 ppm had pale foci in their lungs tha are routinely seen in long-term studies. A pale focus in the spleen was also observed in a dog in the control group and a dog at 80 ppm. In the group of dogs at 80 or 200 ppm there were no observations that were considered to be attributable to exposure to sulfuryl fluoride.

There was a statistically significant decrease in the relative heart weights of dogs at 20 ppm. However, the terminal mean body weights of the dogs at 20 ppm were also greater than those of animals in the the control group and at 80 ppm. Most of the relative organ weights of the male and female dogs at 20 ppm were decreased as a reflection of their heavier mean body weights. Given a lack of dose–response relationship and histopathological correlates, the minor decreased relative heart weight in these animals was considered not to be of toxicological significance.

Exposure to sulfuryl fluoride during the long-term study resulted in exposure-related histopathological effects in the lungs, brain, thyroid gland and canine teeth (Table 18). The histopathological changes in the lungs at 200 ppm corresponded with the severity of laboured respiration in-life and were consistent with the consolidation noted at necropsy. The pulmonary changes appeared to involve primarily the peripheral portions of the lung, without recognizable alterations in the major airways. Associated with a chronic inflammatory reaction was a focal thickening of the pleura and thickening of the interalveolar septae. Special stains to demonstrate fibrin within these areas were not definitive; however, in the chronically inflamed areas the increased thickening of the interalveolar septae and the pleura were caused by collagen deposition. Although the peripheral portions of the lungs of dogs at 200 ppm were adversely affected, their nasal turbinates, larynx, trachea and major portions of the bronchial tree were not.

Microscopic changes in the brain were present in the head of the caudate nucleus of two out of four males and three out of four females in the group at 200 ppm. The microscopic appearance consisted of a focus of malacia (liquefaction necrosis) in which vessels and some neuropil persisted within the lesion. Inflammatory cells were an insignificant feature; however, there were some gitter cells (macrophages) persisting within the malacic foci. Whether the scarcity of cells was attributable to cells lost at the time of tissue processing, or to migration from the site before necropsy was undetermined. The microscopic features of the lesions indicated that they were not of recent vintage. The malacic focus was linear, dorsoventral in orientation and located midway between the lateral ventricles and the internal capsule. There were vessel(s) and their supporting interstitium present within the center of the malacic foci. The cells and neuropil immediately

Table 18. Main histopathology findings in a 1-year study with sulfuryl fluoride in dogs

Site	Finding	Severity	Concentration (ppm)							
			Males				Females			
			0	20	80	200	0	20	80	200
No. of dogs examined			4	4	4	4	4	4	4	4
Bone marrow	Hyperplasia, myeloid:	Slight	0	0	0	0	0	0	0	1
		Moderate	0	0	0	1	0	0	0	0
Brain cerebellum	Within normal limits		4	4	4	2	4	3	4	4
	Inflammation, subacute, ependyma, focal:	Very slight	0	0	0	1	0	0	0	0
Brain cerebrum	Within normal limits		4	4	4	2	4	3	4	1
	Malacia, caudate nucleus, bilateral, focal:	Very slight/slight	0	0	0	2	0	0	0	2
		Moderate	0	0	0	0	0	0	0	1
Liver	Atrophy secondary to inanition, hepatocellular:		0	0	0	2	0	0	0	3
	Pigment—haemosiderin, Kupffer cells, multifocal:	Very slight/slight	0	0	0	0	3	1	4	2
Lungs	Inflammation—chronic active, alveoli, multifocal:	Very slight	0	0	0	2	0	0	2	1
		≥ Moderate	0	0	0	2	0	0	0	3
	Inflammation—subacute to chronic, interstitium, multifocal:	Very slight	4	4	4	0	4	4	2	0
	Aggregates of alveolar macrophages, multifocal:	Very slight	0	0	3	0	0	0	1	0
Lymph node—mesenteric	Atrophy		0	0	0	2	0	0	0	3
Teeth	Fluorosis—dental, canine tooth:	Very slight	0	0	2	0	0	0	1	0
		Slight	0	0	1	4	0	0	0	4
Thyroid gland	Hypertrophy, epithelial cells	Very slight	0	0	0	4	0	0	0	3

From Quast et al. (1993a)

adjacent to the malacic foci were normal in appearance. The character of the microscopic change was considered by the examining pathologist to be indicative of ischaemic tissue damage rather than cytotoxicity to neuronal elements. There were no other recognizable changes in the numerous sections of brain stained with haematoxylin and eosin, Luxol fast blue Cresyl violet or Sevier-Munger. The microscopic effects in the brain were not seen in dogs at 80 or 20 ppm.

The effects in the thyroid gland were limited to the group at 200 ppm in which all male and three female dogs exhibited very slight hypertrophy of the follicular epithelium.

A minor change was observed microscopically in the canine tooth that was evaluated for possible changes suggestive of slight dental fluorosis. The change consisted of concentric rings that stained slightly darker and corresponded with each day of exposure. The teeth of these dogs were found to be unaffected during in-life examination and at gross necropsy. As the teeth reached their maturity it was more difficult to recognize the presence of the rings. Other than the presence of these growth rings in the dentin, there were no other recognizable changes in the canine tooth. All of the dogs at 200 ppm and several at 80 ppm were affected. There were no comparable effects recognized in the canine tooth of dogs at 20 ppm or the controls.

Some other tissues (liver and lymphoid tissue atrophy; bone marrow myeloid hyperplasia) had microscopic changes that were observed more frequently in the group at 200 ppm than in the other groups; however, they were considered to be secondary to the general poor condition of the animals.

In this 1-year study in dogs, the highest exposure level (300 ppm) exceeded the maximum level tolerable for this length of time, and resulted in premature termination of this group (after about 9 months) owing to local effects on the respiratory system. The NOAEC was 80 ppm (approximately equivalent to 2.3 mg/kg bw per day) on the basis of the range of effects including deaths and brain vacuolation seen at 200 ppm (approximately equivalent to 5.8 mg/kg bw per day).

The respiratory tract findings at 80 ppm and above are not relevant to a dietary intake risk assessment. The finding of slight dental fluorosis at microscopic examination is not considered to be a toxicologically adverse effect (Quast et al., 1993a).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female CD-1 mice were exposed by whole-body inhalation to sulfuryl fluoride (purity, 93.6–99.7%; lot Nos WP 880329-752, WP 901011-907, WP 910321-918 and WP 910826-929) at a concentration of , 5, 20 or 80 ppm for 6 h/day, 5 days per week for up to 18 months. Ten females and 10 males from each group were sacrificed after 12 months. Intakes were 0, 0.7, 3.0, 12 mg/kg bw per day. The study complied with GLP and conformed to OECD guideline 451.

Animals were observed daily for assessment of exposure-related effects. Individual body weights were determined weekly for the first 13 weeks and monthly thereafter. Blood samples were obtained for haematological and clinical chemistry determinations from 10 animals of each sex per dose when they were necropsied at 12 months, and from 20 animals of each sex per dose at terminal necropsy after 18 months. Palpation for masses was conducted during the weekly clinical examinations. At each scheduled necropsy, a wide range of tissues was collected and major organs (brain, heart, liver, kidneys, lungs at 18 months only, and testes) weighed. In general, all tissues including gross lesions from mice in the control group and at 80 ppm were examined microscopically. Target tissues and grossly visible lesions were also examined from mice in the groups exposed at lower doses. No special stains were used for the nervous system or brain as these had not previously proved useful. Food and water consumption was not assessed, urine analysis and ophthalmology were not conducted.

A few male mice and a single female mouse scheduled for the necropsy at 12 months were removed from the study because of morbidity or death. The cause of these premature deaths was considered to be unrelated to treatment. In the main study groups, a reduced survival was observed in the group at 80 ppm (Table 19) and this dose was considered to be above the maximum tolerated dose. Overall survival was considered adequate for assessing carcinogenic potential.

There were no clear treatment-related clinical signs of toxicity. By necropsy at 12 and 18 months, the mean body weight of 80 ppm male mice was $< 90\%$ of controls. The mean body weights of the females at 80 ppm were about 85% that of of controls by study termination.

In males at 80 ppm, there was a statistically significant increase in platelet count at 12 months, but not at 18 months; there was also a slight but statistically significant increase in erythrocyte counts. In females at 80 ppm there was also a statistically significant increase in platelet count at 12 months, but not at 18 months. Evaluation of individual mouse differential counts and morphology of blood smears from each time period failed to detect any exposure-related effects. Bone marrow sections appeared normal.

In males at 80 ppm at 12 months, a marginally lower serum potassium concentration was noted; however, it was not evident at 18 months (Table 20). The serum glucose concentrations in the group at 80 ppm and serum albumin concentrations in the group at 5 ppm were also statistically identified as decreased in mice at 18 months. These identified differences in clinical chemistry parameters lacked a consistent dose–response pattern and are not considered to be an adverse effect of exposure to sulfuryl fluoride.

In females at 80 ppm at 12 months there was a statistically significant increase in serum urea nitrogen. Although statistically significant, this value was considered well within the normal physiological range and was not associated with an increase in creatinine that would suggest a renal effect. Furthermore, the alteration did not progress with longer duration of exposure.

At 12 months, there were no gross pathology observations indicative of a target organ effect at any concentration. At 18 months, in all exposed groups of male mice, but especially in the group at 80 ppm, the number of mice with a dilated kidney pelvis was considerably decreased. There were no other exposure-related findings in the kidney in either exposed or control male mice. There were also a number of increases in some macroscopic effects seen in the group at 80 ppm, associated with the moribund condition.

Table 19. Cumulative mortality in mice exposed to sulfuryl fluoride by inhalation for up to 18 months

Cumulative mortality (%) at test day	Concentration (ppm)							
	Males				Females			
	0	5	20	80	0	5	20	80
357	8	8	8	16	10	10	8	8
448	14	20	24	30	20	12	12	32
476	22	24	32	38	26	12	18	50
511	36	34	38	50	32	16	26	64
539	44	38	44	60	34	22	36	68

From Quast, Bradley & Nitschke (1993)

*Statistically different from control by Gehan-Wilcoxon, alpha = 0.05.

Table 20. Clinical chemistry findings in mice exposed to sulfuryl fluoride by inhalation for up to 18 months

Concentration (ppm)	Males				Females			
	Glucose (mg/dl)		Potassium (mmol/l)		Urea nitrogen (mg/dl)		Creatinine (mg/dl)	
	12 months	18 months	12 months	18 months	12 months	18 months	12 months	18 months
0	199	149	5.8	6.0	21	27	0.4	0.5
5	188	129	5.2	6.0	22	32	0.4	0.4
20	164	159	5.5	5.9	20	34	0.4	0.5
80	169	112*	4.9*	6.0	27*	37	0.4	0.5

From Quast et al. (1993b)

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

There were a number of organ-weight changes in the group at 80 ppm that were reflective of the decreased body weights. There was no evidence of any target organ toxicity.

The histopathology findings are summarized in Table 21. At 12 months, in mice at 80 ppm the brain and the thyroid gland were clear target organs. An increased incidence of amyloid in all groups of males did not exhibit a dose-related response and is not considered to be treatment-related. At 18 months, the brain and thyroid gland were again identified as target organs. There were also many statistically significant non-neoplastic changes seen at 80 ppm in a number of other tissues/organs (Table 21). The incidence of a number of age-related lesions was reduced at 80 ppm, possibly associated with the lower survival. At 20 ppm there were statistically significant increases in amyloid deposition in the ileum and jejunum of females, reduced mineralization of brain regions in females and a reduction in dilation of the renal pelvis in males. The reductions in mineralization and dilatation of the renal pelvis are not considered to be adverse findings. The amyloid deposition does not demonstrate a clear dose–response relationship in terms of incidence and severity.

Microscopic vacuolation in the external capsule of the brain of mice from the 18 month study was only observed in mice at 80 ppm. The extent of involvement of the brain was less at 18 months than at 12 months, and less than in mice at 100 ppm in the 13 week study. In addition, a recognizable change was not observed in the caudate putamen or amygdaloid regions of mice exposed for 18 months compared with earlier studies. In the brain sections from the region of the thalamus and hypothalamus, there was a significantly decreased incidence of focal mineralization in the group of males and females at 80 ppm and in females at 20 ppm.

Microscopic changes observed in the thyroid were consistent with the results of the 13-week study, and primarily comprised hypertrophy of follicular epithelial cells. The colloid within the follicles was decreased in amount and stained less intensely eosinophilic. There were no degenerative or inflammatory changes present in the affected thyroids and no indication of increases in tumour rates.

Only in the ileum and jejunum were there statistically significant differences in the incidence of intestinal amyloidosis in females at 20 and 80 ppm. The study authors noted that amyloid deposits are only observed in the ileum, jejunum, duodenum, mesenteric lymph nodes of and ovaries of mice aged 0–8 months. With increasing age, the number of other tissues affected also increased. The test facility conducted only a limited number ($n = 2$) of contemporary studies in CD-1 mice and only one of these used a similar grading scheme for amyloidosis in the intestines, and this shows a very different (less extensive) pattern of amyloid deposition to the controls in the study with sulfuryl fluoride. Taking into account the variable background rate, absence of deposition at other sites and lack of clear dose–response (for severity or incidence) between 20 and 80 ppm, it is considered that the statistically significant findings at 20 ppm in females are not a treatment related adverse effect.

Renal amyloidosis was detected in the glomerulus of all male mice. More of the males at 80 ppm had very slight or slight amounts of amyloid deposited in their glomerulus, in contrast to the females at 80 ppm, which had severe to very severe amounts. The total number of male mice with the two most severe categories of glomerular amyloidosis combined was decreased, although not significantly so, in contrast to the increase in females. The incidence of interstitial amyloid deposits and renal amyloidosis and mineralization was decreased in males at 80 ppm and may have been the result of earlier mortality of some mice in this group during the first year, before the onset of significant age-related changes.

A reduced incidence of peripheral nerve degeneration was noted in mice treated with sulfuryl fluoride.

No increases in tumour incidences were seen in groups of mice treated with sulfuryl fluoride. Additional procedures (Peto, 1974; McConnell et al., 1986) were performed to take account of the reduced survival at the highest concentration. These did not identify any increases in tumour incidences.

Table 21. Histopathology findings in mice exposed to sulfuryl fluoride by inhalation for up to 18 months

Site	Finding	Severity	Concentration (ppm)							
			Males				Females			
			0	5	20	80	0	5	20	80
12 months										
No. of mice examined			10	10	10	10	10	10	10	10
Brain, cerebrum	Vacuolation, caudate putamen, bilateral, focal:	Very slight	0	0	0	1	0	0	0	0
	Vacuolation, external capsule, bilateral, focal:	Very slight	0	0	0	10	0	0	0	9
Brain, thalamus/hypothalamus	Mineralization, unilateral, focal		3	3	3	1	2	3	2	0
	Mineralization, bilateral, focal		3	3	1	0	9	2	0	0
	Mineralization, any symmetry, focal (combined)		6	6	4	1	2	5	2	0
	Mineralization, ependymal canal, focal		1	0	0	0	0	0	0	0
Thyroid gland	Amyloid, interstitium	Very slight	1	2	2	2	4	3	3	3
		Slight	0	2	4	2	0	1	4	1
		Moderate	0	1	0	0	0	0	0	0
		Combined	1	5	6	4	4	4	7	4
	Hypertrophy, epithelial cells:	Very slight	0	0	0	7*	0	0	0	4*
18 months										
No. of mice examined			50	50	50	50	50	50	50	50
Brain cerebellum	Within normal limits		49	50	50	50	50	50	49	50
Brain cerebrum	Within normal limits		48	50	49	37	49	50	47	38
	Vacuolation, external capsule, bilateral, focal:	Very slight	0	0	0	13*	0	0	0	12*
Brain, thalamus/hypothalamus	Within normal limits		11	16	16	39	19	27	40	46
	Mineralization, focal (combined)		39	34	33	11*[a]	30	23	10*	2*[a]
	Mineralization, meninges, focal		1	0	0	0	0	0	0	2
Heart	Within normal limits		9	13	14	20	18	19	18	11
	Amyloid:	Very slight	10	5	8	10	6	4	4	15*[a]
		≥ Slight	15	21	19	7	21	18	21	15
	Thrombus—acute or recent, atrium:		0	1	3	1	0	0	2	9*[a]
	Thrombus—total		2	3	5	2	4	1	5	14*[a]
Ileum	Within normal limits		21	22	20	29	25	25	14	16
	Amyloid	< Slight	0	2	2	2	2	2	2	2
		Moderate	16	10	14	13	14	16	24	23
		≥ Severe	13	16	14	6	9	7	10	9

Site	Finding	Severity	Concentration (ppm)							
			Males				Females			
			0	5	20	80	0	5	20	80
		Combined	29	28	30	21	25	25	36*	34[a]
Jejunum	Within normal limits		23	23	24	30	27	26	17	17
	Amyloid:	Very slight	3	3	5	5	1	2	7*	7*[a]
		Slight	15	13	13	9	7	11	8	15
		≥ Moderate	9	11	8	5	15	11	18	11
		Combined	27	27	26	19	23	24	33*	33*[a]
Kidneys	Within normal limits		1	1	2	4	1	1	1	0
	Anyloid, glomerulus, bilateral, multifocal:	Very slight	15	15	12	14	17	14	10	6*[a]
		Slight	4	3	5	12*[a]	8	7	6	5
		Moderate	5	1	4	4	3	1	3	6
		Severe	4	5	8	5	6	5	8	8
		Very severe	18	20	16	9	14	18	20	24*
	Amyloid, interstitium:	Total	11	7	6	2*[a]	16	18	23	12
	Dilatation, pelvis :	Total	20	17	9*	7*[a]	2	0	0	2
	Infarct, cortex, any symmetry, focal or multifocal: any severity:	Total	1	0	1	0	7	5	6	5
Liver	Atrophy secondary to inanition, hepatocellular:		18	11	18	27	15	11	19	27*[a]
	Pigment-laden macrophages:	Total	3	4	2	2	10	13	4	38[a]
Lymph node—mediastinal	Atrophy:		0	0	1	4[a]	1	1	0	1
Peripheral nerve	Within normal limits		15	20	25	26	11	23	18	18
	Degeneration—individual nerve fibre(s):	Total	35	30	25*	24*[a]	39	27	32	32
Thymus	Atrophy		1	0	1	6*[a]	7	4	8	12
Thyroid gland	Amyloid, interstitium:	Very slight	5	3	1	4	3	7	8	4
		Slight	10	8	11	8	6	8	13	18*[a]
		≥ Moderate	13	16	14	8	14	9	10	9
	Hypertrophy, epithelial cells:	Very slight	1	0	1	20*	1	1	1	6

From Quast et al. (1993b)

*Statistically identified difference from control mean by Yate's chi-squared pairwise test, alpha = 0.05.

[a] $p < 0.05$ for trend]

The overall NOAEC was 20 ppm (approximately equivalent to 3 mg/kg bw per day) on the basis of a range of findings, including reduced survival at 80 ppm (approximately equivalent to 12 mg/kg bw per day). There was no increase in tumour incidences in the treated groups. The NOAEC for carcinogenicity was 80 ppm (approximately equivalent to 3 mg/kg bw per day), the highest concentration tested (Quast et al., 1993b).

Rats

In a long-term study of toxicity (neurotoxicity) and oncogenicity, groups of 50 male and 50 female Fischer 344 rats were exposed by whole-body inhalation to sulfuryl fluoride (purity, 93.6–99.7%; lot Nos WP 880329-752, WP 901011- 907, WP 910321-918, WP 910826-929 and WP 920131-940) at a concentration of 0, 5, 20 or 80 ppm for 6 h/day, 5 days per week for up to 2 years. Intakes were 0, 0.4, 1.4, 5.6 mg/kg bw per day. An additional satellite group of 15 males and 15 females per dose was maintained for 12 months. The study complied with GLP and OECD guideline 453.

Animals were observed daily for clinical signs and were palpated for masses during the weekly clinical examinations. Individual body weights were determined weekly for the first 13 weeks and monthly thereafter. Blood samples were obtained for haematological and clinical chemistry determinations from 10 animals of each sex per dose at approximately 6, 12, 19 and 21 months, and from 20 animals of each sex per dose at the 24-month necropsy. Urine analyses were evaluated from the same animals at approximately the same time intervals. At scheduled necropsies a full range of tissues was collected and major organs weighed. All tissues from rats in the control group and at 80 ppm were examined histopathologically at the 12-month necropsy, and target tissues and grossly visible lesions were also examined from intermediate exposure levels. Because all of the rats at 80 ppm in the 2-year study were removed in a moribund condition or dead before study termination, a complete set of tissues from all groups was prepared and examined microscopically. Food and water consumption was not assessed.

Mortality was increased in the rats at 80 ppm, and survival in rats at 20 ppm was similar to or better than that of controls (Table 22). There were no clear treatment-relatedclinical signs of toxicity, including those associated with the nervous system. Reduced body-weight gain was evident at 80 ppm for much of the study and in males at 20 ppm in the last month of the study (Table 23); the latter finding is not considered to be adverse as the body weight of rats is very variable at this age and the magnitude of the change was small (< 10%). Ophthalmology showed the only notable changes (opacity) in rats at 80 ppm, these changes being considered secondary to kidney failure.

There was an apparent decrease in erythrocyte parameters (erythrocyte counts, haemoglobin and erythrocyte volume fraction) at 19 and 21 months in males at 80 ppm. However these were not considered to be exposure-related because of high values for control males at this time, when compared with values at 6, 12 and 24 months, and the absence of similar effects in females.

Samples for urine analysis were taken at 6 months and approximately 2 weeks later. The results for the second set of samples did not confirm an increase in specific gravity and protein content seen at 6 months. This finding is not considered indicative of kidney disease. In contrast to the first year, the urine specific gravity findings in male and female rats at 19 and 21 months clearly showed biological effects that were statistically significant. The decreased specific gravity in males and females at each time-point for the group at 80 ppm was interpreted to being caused by a loss of functional nephrons, associated with progressive chronic renal disease. A slight increase in urinary blood may have been secondary to renal histopathological changes. There were thus a number of treatment-related effects at 80 ppm that were consistent with kidney disease (Table 24).

Decreased creatinine kinase is not considered to be an adverse effect in isolation and the increase in AST activity was sporadic and not linked to any histopathological findings.

Table 22. Mortality in rats exposed to sulfuryl fluoride by inhalation for up to 2 years

Cumulative mortality (%) at study day	Concentration (ppm)							
	Males				Females			
	0	5	20	80	0	5	20	80
357	2	2	0	2	2	0	0	0
560	10	6	6	22	12	8	4	12
588	10	14	6	46	16	8	6	26
644	18	18	14	86	28	12	12	82
651	20	20	14	90	30	14	12	90
707	36	34	28	100	46	20	22	100
735	42	34	44	100	50	26	24	100

From Quast, Bradley & Nitschke (1993)

Table 23. Cumulative body-weight gain (per cent of control) in rats exposed to sulfuryl fluoride by inhalation for up to 2 years

Study day	Concentration (ppm)					
	5	20	80	5	20	80
	Males			Females		
369	100	102	98	98	98	96
453	101	101	95	96	97	93
537	99	100	86	100	100	84
594	99	100	79	96	98	70
706	99	97	—	105	101	—
734	96	93	—	105	101	—

From Quast, Bradley & Nitschke (1993)

The results of gross pathology examination are summarized in Table 25. At 12 months, an exposure-related effect was observed in the lungs of both sexes at 80 ppm. The pulmonary changes were characterized by multiple, small pale-coloured foci scattered sub-pleurally and located primarily in the diaphragmatic lobes. Also at 12 months, after formalin fixation of tissues the labial surface of the incisor teeth of all rats at 80 ppm was found to be discoloured with a repetitive pale and slightly darker-coloured horizontal line. None of the molar teeth was similarly affected after formalin fixation. The observations on the incisor teeth were only observed in the fixed tissue. At 24 months, findings for the lungs and incisor teeth were consistent with those at 12 months, while the most significant gross observations were related to advanced chronic renal disease at 80 ppm (Table 26). Owing to renal failure there were many other observations in numerous tissues, which represent the spectrum of secondary uraemic changes. For some tissues, the incidence of observations was decreased which was, in part, a reflection of the early onset of morbidity and death.

At 12 months, the relative kidney and liver weights of males at 80 ppm were significantly higher than tose of the controls. This correlated with slight histopathological changes in the kidneys, but not in the liver of these rats. However, a slight increase in relative organ weights was probably linked to slightly decreased, fasted body weight in males at 80 ppm. At 24 months, there were no biologically significant changes in organ weights.

Table 24. Clinical chemistry findings in rats exposed to sulfuryl fluoride by inhalation for up to 2 years

Parameter		Concentration (ppm)							
		Males				Females			
		0	5	20	80	0	5	20	80
Urea nitrogen (mg/dl)	6 months	17	16	18	18	21	20	24*	20
	12 months	13	14	14	17*	15	14	14	17*
	21 months	16	18	20	78#	20	20	23	111#
	24 months	22	23	26	NAR	22	20	21	NAR
AST (mU/ml)	6 months	112	118	136	113	78	75	76	83
	12 months	109	153*	97	105	65	66	69	87#
	21 months	62	93	111	51#	75	58	70	67
	24 months	100	102	148	NAR	108	95	86	NAR
Creatine kinase (mU/ml)	6 months	121	67*	79	73*	158	114	122	109
	12 months	131	168	152	88	124	141	111	98
	19 months	143	91*	63*	47*	161	102#	77$	117#
	21 months	101	75	59*	53*	68	61	54	149
	24 months	204	187	130	NAR	198	133#	101#	NAR
Creatinine (mg/dl)	6 months	0.7	0.7	0.8*	0/8	0.7	0.8	0.7	0.7
	12 months	0.6	0.6	0.7	0.7*	0.6	0.6	0.6	0.6
	19 months	0.8	0.8	0.8	2.4#	0.9	0.8	0.8	1.9#
	21 months	0.7	0.7	0.8	2.8#	0.7	0.7	0.7	2.6#
	24 months	0.8	0.8	0.9	NAR	0.7	0.6*	0.6*	NAR
Chloride (mmol/l)	6 months	118	120	118	118	118	120	119	119
	12 months	107	107	107	105	108	107	107	107
	19 months	114	114	115	108	115	116	116	109#
	21 months	117	118	117	111*	109	109	110	102#
	24 months	113	114	114	NAR	109	110	110	NAR

From Quast, Bradley & Nitschke (1993)

AST, aspartate aminotransferase; NAR, No animals remaining, all dead; * Statistically different from control mean by Dunnett's test, alpha = 0.05; # Statistically different from control mean by Wilcoxon's test, alpha = 0.05.

The animals at 80 ppm that were removed from the study showed evidence of advanced chronic renal disease as a result of exposure to sulfuryl fluoride. They also showed many changes in other organs and measured parameters secondarily affected by renal failure. A spectrum of age-related external and internal neoplastic and non-neoplastic changes was also observed in rats that were removed from the study.

In the satellite group at 12 months, exposure-related effects were observed in the kidneys, lungs and incisor teeth of males and females at 80 ppm, and in the teeth of several males at 20 ppm (Table 25). Severe chronic progressive nephropathy was seen in males and females at 80 ppm. The lung changes were microscopically consistent with gross observations in the group at the highest dose. There were no exposure-related pulmonary changes observed in rats at 5 and 20 ppm. Microscopic changes in the lungs of rats at 80 ppm, diagnosed as aggregates of alveolar macrophages, multifocal, very slight or slight, were considered to differ from the simple alveolar

histiocytosis normally seen in controls. Fluorosis involving the upper incisor teeth of male rats was microscopically very slight to slight in degree, while in females essentially all changes were very slight. Occasional neoplasms were present, with the pattern typical for F344 rats aged 1 year. None of these tumours was considered to be associated with exposure to sulfuryl fluoride.

At 24 months, the kidneys, lungs, and incisor teeth were the clear primary target organs affected after 24 months of exposure to sulfuryl fluoride, as they were after 12 months. There were also numerous changes in other tissues/organs as a secondary consequence of these primary effects (e.g. fibrous osteodystrophy). Most (47 out of 50 males and 45 out of 50 females) of the group at 80 ppm were found to have grades of severe and very severe chronic progressive glomerulonephropathy. In contrast, the kidneys of only 5 out of 50 control males and 1 out of 50 control females were similarly affected. There was no indication that the renal findings resulted in an increase in renal tumours. A range of lesions considered secondary to renal degeneration were seen in a number of tissues. No significant renal changes were evident at 20 or 5 ppm.

The lungs of rats at 24 months showed microscopic changes that were comparable to those observed at 12 months. In general, aggregates of alveolar macrophages were classified as moderate in most of the rats at 80 ppm at study termination, in contrast to a slight degree at 12 months, showing progression with increased duration of exposure. The local exposure-related effects did not result in an increased incidence of pulmonary tumours. There were no effects on the respiratory system at 20 or 5 ppm.

Table 25. Gross pathology findings in rats exposed to sulfuryl fluoride by inhalation for up to 2 years

Finding		Concentration (ppm)							
		0	5	20	80	0	5	20	80
		Males				Females			
12 months									
No. of rats examined		9	10	10	10	10	10	10	10
Lungs	Focus—pale, bilateral, multifocal, very slight	0	0	0	10*	0	0	0	10*
24 months									
No. of rats examined		50	50	50	50	50	50	50	50
Kidneys	Within normal limits	8	17	8	3	42	49	47	3*
	Dark, bilateral:	10	4	7	0*	2	0	1	0
	Pale, bilateral:	0	2	3	18*	1	0	0	10*
	Roughened surface, bilateral	33	28	31	45	4	0	1	40*
Lungs	Within normal limits	39	38	30	1*	43	44	45	0
	Firm	0	0	0	2	0	0	0	0
	Focus—pale, multifocal	0	1	8*	46*	0	0	1	46*
Oral tissues	Within normal limits	49	49	48	36	49	48	49	42
	Malocclusion, tooth	0	0	1	1	0	0	0	0
	Mottled, tooth	0	0	0	12*	0	0	0	8*
	Overgrown incisors, lower incisors	1	1	0	2	0	0	0	0

From Quast, Bradley & Nitschke (1993)

* $p < 0.05$

Table 26. Histopathology findings in rats exposed to sulfuryl fluoride for up to 2 years

Site	Finding	Severity	Concentration (ppm)							
			Males				Females			
			0	5	20	80	0	5	20	80
12 months										
No. of rats examined			10	10	10	10	10	10	10	10
Kidneys	Chronic progressive glomerulonephropathy, bilateral:	Very slight	9	10	10	0	3	1	1	9
		Slight	0	0	0	10	0	0	0	1
Lungs	Alveolar histiocytosis, multifocal:		10	10	10	0	10	10	10	0
	Hyperplasia, alveolar cell, focal:		0	0	1	0	0	0	0	0
	Aggregates of alveolar macrophages, multifocal:	Very slight	0	0	0	2	0	0	0	0
		Slight	0	0	0	8	0	0	0	10
Oral tissues	Fluorosis dental, upper incisors, bilateral:	Very slight	0	0	3	3	0	0	0	0
		Slight	0	0	0	7	0	0	0	0
24 months										
No. of rats examined			50	50	50	50	50	50	50	50
Kidneys	Chronic progressive glomerulonephropathy, bilateral:	Very slight	8	9	7	2	42	46	48*	0*
		Slight	13	16	18	0*	5	1	2	3
		Moderate	24	20	22	1*	0	0	0	2
		Severe	4	2	2	4	0	0	0	5*,[a]
		Very severe	1	2	1	43*	1	0	0	40*
	Mineralization, tubule(s):	Very slight	48	48	49	5*	49	50	50	12*
		Slight	0	0	0	0	0	0	0	1
	Mineralization, secondary to renal disease:		1	2	1	45*	1	0	0	37*
	Adenoma, tubule(s), benign, primary:		0	1	0	0	0	0	0	0
	Myxosarcoma, papilla(e), malignant, primary, no metastasis:		0	0	1	0	0	0	0	0
Lung	Alveolar histiocytosis, multifocal:	Very slight	41	46	43	1*	46	47	45	0*
		Slight	1	1	3	0	0	0	1	1
	Mineralization, blood vessels, multifocal:	Focal	50	50	50	28*	42	41	34	38
		Multifocal	0	0	0	22*	2	1	0	9*
	Mineralization, secondary to renal disease, alveoli/septa:		0	2	1	42*	1	0	0	37*
	Aggregates of alveolar macrophages, multifocal:	Very slight	3	1	1	0	2	0	3	0
		Slight	1	0	1	15*	0	0	0	6*,[a]
		Moderate	1	0	0	34*	0	0	0	42*
Oral tissues	Within normal limits:		13	24	9	0	35	37	30	0
	Inflammation acute, gingiva focal:	Unilateral	1	3	3	7	1	1	2	1

		Bilateral	0	0	0	0	0	1	0	1
	Inflammation—chronic active, focal:	Unilateral	6	5	7	1	3	1	3	0
		Bilateral	5	1	2	0	0	0	0	0
	Fluorosis—dental, upper incisors, bilateral:	Very slight	0	0	10*	12*,[a]	0	0	2	4[a]
		Slight	0	0	0	38*	0	0	0	46*
	Squamous cell carcinoma/papilloma:		4	1	6	0	0	1	1	0
Brain	Cerebral cortex vacuolation:	Very slight	2	0	1	1	1	3	3	22*
Bone	Fibrous osteodystrophy		0	2	1	44*	1	0	0	45*

From Quast, Bradley & Nitschke .(1993)

*Statistically identified difference from control mean by Yate's chi-squared pairwise test, alpha = 0.05.

[a] Linear trend by Cochran-Armitage linear trend test, alpha = 0.02, two-sided.

Dental fluorosis, slight or very slight, was present in the incisor teeth of all male and female rats at 80 ppm at 24 months, and was statistically increased in males at the intermediate dose. This finding is not considered to be toxicologically adverse. There was no evidence of effects other than dental fluorosis on teeth or oral tissues.

There was no evidence of effects on the brain or other parts of the nervous system after 24 months at 20 or 5 ppm. At 80 ppm there was an increase (very slight) in vacuolation of the cerebral cortex in females.

No increases in tumour incidences were seen in groups of rats exposed to sulfuryl fluoride for 24 months, even when additional procedures (McConnell et al., 1986; Peto, 1974) were performed to take account of the reduced survival at the highest concentration.

In this 2-year long-term study of toxicity/oncogenicity with sulfuryl fluoride administered by inhalation in Fischer 344 rats, no evidence for carcinogenicity was observed in either sex at any concentration up to 80 ppm (approximately equivalent to 5.6 mg/kg bw per day), the highest concentration tested. The NOAEC for toxicity was 20 ppm (approximately equivalent to 1.4 mg/kg bw per day) on the basis of effects on the kidney, brain and survival of the respiratory tract at 80 ppm (approximately equivalent to 5.6 mg/kg bw per day) (Quast, Bradley & Nitschke, 1993a).

Dogs

No long-term studies of toxicity were submitted.

2.4 Genotoxicity

Sulfuryl fluoride has been investigated in vitro for its ability to induce mutations in bacterial and mammalian cells, for clastogenicity and the induction of unscheduled DNA synthesis, and in a study of micronucleus formation in vivo (Table 27). The highest concentrations used were justified based on the results of screening for toxicity/cytotoxicity. All studies complied with GLP and the main principles of the OECD guidelines extant at the time of performance, and included positive controls that gave acceptable results. Positive results were seen in studies with mouse lymphoma cells and rat lymphocytes. These positive results were consistent with findings with fluoride ion that are considered not to have a direct action on DNA (see IPCS, 2002). Analytical work in the assay in mouse lymphoma cells demonstrated almost complete hydrolysis of sulfuryl fluoride to fluoride ion and fluorosulfate. The overall extent of the genotoxicity database is considered adequate.

Table 27. Results of studies of genotoxicity with sulfuryl fluoride

End-point	Test object	Concentration or dose	Purity (%) [Batch/lot]	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537	300–30 000 ppm[a]	96.5 [874]	Negative +S9 Negative –S9	Gollapudi et al. (1990a)
Reverse mutation	*E. coli* WP2 *uvrA*	100–50 000 ppm[a]	99.8 [OC16160101]	Negative + S9 Negative –S9	Mecchi (2002)
Chromosome aberrations	Rat lymphocytes	10 000–38 000 ppm[a]	99.8 [OC16160101]	Positive +S9 [b] Positive –S9[b]	Gollapudi et al. (2002a)
Unscheduled DNA synthesis	Sprague Dawley rat hepatocytes	102–1 020 ppm[c]	96.5 [874]	Negative	Gollapudi et al. (1991)
Gene mutation	Mouse lymphoma L5178Y $Tk^{+/-}$	100–7 000 ppm[d]	99.8 [OC16160101]	Positive +S9 [e] Positive –S9[e]	Gollapudi et al. (2002b)
In vivo					
Micronucleus formation	Bone marrow from CD-1 mice (five of each sex per group per time-point)	50, 175, 520 ppm for 4 h (sacrifice at 24, 48 or 72 h)	99.6 [WP880329]	Negative	Gollapudi et al. (1990b)

[a] Plates were exposed by placing them in bags containing sulfuryl fluoride atmospheres or an equivalent amount of compressed air for 4 h.

[b] The NOAEC was 2500 ppm. Findings show some consistency with those associated with the generation of fluoride ion in the culture medium.

[c] Exposure was by replacing some of the headspace in the "Leighton tube" culture vessel with sulfuryl fluoride; for 18–19 h.

[d] Exposure was by flushing the headspace in the culture vessel with sulfuryl fluoride, for 4 h.

[e] The NOAEC was 1000 ppm. Findings show some consistency with those associated with the generation of fluoride ion in the culture medium.

2.5 Reproductive toxicity

(a) Multigeneration study

In a two-generation study, groups of 30 male and 30 female Sprague-Dawley rats were exposed by whole-body inhalation to sulfuryl fluoride (purity, 93.6–98.8%; the two impurities were air and water; lot Nos WP901011-907, WP910321-918 and WP910826-929) at a concentration of 0, 5, 20 or 150 ppm, 6 h/day, 5 days per week before mating. Males and females were subsequently exposed at these levels for 6 h/day, 7 days per week during mating, gestation and lactation (except day 21 of gestation to day 4 of lactation, for animal welfare reasons) and until sacrifice. Intakes were 0, 0.4, 1.4, 10.8 mg/kg bw per day. The study complied with GLP and conformed OECD guideline 416.

The exposure of F_0 rats began at approximately 6 weeks of age. After 10 weeks of exposure (5 days per week, excluding holidays), F_0 rats were mated, one male to one female, to produce the F_1 litters. After weaning of the F_1 litters (at age 3 weeks), 30 males and 30 females from each

treatment group were randomly selected and assigned to the respective treatment group to become the parents (F_1) for the next generation. After approximately 12 weeks of exposure following weaning of the last F_1 litter, the F_1 adults were bred to produce the F_2 litters. All rats were housed continuously in exposure chambers after the initial exposure to sulfuryl fluoride except during late gestation and early lactation, when the females were housed outside of the exposure chambers. Maternal rats were not exposed to sulfuryl fluoride after day 20 of gestation to postnatal day 4, in order to allow for parturition and initiation of lactation. Non-pregnant females were excluded from exposure for a period to equalize the number of exposure days between non-pregnant and pregnant rats. During lactation, pups were not placed in the exposure chambers, but remained in the nesting cages separated from the dam for 6 h/day on days 5–21 of lactation. Each rat was observed at least daily for changes in behaviour or demeanour. All adult rats found dead or moribund were submitted for a gross pathology examination; pups found dead during the lactation phase were examined grossly. Evaluations of litter parameters and body weights were performed regularly. A complete necropsy was conducted on all F_0 and F_1 adults. Histological examination of potential target organs and reproductive tissues was performed on controls and groups at the highest dose. Examination of tissues from groups at the lowest and intermediate doses was limited to those tissues which demonstrated treatment-related histological changes in the group at the highest dose. At the time of weaning, 10 pups of each sex per dose from the F_1 and F_2 litters were randomly selected for a complete necropsy. Terminal body weights were not recorded.

In the F_1 generation,five rats died and one rat in a moribund condition was euthanized before scheduled necropsy. None of the deaths or morbidities were considered to be treatment-related. No treatment-related effects were observed in any exposure group. Body weights of F_0 males at 150 ppm were significantly lower than controls from day 14 of this study. The changes in body weight were progressive over the duration of exposure. The body weights of F_0 females at 150 ppm were lower than those of the controls throughout most of the study, achieving statistical significance during premating, gestation and lactation. Body-weight gains in females at 150 ppm were significantly increased during lactation commensurate with a compensatory response.

No significant treatment-related effects were observed on the F_0 male or female fertility indices, length of gestation, pup survival indices or pup sex ratio in any exposure group (Table 28). The statistically significant increases in the female conception index, female fertility index and male conception index of animals at 5 ppm were considered to be related to the relatively low control values. A slight increase in time to mating at 150 ppm had no effect on overall reproductive outcome.

Gross pathological changes observed in F_0 adults included dental fluorosis and grey or pale foci in the lungs. Dental fluorosis was described as very narrow brown and white horizontal bands on the upper and lower incisors. Dental fluorosis occurred in almost all F_0 adults at 150 ppm but not at lower concentrations. Teeth were not examined histologically, given the lack of histological correlates for dental fluorosis in the 2 year study in rats.

In the lungs, aggregates of alveolar macrophages were observed grossly as multiple, round, pale or grey foci. In adults of the F_0 generation, lung foci were noted in five males at 20 ppm, and in all males and eighteen females at 150 ppm. Pale or grey foci in the lungs were not observed in F_0 males or females at 5 ppm (Table 29).

Organ weights were not recorded. Histopathological changes attributable to exposure to sulfuryl fluoride were observed in the lungs of adult males and females at 150 or 20 ppm and in the brain of males and females at 150 ppm (Table 30).

Litter data, F_1 pups

There were no treatment-related effects on the number of F_1 pups born alive or dead, or on the litter size at any exposure. The litter size for live pups at birth and on days 1 and 4 before culling was increased slightly at 5 and 20 ppm (Table 31). The body weights of F_1 male and female pups from dams at 150 ppm were lower than those of the controls throughout most of lactation. These effects were thought secondary to maternal toxicity as evidenced by the decreased

maternal growth observed throughout the premating and gestation periods. Sporadic deficits in the pup weight at 5 and 20 ppm correlated with increased numbers of pups in the litters and are not considered to be an adverse effect of treatment (Table 31).

No clinical signs of toxicity attributable to exposure were observed in F_0 dams or F_1 pups at 5 or 20 ppm during gestation or lactation. The number of F_1 pups with no milk in their stomachs was increased in the group at 150 ppm. The increased incidence of no milk in the stomach was consistent with the lower body weights of the dams and pups from the group at the highest dose and was considered to be treatment-related, however, it had no effect on pup survival.

No alterations in gross pathology of F_1 weanlings were attributed to treatment were observed at any exposure. Organ weights of F_1 pups were not recorded and no histology was carried out.

Table 28. Principal reproductive indices for F_0 rats exposed to sulfuryl fluoride by inhalation

Indice	Concentration (ppm)			
	0	5	20	150
No. of males	30	30	30	30
No. of females	30	30	30	30
Females mating	30	30	30	30
Males siring a litter	19	26	24	22
Females giving birth	20	29	26	26
Days to mating	3.1	3.3	3.0	3.9
Duration of gestation (days)	22.1	21.8	21.7	21.9

From Breslin et al. (1992)

Table 29. Gross pathology findings in F_0 rats exposed to sulfuryl fluoride by inhalation

Site	Finding	Concentration (ppm)							
		Males				Females			
		0	5	20	150	0	5	20	150
No. of rats examined		30	30	30	30	30	30	30	30
Lungs	Within normal limits	30	29	24	0	30	30	29	12
	Focus—grey, multifocal	0	0	5	30	0	0	0	18
Oral tissues	Within normal limits	30	30	30	3	29	28	29	1
	Dark, lower incisors	0	0	0	27	0	0	0	29
	Overgrown incisors, lower incisors	0	0	0	1	0	0	1	0
	Worn/broken, upper incisors	0	0	0	4	1	1	1	0
	Malformation, upper incisors	0	0	0	1	0	1	0	0

From Breslin et al. (1992)

Table 30. Histopathology findings in F_0 rats exposed to sulfuryl fluoride by inhalation

Site	Finding	Severity	Concentration (ppm)							
			Males				Females			
			0	5	20	150	0	5	20	150
No. of rats examined			30	30	30	30	30	30	30	30
Brain	Within normal limits		30	30	29	18	30	30	30	16
	Vacuolation, cerebrum, bilateral:	Very slight	0	0	0	11*	0	0	0	0
		Slight	0	0	0	0	0	0	0	14*
	Granular cell tumour, meninges, benign, primary		0	0	0	1	0	0	0	0
Lungs	Within normal limits		25	19	16	0	23	20	8	0
	Granuloma(s) foreign body multifocal		0	0	0	0	0	0	0	1
	Inflammation—acute:	Very slight	2	3	0	0	0	0	0	0
	Inflammation—chronic:	Very slight	2	2	1	9	2	2	1	19*
		Slight	0	0	0	5	0	1	0	6
	Aggregates of alveolar macrophages:	Very slight	3	5	10	6	7	9	19	1
		≥ Slight	0	0	1	24*	0	1	0	29*

From Breslin et al. (1992)

* $p < 0.05$

Table 31. Principal indices for F_1 pups of parents exposed to sulfuryl fluoride by inhalation

Indice	Time-point	Concentration (ppm)			
		0	5	20	150
Mean size of litter	Born live	13.6	15.6	15.1	13.8
F_1 litters	Lactation day 1	13.3	15.1	14.8	13.5
	Day 4 pre-cull	13.1	14.8	14.7	13.3
	Day 21	7.7	7.9	8.0	7.9
Mean pup body weight	Day 1—female	7.1	6.6	6.7	6.6*
	Day 1—male	7.2	7.1	7.2	7.0
	Day 4 post-cull—female	10.3	9.3	9.8	8.5*
	Day 4 post-cull—male	10.7	9.7	10.4	9.2*
	Day 21 (weaning)—female	41.4	38.1	41.0	34.7*
	Day 21 (weaning)—male	42.6	40.7	43.0	35.6*

From Breslin et al. (1992)

* $p < 0.05$

F_1 generation

Two rats died and two rats were euthanized after being found in a moribund condition before the scheduled necropsy. None of the deaths or morbidities were considered to be treatment-related. No clinical signs of toxicity or treatment-related effects were observed in F_1 males or females at any exposure. The body weights of F_1 rats at 150 ppm were significantly lower than those of the controls. The statistically significant increases and decreases in the lactational body-weight gain of dams at 5, 20 or 150 ppm were not considered to be treatment-related as weights during lactation are usually highly variable, the changes observed did not correspond to a dose–response relationship and the changes were inconsistent, being both significantly increased and decreased.

No significant effects were observed on the F_1 male or female fertility indices, duration of gestation, time to mating, pup survival indices or pup sex ratio at any exposure (Table 32).

Gross pathology changes that were observed in F_1 adults, and that were attributable to exposure to sulfuryl fluoride, were limited to dental fluorosis and grey or pale foci in the lungs and were similar to the observations made for F_0 adults. Dental fluorosis occurred in most adult F_1 rats of both sexes at 150 ppm and appeared as very narrow brown and white horizontal bands on the upper and lower incisors. The lesion was not observed at 5 or 20 ppm or in the controls (Table 33).

Organ weights were not recorded.

Table 32. Principal reproductive indices for F_1 rats exposed to sulfuryl fluoride[a]

Indice	Concentration (ppm)			
	0	5	20	150
No. of males	30	30	30	30
No. of females	30	30	30	30
Females mating	29	30	27	29
Males siring a litter	20	19	20	21
Females giving birth	20	23	23	26
Days to mating	3.9	4.5	3.9	4.8
Gestation period (days)	22.0	21.8	21.8	21.8

From Breslin et al. (1992)

[a]For females taking litters to weaning . For pup data see Table 31.

Table 33.Gross pathology findings in F_1 rats exposed to sulfuryl fluoride

Site	Finding	Concentration (ppm)							
		Males				Females			
		0	5	20	150	0	5	20	150
No. of rats examined		30	30	30	30	30	30	30	30
Lungs	Within normal limits	27	28	24	16	29	29	23	6
	Focus—pale	1	0	5	12	0	0	5	24
	Aspirated blood secondary to decapitation	1	2	0	2	0	1	2	0
Oral tissues	Within normal limits	29	29	30	8	30	30	30	10
	Dark, tooth	0	0	0	22	0	0	0	20

From Breslin et al. (1992)

Table 34. Histopathology findings in F_1 rats exposed to sulfuryl fluoride

Site	Finding	Severity	Concentration (ppm)							
			Males				Females			
			0	5	20	150	0	5	20	150
No. of rats examined			30	30	30	30	30	30	30	30
Brain	No. of tissues examined		30	30	30	30	30	30	30	30
	Within normal limits		30	30	30	28	30	29	30	23
	Hypoplasia, cerebellum, unilateral, focal		0	0	0	0	0	1	0	0
	Vacuolation, cerebrum, bilateral:	Very slight	0	0	0	1	0	0	0	5
		Slight	0	0	0	1	0	0	0	2

From Breslin et al. (1992)

Histopathological changes observed in adults, that were attributable to exposure to sulfuryl fluoride, were observed in the lungs of males and females at 150 or 20 ppm and in the brain of males and females at 150 ppm. Pulmonary changes were characterized as aggregates of alveolar macrophages and were observed most commonly in subpleural or peribronchial locations as described in F_0 adults. Aggregates of alveolar macrophages were more prevalent at 20 and 150 ppm (Table 34). The aggregates of alveolar macrophages observed commonly at 20 and 150 ppm were morphologically the same as the normal, spontaneous aggregates of alveolar macrophages observed in the lungs of several control rats, except that the lesion was more frequently accompanied by chronic inflammation in the group at the highest dose. In the brain, very slight to slight, bilaterally symmetrical, vacuolation of the caudate putamen myelinated fibre tracts was observed in F_1 males and females at 150 ppm. The pattern of brain lesions was similar to that described for F_0 animals (Table 34).

Litter data, F_2 pups

Litter parameters and pup weights at birth were unaffected by treatment. F_2 pups from dams at 150 ppm had lower body weights throughout lactation, with statistically significant differences on days 14 and 21 of lactation (Table 35).

The number of F_2 pups with no milk in their stomachs was increased at 150 ppm, but this had no effect on overall litter size or pup survival.

No gross pathology alterations attributable to treatment were observed in F_2 weanlings at any exposure. No histology was conducted, and Organ weights were not recorded.

The NOAEC for reproductive effects was 150 ppm (approximately equivalent to 10.8 mg/kg bw per day), the highest concentration tested. Parental toxicity was evident at 150 ppm, toxicity comprised reduced body weights, overt dental fluorosis, alveolar macrophages and brain vacuolation. These findings are consistent with those from short-term studies. The NOAEC for parental toxicity was 20 ppm (approximately equivalent to 1.4 mg/kg bw per day). Reduced growth of F_1 and F_2 pups during lactation was noted at 150 ppm and was the only effect in offspring. The NOAEC in offspring was 20 ppm (approximately equivalent to 1.4 mg/kg bw per day) (Breslin et al., 1992).

Table 35. Principal pup indices for F_2 rats of parents exposed to sulfuryl fluoride

Pup data	Time-point	Concentration (ppm)			
		0	5	20	150
Mean size of F_1 litters	Born live	15.2	16.0	14.1	14.7
	Lactation day 1	15.0	15.9	14.1	14.6
	Day 4 pre-cull	14.9	15.8	13.8	14.2
	Day 21	7.6	8.0	7.7	7.8
Mean pup body weight	Day 1—female	6.4	6.6	6.8	6.2
	Day 1—male	6.8	7.0	7.2	6.7
	Day 4 post-cull—female	9.2	9.7	9.6	8.6
	Day 4 post-cull—male	9.7	10.2	10.3	9.2
	Day 21 (weaning)—female	39.8	42.4	41.7	35.6*
	Day 21 (weaning)—male	41.5	43.8	42.9	38.3

From Breslin et al. (1992)

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

(b) Developmental toxicity

Rats

In a range-finding study of developmental toxicity, groups of seven to nine pregnant Fisher F344 rats were exposed by inhalation to sulfuryl fluoride at a concentration of 0, 30, 100 or 300 ppm for 6 h/day on days 6–15 of gestation. There was evidence of maternal toxicity only at 300 ppm, consisting of reduced food consuption and slight pathological changes to the liver and kidneys. There was no clear evidence of fetotoxicity on the basis of the limited examinations performed (Hanley et al., 1980).

In a study of teratology, groups of 35–36 Fischer 344 rats and New Zealand White rabbits were exposed by inhalation to sulfuryl fluoride (purity, 99.8%; Vikane fumigant, lot No. 217) at a concentration of 0, 25, 75 or 225 ppm for 6 h/day on days 6–15 of gestation. Intakes were 0, 1.8, 5.4, 16.2 mg/kg bw per day. The study complied with GLP. It was not stated whether the study was conducted according to any guideline. Animals were observed daily. Body weights were recorded on days 6, 9, 12, 16 and 21; food and water consumption of rats were recorded at 3-day intervals, beginning on day 6 of gestation. Test animals were terminated by inhalation of carbon dioxide on day 21 of gestation. The uterine horns were exterioried through a mid-line incision in the abdominal wall. The following data were recorded: number and position of fetuses in utero; number of live and dead fetuses; number and position of resorption sites; number of corpora lutea; sex, body weight and crown–rump length of each fetus; and any gross external alteration. In addition, maternal liver and kidney weights were recorded. The uteri of apparently non-pregnant animals were stained with a 10% solution of sodium sulfide and examined for evidence of implantation sites. One half of each litter selected using a table of random numbers was examined by dissection under a low power microscope for evidence of soft tissue alterations. The heads of rat fetuses examined by dissection were removed, placed in Bouin fixative and examined by a serial sectioning technique. All fetuses were then eviscerated, preserved in alcohol and subsequently cleared and stained with alizarin red S and examined for skeletal alterations.

No overt signs of maternal toxicity or effects on general demeanour were observed at any exposure. Maternal body weights measured during gestation were comparable to those of the

controls at all exposures, and there were no significant differences from control values with respect to maternal body-weight gains. At 225 ppm, water consumption was significantly increased on days 6–17 of gestation. No effects on absolute or relative weights of the liver or kidney were evident

There were no effects of sulfuryl fluoride exposure on litter parameters (Table 36). At 225 ppm, fetal body weights and crown–rump lengths were statistically significantly higher than concurrent control values, but differences were considered to be biologically negligible (mean, < 4%) and of no relationship to treatment.

The number of fetuses that exhibited major malformations was higher in the control group (four fetuses from two litters) than in the exposed groups (two fetuses in two litters in each exposure group). The majority of malformations were microphthalmia or anophthalmia (Table 37).

A number of minor alterations or instances of delayed development were observed among both control and treated groups, with no evidence of a relationship with treatment.

Minor skeletal variations that were observed included statistical increases in the incidence of bilobed thoracic centra in fetuses from the groups at 25 and 225 ppm and an increased incidence of unfused thoracic centra in the group at 75 ppm when compared with controls. However, there was no dose–response relationship and other than these, the incidence of skeletal variations observed in fetuses from the groups at 25, 75 and 225 ppm were comparable that among control fetuses. Therefore, the statistical increases of these minor skeletal variants that occur spontaneously in rats were not considered to be evidence of fetotoxicity.

There was no evidence of maternal toxicity, embryolethality, or fetotoxicity with Vikane at concentrations of up to 225 ppm. The incidences of major malformations, whether considered

Table 36. Litter data for pregnant rats exposed to sulfuryl fluoride

Parameter	Concentration (ppm)			
	0	25	75	225
No. bred	36	35	35	36
Non-pregnant	5	6	3	5
No. dead/sacrificed	0	0	0	0
No. of total resorptions	0	0	0	1
No. of viable litters	31	29	32	31
No. of corpora lutea/dam	12	12	12	12
No. of implantations/dam	10	10	10	10
Preimplantation loss (%)	18	13	19	23
Fetuses/litter	9	8	8	8
No. of resorptions/litter	1.52	2.07	2.03	1.94
Implantations resorbed (%)	15	20	21	20
Litters with resorptions (%)	74	93	84	81
Fetal body weights (g)	4.32	4.28	4.45	4.48
Fetal sex ratio, M:F	51:49	45:55	52:48	49:51
Fetal crown–rump length	40.7	40.7	41.1	41.3

From Hanley et al. (1981)

Table 37. Malformations and alterations in fetuses from female rats exposed to sulfuryl fluoride

Observation	Concentration (ppm):							
	0		25		75		225	
No. of fetuses (No. of litters) examined								
External examination	264 (31)		244 (29)		245 (32)		245 (30)	
Bouins head examination	118 (28)		112 (26)		111 (29)		108 (23)	
Visceral examination	144 (31)		132 (29)		134 (32)		136 (30)	
Skeletal examination	264 (31)		244 (29)		245 (32)		245 (30)	
Observations[a]	Fetus	Litter	Fetus	Litter	Fetus	Litter	Fetus	Litter
Total major malformations[a]	4	2	2	2	2	2	2	2
Soft tissue alterations:[a]								
Anophthalmia/ microphthalmia	2	2	2	2	2	2	2	2
Skeletal alterations:								
Sternebrae—delayed ossification	90	27	83	28	70	29	76	26
Sternebrae—unfused	9	8	7	7	6	6	5	5
Sternebrae—fused	1	1	0	0	2	1	0	0
Sternebrae—extra site of ossification	0	0	0	0	1	1	0	0
Vertebrae—delayed ossification—centra	73	25	80	28	65	28	61	25
Vertebrae—bilobed centra	11	10	22*	16	12	9	24*	17
Vertebrae—unfused centra	2	2	2	2	7*	6	2	2
Ribs—spurs	1	1	0	0	3	2	1	1

From Hanley et al., (1981)

[a]No. of fetuses or litters

*Statistically different from control values by Wilcoxon's test, alpha = 0.05.

individually or collectively at each concentration were not significantly increased in any treatment group when compared with control values. The incidence of resorptions was not significantly increased among rats in any exposure group, indicating that Vikane was not embryolethal at concentrations as high as 225 ppm.

The NOAEC for maternal and fetotoxicity was 225 ppm (approximately equivalent to 16.2 mg/kg bw per day), the highest dose tested (Hanley et al., 1981).

Rabbits

In a range-finding study of developmental toxicity, groups of five to seven pregnant New Zealand White rabbits were exposed to sulfuryl fluoride at a concentration of 0, 30, 100 or 300 ppm for 6 h/day on days 6–18 of gestation. There was evidence of maternal toxicity only at 300 ppm, consisting of reduced body-weight gain, increased liver weight and slight pathological changes to the liver. There was no clear evidence of fetotoxicity on the basis of the limited examinations performed (Hanley et al., 1980).

In a study of teratology, groups of Fischer 344 rats and New Zealand White rabbits wree exposed by inhalation to sulfuryl fluoride (purity, 99.8%; Vikane fumigant, lot No. 217) at a concentration of at a concentration of 0, 25, 75 or 225 ppm for 6 h/day on days 6–15 of gestation. Intakes were 0, 1.4, 4.3, 13 mg/kg bw per day. The study complied with GLP. It was not stated whether the study was conducted according to any guideline.

Animals were observed daily throughout the experiment. Body weights were recorded on days 6, 9, 12, 15, 19 and 29 of gestation. Test animals were killed by inhalation of carbon dioxide on day 29 of gestation, and maternal liver and kidney weights were recorded. The uterine horns were exposed and the following data were recorded: number and position of fetuses in utero; number of live and dead fetuses; number and position of resorption sites; number of corpora lutea; the sex, body weight and crown-rump length of each fetus; and any gross external alteration. The uteri of apparently non-pregnant animals were stained with a 10% solution of sodium sulfide and examined for evidence of implantation sites. One half of each litter selected using a table of random numbers was examined immediately by dissection under a low-power microscope for evidence of soft tissue alterations. All fetuses were then eviscerated, preserved in alcohol and subsequently cleared and stained with alizarin red S and examined for skeletal alterations.

No animals in the control group died or were terminated early. Among the rabbits exposed to Vikane fumigant at 25 ppm during gestation, there were two deaths recorded. The cause of death of one animal could not be ascertained upon gross pathology examination. Gross necropsy of the second rabbit at 25 ppm revealed pulmonary inflammation consistent with pneumonia, most probably caused by *Pasteurellosis*. A single death occurred among maternal rabbits at 75 ppm; however, the cause of death could not be determined. Three deaths occurred among maternal rabbits at 225 ppm. Upon gross necropsy, these were attributed to severe pulmonary inflammation consistent with pneumonia, most probably caused by *Pasteurellosis;* pulmonary inflammation is an effect of sulfuryl fluoride and these findings at 225 ppm are possibly related to treatment. At 225 ppm, dams lost weight as from day 9 of gestation (Table 38). There were no differences in absolute or relative weights of the liver or kidneys of exposed rabbits when compared with controls.

Control and treated animals exhibited a similar pattern of implantation loss and early delivery. No adverse effects on average litter size, fetal sex ratio, or the incidence of resorptions among rabbits exposed to Vikane were observed and values that were observed (Table 39) are within the historical control range for this species in this laboratory. At 225 ppm, fetal body weights were significantly decreased when compared with control values, and a trend toward decreased fetal crown–rump length was also noted. These parameters were unaffected in rabbits at 25 and 75 ppm (Table 39).

Table 38. Mean maternal body weights (g) in pregnant rabbits exposed to sulfuryl fluoride

Time-point	Concentration (ppm)			
	0	25	75	225
No. of dams	26	22	21	21
Day of gestation:				
6	4036	4161	3945	4025
9	4036	4169	3946	4065
12	4031	4187	3952	4036
15	4100	4256	3997	4016
19	4116	4285	4065	3974
29	4276	4360	4109	3965*

From Hanley et al. (1981)

*Statistically significantly different from control mean by Dunnett's test, alpha = 0.05.

Table 39. Effect on gestation for pregnant rabbits exposed to sulfuryl fluoride

Parameter	Concentration (ppm)			
	0	25	75	225
No. bred	28	29	28	29
Non-pregnant	5	3	6	3
No. dead/sacrificed	0	2	1	3
No. of total resorptions	1	0	3	0
No. of viable litters	19	22	18	21
No. of corpora lutea/dam	10	10	10	10
No. of implantations/dam	9	9	8	8
Preimplantation loss (%)	11	15	14	11
Fetuses/litter	8	8	7	8
No. of resorptions/litter	1.1	0.9	2.1	1.0
Implantations resorbed (%)	13	11	16	8
Litters with resorptions (%)	45	36	57	33
Fetal body weights (g)	38.22	38.18	38.44	32.95*
Fetal sex ratio, M:F	50:50	52:48	42:58	44:56
Fetal crown–rump length	93.6	94.5	93.8	89.7

From Hanley et al. (1981)

*Significantly different from control mean using binomial distribution test, alpha = 0.05.

The incidence and pattern of malformations and external, soft tissue and skeletal alterations were similar in fetuses from control and treated groups (Table 40). Low incidences of small spleen, dilated renal pelves and pale liver in the group at 225 ppm were possibly treatment-related, given the background incidence of zero in other groups, but are likely to be secondary to low fetal weight and maternal toxicity.

Pregnant rabbits at 225 ppm showed maternal toxicity, as evidenced by a loss of body weight during gestation. Decreased fetal body weight, indicative of a fetotoxic effect, was also observed at 225 ppm. No evidence of maternal toxicity or fetotoxicity was observed among rabbits given Vikane at 25 or 75 ppm. The incidence of resorptions was not significantly increased among rabbits in any exposure group, indicating that Vikane was not embryolethal at concentrations as high as 225 ppm. There was no evidence of teratogenicity.

The NOAEC for maternal and fetotoxicity was 75 ppm (approximately equivalent to 4.3 mg/kg bw per day) (Hanley et al., 1981).

2.6 Special studies

(a) Study screening for acute neurotoxicity

The protocol was designed to evaluate the effects of short-term exposure to high levels of sulfuryl fluoride on the function of the central nervous system of Fischer 344 rats. Dose selection was based primarily on the observation that changes in evoked potentials occurred in the absence

Table 40. Findings in fetuses from pregnant rabbits exposed to sulfuryl fluoride

Finding	Concentration (ppm):							
	0		25		75		225	
No. of fetuses (No. of litters) examined								
External examination	153 (19)		167 (22)		145 (18)		163 (21)	
Visceral examination	84 (19)		89 (22)		78 (18)		90 (21)	
Skeletal examination	153 (19)		167 (22)		138 (18)		152 (20)	
Observations	*Fetus*	*Litter*	*Fetus*	*Litter*	*Fetus*	*Litter*	*Fetus*	*Litter*
Total major malformations[a]	2	2	4	4	3	3	2	2
External alterations:[a]								
Exencephaly	1	1	0	0	0	0	1	1
Soft tissue alterations:[a]								
Persistent *truncus arteriosis* and ventricular septal defect	0	0	0	0	0	0	1	1
Patent *ductus arteriosis*	0	0	1	1	2	2	1	1
Retro-oesophageal subclavian artery	1	1	0	0	0	0	0	0
Extra vessel(s) off major arteries	4	3	3	3	1	1	6	4
Satellite vessel(s) off major arteries	60	19	46*	18	50	17	43*	20
Hamartoma adjacent to gallbaldder	2	2	5	4	6	4	7	4
Small spleen	0	0	0	0	0	0	1	1
Dilated renal pelvis	0	0	0	0	0	0	1	1
Pale liver	0	0	0	0	0	0	3	2
Skeletal alterations:								
Skull—delayed ossification	2	2	0	0	0	0	2	2
Skull—foramen	3	3	2	2	2	1	5	3
Ribs—extra ribs	63	17	75	21	65	17	58	18
Ribs—spurs	3	3	8	7	3	2	3	3
Sternebrae—unfused	1	1	2	2	1	1	2	2
Cartilage—extra cartilage	1	1	1	1	0	0	0	0
Cartilage—missing cartilage	14	5	9	7	4	4	5	1

From Hanley et al. (1981)

[a] No. of fetuses or litters

*Statistically different from control values by Wilcoxon's test, alpha = 0.

of neuropathological changes in F344 rats exposed to sulfuryl fluoride at 100 ppm for 6 h/day, 5 days per week, for 13 weeks (see Mattson et al., 1986). The study was conducted in female rats, considered to be the most sensitive sex on the basis of flash evoked potential data, in the previous 13-week study of neurotoxicity. The study complied with GLP and US EPA guideline 82-7 (addendum 10, 1991).

Groups of 12 female Fischer 344 rats were exposed by inhalation to sulfuryl fluoride (purity, 99.8%; lot WP920619-953) at a concentration of 0, 100 or 300 ppm for 6 h/day on two consecutive days (this was considered to effectively represent an acute exposure of 12 h during 30 h). Intakes were 0, 10, 31 mg/kg bw per day. The animals were evaluated before exposure and

after exposure by FOB, grip performance, landing foot splay, motor activity (6 × 8 min blocks) and a battery of electrodiagnostic tests.

The electrodiagnostic tests consisted of flash evoked potentials (FEP), auditory brainstem response (ABR) to clicks and somatosensory evoked responses (SEP) to tail stimulation. Investigations were performed on both early and mid-latency responses. These were detected using epidural electrodes placed into the skull and secured with dental cement. The somatosensory electrode was placed 2.0 mm posterior and 2.0 mm lateral left of bregma, the visual cortex electrode was placed 1.5 mm anterior and 3.0 mm lateral right of lambda, and the cerebellar electrode (for ABR) was located 3.0 mm posterior and 0.0 mm lateral of lambda. A reference electrode was placed 8.0 mm anterior and 1.0 mm lateral left of bregma. No histopathological examinations were performed.

There were no exposure-related effects on body weights.

Minor differences relating to cage-side, hand-held and open-field observations were reported for the group at 300 ppm before and after exposure. Before exposure, 10 out of 12 animals in the group at 300 ppm exhibited virtually no resistance to removal from the home-cage compared with the controls for which 6 out of 12 showed no resistance and 6 out of 12 showed minimal resistance. During the FOB conducted after exposure, all groups were comparable for 'resistance to removal', but the group at 300 ppm was slightly less reactive to handling than were the controls. Of the observations that were possibly distributed differently according to treatment, reactivity to handling after exposure had the greatest difference; 5 out of 12 controls showed no resistance to handling while at 300 ppm 10 out of 12 showed no resistance. However, this result was not statistically significant and is consistent with the differences in resistance to removal from the cage before exposure.

There were no exposure-related differences in hind limb or forelimb grip performance, hindlimb landing foot splay, body temperature or motor activity results. Interpretation of the evoked potential data is confounded by marked inter-animal differences before exposure and between results for controls before and after exposure. Within this variable background, there were no statistically significant differences associated with exposure to sulfuryl fluoride in potency or amplitude of any of the parameters measured.

In this study, there were no adverse exposure-related effects in any of the parameters. The NOAEC was 300 ppm (approximately equivalent to 31 mg/kg bw per day), the highest concentration tested (Albee et al., 1993).

(b) Short-term study of neurotoxicity with sulfuryl fluoride

Groups of seven male and seven female Fischer 344 rats (aged 5 weeks) were exposed by inhalation to sulfuryl fluoride (purity, 99.8%; lot No. TWP 830919-408) at a concentration of 0, 30, 100 or 300 ppm for 6 h/day, 5 days per week for 13 weeks. Intakes were 0, 2.2, 7.2, 22 mg/kg bw per day. The study complied with GLP and US EPA guideline 82-5.

Groups of rats had surgery 2 months after the start of the study (they were too young at the start of the study) to implant brain electrodes. Epidural electrodes were placed into the skull and secured with dental cement: the somatosensory electrode was placed 1.5 mm posterior and 3.0 mm lateral left of bregma, the visual-cortex electrode was placed 6.8 mm posterior and 3.0 mm lateral right of bregma, and the cerebellar electrode was located 12.0 mm posterior and 0.0 mm lateral of bregma. A reference electrode was placed 7.0 mm anterior and 1.0 mm lateral left of bregma. The following responses were measured: auditory brainstem response (ABR) to clicks and pips; cortical flicker fusion (CFF) to light flashes; visual evoked response (VER) to light flash; somatosensory evoked response (SER) to tail stimulation and caudal nerve action potentials (CNAP) in the tail. During data gathering in the electrophysiological tests, the rats were fully conscious but physically restrained. Electrophysiological investigations and a limited FOB were performed after 13 weeks exposure; no investigations were performed at intermediate time-points.

Two male and two female rats at 0 ppm or 300 ppm were placed on recovery for approximately 2 months after 13 weeks of exposure. Because ABR was clearly affected by treatment, and was considered to be a highly reliable response, only ABR was evaluated during recovery. Only limited data on the recovery animals were presented.

At necropsy, the rats were euthanized and examined for alterations in gross pathology. The necropsy included examination of the eyes in situ by a glass-slide technique with fluorescent illumination. A complete set of tissues was collected from each animal and preserved in neutral, phosphate-buffered 10% formalin. The sciatic, tibial, caudal and optic nerves were collected. Lungs were infused with formalin to their approximate normal inspiratory volume and the nasal cavity was flushed with formalin via the pharyngeal duct.

Representative sections of ear tissues were prepared for histology. Sections of the middle ear were prepared from one female and six males in the control group, as well as seven males in the group at 100 ppm. Sections of the middle ear were also prepared from three control males from the standard 13-week study; these provided control tissue from animals that did not have surgical implants. A section of cerebrum was prepared from the brain of a control male and a male at 300 ppm, both of which died as a result of implant surgery. Tissues were routinely processed and stained with haematoxylin and eosin. Two male and two female rats from the groups at 0 and 300 ppm (eight rats) constituted a recovery group and were necropsied 2 months after exposure.The brains were sent to another laboratory for histopathology examination. Each brain was bisected on the midline. The right hemisphere (half) of the brain was again bisected longitudinally. The left hemisphere was cut into five equal cross sections yielding seven tissue specimens per brain. Each longitudinally-cut specimen was embedded in a paraffin block. The five transverse specimens were embedded, in groups of two and three, into two paraffin blocks. All tissue sections were cut to a thickness of 10 µm and stained with haematoxylin and eosin. Special stains were performed with tissue sections from the group at the highest concentration (300 ppm) and control animals. Three special stains were performed: gallocyanine, for detection of any changes in the neuronal Nissl substance; Luxol fast blue for detection of any changes in the myelin sheaths; and Bodian, for detection of any axonal changes. In addition, another special stain, Holzer, was also performed on the long-term recovery animals to detect any scarring in damaged brain areas. Because vacuoles were seen in the caudate-putamen of 300 ppm rats in the standard study, step-sections assured critical re-examination of the caudate-putamen. Only Luxol fast blue–haematoxylin and eosin staining was performed on the sections.

Four males died, three from anaesthetic accidents and one from a handling accident. The only FOB findings were that rats at 300 ppm weighed less than did the controls and some had unkempt fur and excessive lachrymation. Evoked responses exhibited marked inter-animal variation, but based on statistical analysis were clearly altered at 300 ppm and slightly altered at 100 ppm (Table 41). The principal effect was a decrease in cortical flicker fusion (CFF) and a slowing of all waveforms at 300 ppm, and a slowing of the VER and SER of female rats at 100 ppm. An apparent effect on SER at 30 ppm in females was not statistically significant with mean values well within the control range. Visual inspection of ABRs indicated that, although not statistically significant, the ABRs of male rats at 100 ppm were also slow (although three males accounted for nearly all the poor ABR-tone responses in this group). Rectal temperatures of rats at 300 ppm were slightly ($p < 0.05$) lower than those of control rats during testing for VER, Cry, and ABR tone. Temperature differences diminished to non-significant during the 35 min of electrophysiological testing. The ABR results for the recovery groups showed evidence of recovery at 1 month and were within the normal ranges of response after 2 months (Figure 1).

At necropsy, all rats from the groups at 100 and 300 ppm had mottled incisor teeth. This mottling was presumed to be due to fluorosis. Pale foci were observed on the pleural surfaces of all rats at 300 ppm and one male and one female at 100 ppm. These pleural foci probably resulted from chronic inflammation. Histopathological changes were seen in the brains of rats at 300 ppm

Table 41. Body weights, electrophysiological data and grip strength in rats exposed to sulfuryl fluoride for 13 weeks

Parameter	Concentration (ppm)							
	0	30	100	300	0	30	100	300
	Males				Females			
Body weight (g)	251.10	275.75	270.06	213.80	168.14	162.84	163.57	131.29
Grip strength (g force)	560.00	611.67	684.29	563.33	369.29	395.71	376.43	340.00
VER latency (msec)	–0.18	–2.05	–2.38	5.91	0.00	0.05	11.87	10.90
VER power (μV^2)	26.40	30.98	41.09	37.42	28.49	28.90	27.87	26.71
CFF (Hz)	45.26	48.33	46.86	42.67	47.71	48.57	45.14	42.67
ABR latency (msec)	0.00	0.00	0.01	0.04	0.00	0.00	–0.03	0.05
ABR power (μV^2)	1.69	1.80	1.36	1.41	1.62	1.71	1.86	1.40
ABR-III/V latency (msec)	0.02	0.04	0.14	0.18	0.00	0.04	–0.03	0.16
ABR-III/V power (μV^2)	1.20	1.09	0.88	0.88	1.15	1.16	1.27	0.90
CER-S[a] latency (msec)	–0.07	0.07	0.64	0.89	0.25	0.55	0.72	1.27
CER-S[a] power (μV^2)	11.89	13.56	11.91	12.08	15.47	14.46	15.07	16.65
CER-L[b] latency (msec)	–0.54	0.67	1.20	2.75	0.51	0.86	–1.11	4.05
CER-L[b] power (μV^2)	29.45	33.08	29.97	29.93	28.61	27.27	34.43	36.86
SER-S[c] latency (msec)	0.06	–0.82	0.24	0.24	0.09	0.60	–0.04	–1.12
SER-S[c] power (μV^2)	15.20	20.95	16.90	12.78	21.81	20.67	26.12	18.08
SER-L[d] latency (msec)	–0.36	–0.65	0.60	3.90	0.17	2.56	4.44	5.19
SER-L[d] power (μV^2)	49.70	49.07	45.32	43.16	51.27	49.67	62.68	51.76
CNAP ratio	0.91	0.94	0.92	0.92	0.92	0.94	0.93	0.94
Temp-VER	37.46	37.33	37.53	36.72	37.37	37.79	37.73	37.03
Temp-CFF	37.52	37.33	37.80	37.00	37.83	38.04	38.24	37.39
Temp-ABRT	37.54	37.53	38.00	37.27	38.07	37.87	38.29	37.40
Temp-ABRC	37.82	37.85	37.87	37.73	38.20	38.04	38.27	37.61
Temp-C/S	38.18	38.03	38.07	37.87	38.23	38.21	38.27	37.77

From Mattson et al. (1986)

ABR, auditory brainstem response; CER, cerebellar evoked response; CFF, cortical flicker fusion; CNAP, caudal nerve action potential; SER, somatosensory evoked response; VER, visual evoked response.

[a] III /V, peaks 3 to 5

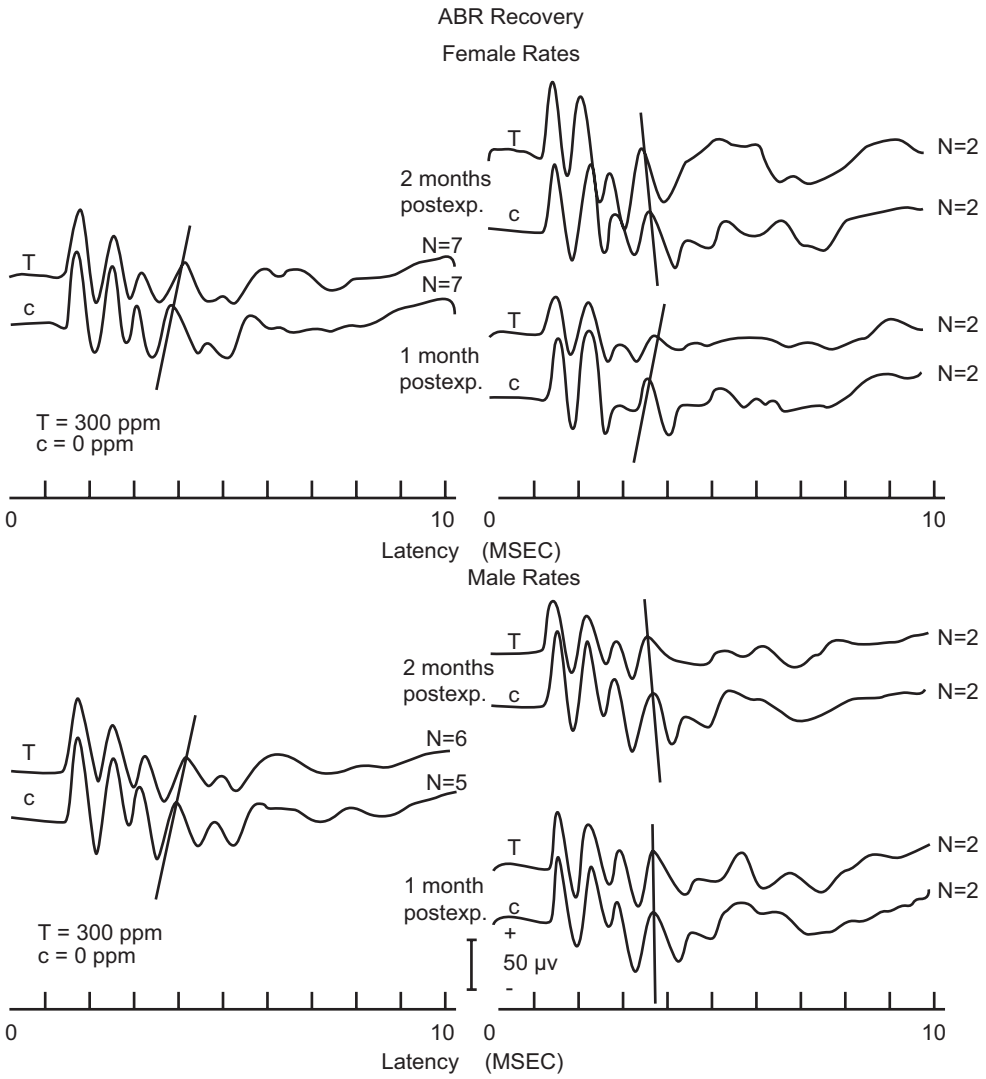
[b] S, 4–25 ms

[c] L, 25–70 ms

[d] S, 2.5–20 ms

[e] L, 20–90 ms

Figure 1. Auditory evoked response in rats exposed to sulfuryl fluoride for 13 weeks with 2 months recovery.



From Mattson et al. (1986)

(Table 42). Vacuoles were present in the white fibre tract of the caudate-putamen. No necrosis or neuronal destruction was noted. Rats allowed to recover from exposure at 300 ppm for 2 months had normal brain histology.

Three male rats from the group at 100 ppm had poor click ABRs and poor ABR responses to tone pips at 4 kHz and 16 kHz. One rat was unresponsive to a sharp noise (observational battery) and did not register an ABR. The other two rats did respond to a sharp noise on the observational battery; however, one of these registered a poor ABR and the other had a flat ABR.Middle-ear histopathology did not reveal a reason for the poor ABRs. A comparable problem did not occur in female rats at 100 ppm or in male or female rats at 300 ppm. The anomalous click ABRs from these three rats were, therefore, not attributed to treatment, and were removed from data analysis. The VER of one female at 30 ppm and one female at 300 ppm was topographically so dissimilar from controls that meaningful measures of latency and correlation could not be made. For this reason, VER data from these two rats were not included in the data analysis.

Inhalation exposure of male and female Fischer 344 rats to sulfuryl fluoride at 300 ppm for 6 h/day, 5 days per week for 13 weeks caused diminished weight gain, dental fluorosis, a slight decrease in grooming, slowing of visual, auditory and somatosensory-evoked potentials, mild pulmonary inflammation, and mild vacuolation in the brain. Auditory brainstem responses (ABRs) and brain histopathology were evaluated 2 months after exposure in two male and two female rats. Both the ABRs and brain histopathology appeared normal at this time, indicating that these treatment effects were essentially reversible. Exposure to 100 ppm resulted in slight dental fluorosis and minor slowing of some evoked responses. All other measurements, including brain histopathology, were normal. The NOAEC was 30 ppm (approximately equivalent to 2.2 mg/kg bw per day) on the basis of alterations in evoked potentials (Mattson et al., 1986).

(c) A long-term study screening for neurotoxicity in rats

This study was conducted as a segment of the 2-year study in rats treated by inhalation, described earlier (Quast et al., 1993c). Groups of 15 male and 15 female Fischer 344 rats were given sulfuryl fluoride (purity, 93.6–99.7%; lot Nos WP 880329-752, WP 901011-907, WP 910321-918, WP 910826-929 and WP 920131-940) at a concentration of 0, 5, 20 or 80 ppm by inhalation for 6 h/day, 5 days per week for approximately 12 months. The intakes were 0, 0.4, 1.4, 5.6 mg/kg bw per day. The study complied with GLP and with US EPA neurotoxicity guideline, (Federal Register, 50: 1985 39458–39463, Paragraphs 798.6050, 798.6200 and 798.6400).

The FOB, hindlimb-grip performance and assay for motor activity were conducted on 15 animals of each sex per group, once during the period before study and then at months 3, 6, 9 and 12 during the exposure period. Forelimb-grip performance and hindlimb landing foot splay were evaluated at month 12. After 12 months of exposure, groups of five rats of each sex group were fasted overnight and perfused in situ. A complete gross examination was conducted on all animals by the study pathologist. The necropsy consisted of an examination of the external tissues and orifices. The head was removed, the cranial cavity opened and the brain, pituitary and adjacent cervical tissues were examined. The nasal cavity was flushed with the same fixative. The skin was

Table 42. Summary of histopathology findings in rats exposed to sulfuryl fluoride for 13 weeks

Finding			Concentration (ppm)							
			0	30	100	300	0	30	100	300
			Males				Females			
No. of rats examined			3	6	7	4	5	7	7	5
Lungs	Within normal limits		3	6	6	0	5	7	6	0
	Focus—pale, pleura:	Focal	0	0	0	0	0	0	1	0
		Multifocal	0	0	1	4	0	0	0	5
Oral tissues	Within normal limits		3	6	0	0	5	7	0	0
	Mottled, lower incisors		0	0	7	4	0	0	7	5
	Mottled, upper incisors		0	0	7	4	0	0	7	5
Brain	Within normal limits		3	6	6	0	5	7	6	0
	Vacuoles, caudate-putamen		0	0	0	4	0	0	0	5
	13 weeks plus 2-month recovery									
Brain	Within normal limits		2	—	—	2	2	—	—	2
	Vacuoles, caudate-putamen		0	—	—	0	0	—	—	0

From Mattson et al. (1986)

reflected from the carcass, the thoracic and abdominal cavities were exposed and the viscera were examined in situ. All visceral tissues were dissected from the carcass and re-examined. The brain, head, spinal column with spinal cord, fore- and hindlimbs, and tail were trimmed to remove excessive skin and muscle as necessary and immersed in fixative (phosphate-buffered solution of 1.5% glutaraldehyde, 4% formaldehyde). Muscles from the hindlimbs were reflected to further expose the nerves. Tissues from the central nervous system were stained with haematoxylin and eosin. Peripheral nerves (sciatic, tibial and sural) were osmicated, embedded in plastic, cut at 2–3 μm and stained with toluidine blue for examination by a Board-certified pathologist.

The results of FOB showed that sulfuryl fluoride had no effect at any time on hand-held or open-field observations, grip performance or landing foot splay. Although there were some statistically significant differences in the mean body weights of treated females, there was no clear dose–response relationship and the differences were considered to be unrelated to treatment.

Sulfuryl fluoride had no effect at any time on any aspect of motor activity.

Results of the gross and histopathological evaluations of perfusion-fixed tissues for neuropathological assessment of rats exposed to sulfuryl fluoride for up to 12 months were found to be unaffected by exposure

The NOAEC for this segment of the long-term study of toxicity/carcinogenicity in rats was ≥ 80 ppm (approximately equivalent to 5.6 mg/kg bw per day), the highest concentration tested (Spencer et al., 1994).

(d) Acute incapacitation in rats

In order to estimate the rapidity with which sulfuryl fluoride might incapacitate an individual, groups of five Fischer 344 rats were exposed to Vikane (purity, 99.8%; lot No. 217) at a concentration of 4000, 10 000, 20 000 or 40 000 ppm by inhalation in a 14-l cylindrical chamber that contained a motor-driven activity wheel. Intakes were not calculated as the study was not relevant to the assessment of dietary intake. The study complied with GLP but was not conducted according to any guideline. The following parameters were evaluated: the time to incapacitation, gross examination at necropsy and histological examination of selected tissues. The rats were pre-trained to walk in a self-propelled activity wheel. When an animal was unable to walk, the exposure was terminated and the timer stopped.

There was a clear dose-related decrease in the time the animals maintained the ability to walk as the concentration of sulfuryl fluoride increased (Table 43). All rats, except those at 4000 ppm, appeared to be slightly cyanotic shortly after exposure started. The blueish skin discolouration disappeared within 10 min after the purging of the chamber with room air (in rats at 10 000 ppm). There was mild diarrhoea in all rats at 4000, 10 000 or 20 000 ppm. Tonic convulsions resulted in termination of the exposure in 3 out of 20 rats, while the remainder of the animals were incapacitated before any convulsive activity. All rats, except two, exhibited tonic convulsions at which time respiration ceased and did not return for 15–30 s after the convulsion. Several rats died immediately after the tonic convulsion. Rats exposed to Vikane at the three higher doses had increased salivation and lachrymation (red-stained). When the exposed rats approached death, their respiratory pattern changed from shallow and rapid to slow and deep; also, some gasping was noted.

The primary findings of gross pathology and histopathology examinations were of vascular congestion of several tissues, including the lung. The pathogenesis of the lesions and the cause of death of animals exposed to sulfuryl fluoride at high concentrations remain to be defined (Albee et al., 1983).

(e) Acute physiological parameters in rats

Groups of three male Fischer 344 rats were exposed to Vikane (purity not stated) at 4000 or 20 000 ppm and one female rat exposed at 20 000 ppm by inhalation to investigate electroencephalogram (EEG), ECG, blood pressure, heart rate, body temperature (with and

without water jacket) and rate of respiration. Owing to the short survival at 20 000 ppm, some measurements could not be performed. Two female rats were exposed at 4000 ppm for determination of body temperature. The intakes were not calculated as the study was not relevant

Table 43.Time to incapacitation and death in rats exposed to sulfuryl fluoride

Concentration (ppm)	No. of rats	Time to incapacity and cessation of exposure (min)	Time to death (min)
4 000	5	41.5	Approximately 200
10 000	5	16.3	Approximately 75
20 000	5	10.3	< 22
40 000	5	6.4	< 20

From Eisenbrandt et al. (1987)

to the assessment of dietary intake. The study complied with GLP but was not conducted according to any guideline.

Five rats at 4000 ppm survived for 79 ± 10 min, with a range of 65–90 min. The mean survival time ± standard deviation for the group at 20 000 ppm ($n = 4$) was 14 ± 4 min with a range of 9–19 min. The manner of death was gradual without any predictable abrupt bodily contortions characteristic of convulsive seizures. Grossly, the ears and extremities became pale, suggesting vasoconstriction in an attempt to maintain body core temperature

The response to sulfuryl fluoride was similar at the two concentrations and included a decrease in heart rate, a gradual increase in blood pressure (culminating in a rapid increase in systolic and pulse pressure just before death), decreased respiration, occasional spiking and high frequency loss in the EEG, and power loss in the EEG. Grossly, the rats exhibited paleness of the extremities and felt cold when removed from the exposure restraint. All physiological parameters ceased to function at about the same time the animals expired regardless of the dose. No parameter was affected first and subsequently precipitated the death of the animal. Artificial maintenance of body temperature did not increase survival time; therefore, the loss of body temperature did not contribute significantly to the cause of death. The authors considered that death may be associated in some manner with the blocking of the endogeneous energy-producing process of oxidative phosphorylation in glycolysis (Gorzinski & Streeter, 1985).

(f) Ultrastructure of the lungs of rats exposed to sulfuryl fluoride at high concentrations

Groups of two male Fischer 344 rats were exposed to sulfuryl fluoride (purity, 99.8%; lot No. WP 030680 217) at a concentration of 4000 or 20 000 ppm by inhalation while walking on a motorized activity wheel. Three control rats were also included in the study. Intakes were not calculated as the study was not relevant to the assessment of dietary intake. Treated rats were exposed until incapacitated. The rats were submitted for necropsy soon after exposure and the lungs were fixed by vascular perfusion. Representative sections of lung were evaluated by light and electron microscopy. The study complied with GLP but was not conducted according to any guideline.

Rats at 4000 ppm were incapacitated after 42–43 min of exposure; one rat subsquently became moribund 83 min after initiation of the exposure. Rats at 20 000 ppm were incapacitated after 12–13 min; one of these animals was moribund at that time (13 min) while the other rat was moribund 31 min after initiation of the exposure.

Histopathological examination of lung tissue by light microscopy revealed minimal changes in rats at 4000 ppm. These rats had congestion of pulmonary vessels and alveolar or interstitial oedema with or without haemorrhage. Rats at 20 000 ppm had more extensive congestion, oedema and haemorrhage.

Viewed by electron microscopy, the ultrastructure of pulmonary tissue from control rats was normal. Lungs of rats at 4000 ppm had multifocal alterations in the alveoli. Segmental swelling and occasional focal protrusions (blebs) from the basement membrane were observed in type I (squamous) epithelial cells of some alveoli. Minimal focal disruptions of type I epithelial cells were present in a few alveoli. The lungs of rats at 20 000 ppm had more extensive ultrastructural changes. The most severe lesions consisted of multifocal destruction of the alveolar wall characterized by disruption of epithelial (type I) and endothelial cells and the subjacent basement membrane. Some type II epithelial cells also were swollen (Eisenbrandt et al., 1987).

(g) Effects in rats treated with calcium gluconate or anticonvulsants

This study was designed to investigate the protective and therapeutic effectiveness of calcium gluconate (CaG)—an antagonist to toxicity caused by fluoride ion—and three anticonvulsants (phenobarbital, diazepam, or diphenylhydantoin) in animals exposed by inhalation to sulfuryl fluoride (purity, 99.8%; lot No. WP 030680 217) at a lethal concentration. Groups of Fischer 344 rats were: (1) exposed to sulfuryl fluoride; or (2) pretreated with one of the above substances and exposed to sulfuryl fluoride; or (3) exposed to sulfuryl fluoride and then treated with one of the above substances. Sulfuryl fluoride was administered at a concentration of 0, 4000 or 10 000 ppm for 55, 45 or 16 min, respectively. The exposure interval for rats at 4000 or 10 000 ppm was sufficient to cause incapacitation or convulsions in at least some animals. CaG was administered at an intraperitoneal dose of 500 mg/kg bw. The anticonvulsants were administered at the following doses: phenobarbital, 35 mg/kg bw, intraperitoneally; diazepam, 5 mg/kg bw, intraperitoneally; diphenylhydantoin 80 mg/kg bw, intraperitoneally). Intakes were not calculated as the study was not relevant to the assessment of dietary intake. The study complied with GLP but was not conducted according to any guideline. The study design and results are summarized in Table 44.

Calcium gluconate and phenobarbital (given before or after exposure to sulfuryl fluoride) and, to a lesser extent, diazepam (given before and/or after exposure to sulfuryl fluoride) appeared to ameliorate the acute toxic effects (seizures) and lethality in rats exposed to sulfuryl fluoride at 4000 ppm but not at 10 000 (Table 44). Diphenylhydantoin is contraindicated, as it induces more severe seizures than seen in animals treated with sulfuryl fluoride only (Nitschke et al., 1985).

(h) Studies with metabolites

The notifier did not submit any studies on metabolites. The primary residue in commodities treated with sulfuryl fluoride is fluoride ion. The toxicity of and exposures to compounds containing fluoride has been reviewed by the IPCS (2002). The following text contains excerpts from this summary:

> *Effects in mammals and in vitro*
>
> In humans and laboratory animals, the absorption of ingested fluoride into the general circulation occurs primarily in the stomach and intestine and is dependent upon the relative aqueous solubility of the form consumed. Soluble fluorides are almost completely absorbed from the gastrointestinal tract; however, the extent of absorption may be reduced by complex formation with aluminium, phosphorus, magnesium or calcium. There is partial to complete absorption of gaseous and particulate fluorides from the respiratory tract, with the extent of absorption dependent upon solubility and particle size.
>
> Fluoride is rapidly distributed by the systemic circulation to the intracellular and extracellular water of tissues; however, in humans and laboratory animals, approximately 99% of the total body burden of fluoride is retained in bones and teeth. In teeth and skeletal tissue, fluoride becomes incorporated into the crystal lattice.
>
> Fluoride crosses the placenta and is transferred from mother to fetus. Fluoride is eliminated from the body primarily in the urine. In infants, about 80–90% of a fluoride dose is retained; in adults, the corresponding figure is approximately 60%. These values can be altered by alterations in urinary flow and urinary pH.

Table 44. Survival of rats treated with calcium gluconate or anticonvulsants before or after exposure to sulfuryl fluoride

Group	Pretreatment	Exposure to sulfuryl fluoride (ppm)	Treatment after exposure to sulfuryl fluoride	No. of surviving/No. of treated rats
1	None	4 000	None	0/10
2	CaG (500 mg/kg bw, intraperitoneal)	4 000	None	6/10
3	None	4 000	CaG (500 mg/kg bw, intraperitoneal)	4/10
4	None	10 000	None	0/5
5	CaG (500 mg/kg bw, intraperitoneal)	10 000	None	0/5
6	None	10 000	CaG (500 mg/kg bw, intraperitoneal)	0/5
7	None	4 000	None	0/5
8	Phenobarbital (35 mg/kg bw, intraperitoneal)	4 000	None	5/5
9	Diphenylhydantoin (80 mg/kg bw, intraperitoneal)	4 000	None	0/5
10	Diazepam (5 mg/kg bw, intraperitoneal)	4 000	Diazepam (2.5 mg/kg, intraperitoneal)	4/5
11	None	4 000	Phenobarbital (35 mg/kg, intraperitoneal, and 20 mg/kg, intravenous	5/5
12	None	4 000	Diazepam (5 mg/kg, intraperitoneal)	2/5
13	None	10 000	Phenobarbital (35 mg/kg, intraperitoneal, and 20 mg/kg, intravenous)	0/5

From Nitschke & Miller (1985)

CaG, calcium gluconate

Fluoride is present in body organs, tissues and fluids. Concentrations of fluoride in whole blood of individuals residing in a community in the USA receiving fluoridated drinking-water ranged from 20 to 6 µg/l. The mean plasma level in 127 subjects with 5.0 mg fluoride/l in their drinking-water was 106 ± 76 (SD) µg/litre. Serum and plasma contain virtually the same amount of fluoride. Levels of fluoride in calcified tissues are generally highest in bone, dentine and enamel. The concentration of fluoride in bone varies with age, sex and the type and specific part of bone and is believed to reflect an individual's long-term exposure to fluoride. The concentration of fluoride in dental enamel decreases exponentially with the distance from the surface and varies with site, surface attrition, systemic exposure and exposure to topically applied fluoride. The concentration of fluoride in soft tissues is reflected by that in blood. Levels of fluoride in the urine of healthy individuals are related to the intake of fluoride. Increased levels of urinary fluoride have been measured in individuals following occupational exposure to airborne fluoride and among those residing in areas associated with endemic fluorosis.

Effects on the skeleton, such as inhibition of bone mineralization and formation, delayed fracture healing and reductions in bone volume and collagen synthesis, have been observed in a variety of studies in which rats received fluoride orally for periods of 3–5 weeks. In medium-term exposure studies, altered bone remodelling, hepatic megalocytosis, nephrosis, mineralization of the myocardium, necrosis and/or degeneration of the seminiferous tubules

in the testis were observed in mice administered fluoride in drinking-water (> 4.5 mg/kg body weight per day) over a period of 6 months.

In a comprehensive carcinogenicity bioassay in which groups of male and female F344/N rats and $B6C3F_1$ mice were administered drinking-water containing up to 79 mg fluoride/litre as sodium fluoride for a period of 2 years, there was no statistically significant increase in the incidence of any tumour in any single exposed group. There was a statistically significant trend of an increased incidence of osteosarcomas in male rats with increasing exposure to fluoride. However, the incidence was within the range of historical controls.

Another 2-year carcinogenicity bioassay involving Sprague-Dawley rats exposed to up to 11.3 mg/kg body weight per day in the diet also found no statistically significant increase in the incidence of osteosarcoma or other tumours. Another study, which reported an increased incidence of osteomas in mice receiving up to 11.3 mg/kg body weight per day, is difficult to interpret, because the animals were infected with Type C retrovirus.

In general, fluoride is not mutagenic in prokaryotic cells. Although fluoride has been shown to increase the frequency of mutations at specific loci in cultured mouse lymphoma and human lymphoblastoid cells, these mutations are likely due to chromosomal damage rather than point mutations. Fluoride has been shown to be clastogenic in a variety of cell types. The mechanism of clastogenicity has been attributed to the effect of fluoride upon the synthesis of proteins involved in DNA synthesis and/or repair, rather than direct interaction between fluoride and DNA. In most studies in which fluoride was administered orally to rodents, there was no effect upon sperm morphology or the frequency of chromosomal aberrations, micronuclei, sister chromatid exchange or DNA strand breaks. However, cytogenetic damage in bone marrow or alterations in sperm cell morphology were reported when the substance was administered to rodents by intraperitoneal injection.

Reproductive or developmental effects were not observed in recent studies in which laboratory animals were administered fluoride in drinking-water. However, histopathological changes in reproductive organs have been reported in male rabbits administered (orally) 4.5 mg fluoride/kg body weight per day for 18–29 months, in male mice administered (orally) > 4.5 mg fluoride/kg body weight per day for 30 days and in female rabbits injected subcutaneously with > 10 mg fluoride/kg body weight per day for 100 days. Adverse effects on reproductive function have been reported in female mice administered (orally > 5.2 mg fluoride/kg body weight per day on days 6–15 after mating and in male rabbits administered (orally) > 9.1 mg fluoride/kg body weight per day for 30 days.

Effects on humans

Epidemiological investigations on the effects of fluoride on human health have examined occupationally exposed workers employed primarily in the aluminium smelting industry and populations consuming fluoridated drinking-water. In a number of analytical epidemiological studies of workers occupationally exposed to fluoride, an increased incidence of lung and bladder cancer and increased mortality due to cancer of these and other sites have been observed. In general, however, there has been no consistent pattern; in some of these epidemiological studies, the increased morbidity or mortality due to cancer can be attributed to the workers' exposure to substances other than fluoride.

The relationship between the consumption of fluoridated drinking-water and morbidity or mortality due to cancer has been examined in a large number of epidemiological studies, performed in many countries. There is no consistent evidence of an association between the consumption of controlled fluoridated drinking-water and increased morbidity or mortality due to cancer.

Fluoride has both beneficial and detrimental effects on tooth enamel. The prevalence of dental caries is inversely related to the concentration of fluoride in drinking-water. The prevalence of dental fluorosis is highly associated with the concentration of fluoride, with a positive dose–response relationship.

Cases of skeletal fluorosis associated with the consumption of drinking-water containing elevated levels of fluoride continue to be reported. A number of factors, such as nutritional status and diet, climate (related to fluid intake), concomitant exposure to other substances and the intake of fluoride from sources other than drinking-water, are believed to play a significant role in the development of this disease. Skeletal fluorosis may develop in workers

occupationally exposed to elevated levels of airborne fluoride; however, only limited new information was identified.

Evidence from several ecological studies has suggested that there may be an association between the consumption of fluoridated water and hip fractures. Other studies, however, including analytical epidemiological investigations, have not supported this finding. In some cases, a protective effect of fluoride on fracture has been reported.

Two studies permit an evaluation of fracture risk across a range of fluoride intakes. In one study, the relative risks of all fractures and of hip fracture were elevated in groups drinking water with > 1.4 mg fluoride/litre (total intake > 6.5 mg/day); this difference reached statistical significance for the group drinking water containing > 4.32 mg fluoride/litre (total intake 14 mg/day). In the other study, an increased incidence of fractures was observed in one age group of women exposed to fluoride in drinking-water in a non-dose-dependent manner.

Epidemiological studies show no evidence of an association between the consumption of fluoridated drinking-water by mothers and increased risk of spontaneous abortion or congenital malformation. Other epidemiological investigations of occupationally exposed workers have provided no reasonable evidence of genotoxic effects or systemic effects upon the respiratory, haematopoietic, hepatic or renal systems that may be directly attributable to fluoride exposure per se.

Evaluation of human health risks

Fluoride has both positive and negative effects on human health, but there is a narrow range between intakes that are associated with these effects. Exposure to all sources of fluoride, including drinking-water and foodstuffs, is important.

There is little information to characterize the dose–response relationships for the different adverse effects. In particular, there are few data on total exposure, particularly with respect to intake and fluoride absorption.

The most serious effect is the skeletal accumulation of fluoride from long-term excessive exposure to fluoride and its effect on non-neoplastic bone disease — specifically, skeletal fluorosis and bone fractures. There is clear evidence from India and China that skeletal fluorosis and an increased risk of bone fractures occur at total intakes of 14 mg fluoride/day and evidence suggestive of an increased risk of bone effects at total intakes above about 6 mg fluoride/day….

Reviews of fluoride toxicity and proposals for upper levels of intake have been performed by the US National Academy of Sciences (1997) and the European Food Safety Authority (2004).

3. Observations in humans

3.1 Plant workers

Sulfuryl fluoride has been registered for use as a structural fumigant in the USA since 1959, and marketed for that use since 1961. Before 1975, Allied Chemical produced sulfuryl fluoride that was packaged and sold by Dow under the Vikane label. Dow began producing Vikane at its Pittsburg, California, plant in 1975; this plant is the only large-scale production facility for sulfuryl fluoride in the world today. The plant has 21 employees who participate in health surveillance examinations every 2 years. No significant sulfuryl fluoride-related health problems have been detected in these examinations. Review of safety records and on-site medical clinic records for 1992–2001 disclosed 10 exposures by inhalation and seven skin burns, primarily related to exposure to hydrogen fluoride used in the process.

No epidemiology studies focused on the health effects of sulfuryl fluoride have been carried out on this group of employees.

3.2 Poisoning incidents

Sulfuryl fluoride is used as a structural fumigant, primarily for the control of dry wood termites in the warmer climates of the USA. Its most common application is in building fumigations, where the building is first wrapped in plastic tarpaulins, and the gas is then released into the sealed structure. Chloropicrin is used as warning agent (lachrymator) when sulfuryl fluoride is used in dwelling fumigations (i.e. in homes), but it is not intended to use chloropicrin with ProFume fumigations in the USA or the European Union. Sulfuryl fluoride also has a label use in the USA for fumigation of vehicles such as rail cars, buses and ships.

In the USA since 1993 there have been 335 reports (included incidents from previous years that were cited in subsequent litigation) of alleged human health effects associated with sulfuryl fluoride reported to the US EPA by Dow AgroSciences. These allegations were received from PROSAR, a human health poison control centre, employees of Dow AgroSciences, customers, and other sources. Thirteen people died, primarily subsequent to unauthorized entry into the tented fumigated structure, as apparent break-ins or one case in attempt to rescue a cat. The majority (60%) of the non-fatal incidents involved symptoms of eye and respiratory irritation, sore throat and cough; in some of these cases the symptoms may have been related to residual chloropicrin in the dwellings subsequent to ventilation and clearing of sulfuryl fluoride from the structure. The next most common (9%) complaint was flu-like symptoms of nausea, diarrhoea, fever, and headache. Approximately 6% complained primarily of shortness of breath or respiratory distress. From the PROSAR records of August 1997 to May 2001, 2.2% of cases required treatment in a health-care facility or emergency room; for June 2001 to June 2002, 3.1% of cases required such treatment. Of the cases in PROSAR records since 1997 to the present, 87% were either asymptomatic or had only minor symptoms.

A relatively recent incident involving sulfuryl fluoride happened in 2002 in Germany during a church fumigation. A fatality was caused by gas leaking into a neighbouring house, which was connected to the church by a closed passageway. The incident involved a fumigator who did not adhere to the German guidelines TRGS 512. No risk area was defined nor monitored, the monitoring equipment was not properly calibrated, the size of the church and the gas volume were incorrectly calculated, the residents of the neighbouring grounds were not informed that the fumigation was taking place.

Two epidemiology studies of fumigators who use sulfuryl fluoride have been published. A study comparing the health and neurological performance of male, Californian sulfuryl fluoride (n = 24) and methyl bromide fumigators (n = 32) with a referrent group (n=29) of non-fumigators was published in 1986. The investigations included a clinical neurological examination, nerve conduction measurements, neurobehavioural tests and a general medical questionnaire. A number of differences between the group exposed to sulfuryl fluoride and the referrent group were identified as potential confounding factors (age, level of education, alcohol consumption, illegal drug use and racial mix). These factors were noted by the study authors and investigated to an extent, but it was not possible to perform statistical corrections owing to the small group sizes. The group exposed to sulfuryl fluoride had more general symptoms than did the referrent group, but fewer than the group exposed to methyl bromide; it is unclear whether the symptoms are linked with the process of fumigation, exposure to the chemicals involved or factors unrelated to fumigation. There were no significant differences betweeen the sulfuryl fluoride fumigators and referrents in: nerve conduction velocity; grip strength; tests for coordination; neurological, visual and cognitive examinations. Some significant effects were identified in the group exposed to methyl bromide, and in some tests the results for the group exposed to sulfuryl fluoride fell between those for the referents and the methyl bromide fumigators. The study authors concluded that there was a pattern of effects in the group exposed to sulfuryl fluoride that merited further investigation (Anger et al., 1986).

A cross-sectional study of 123 fumigation workers using methyl bromide or sulfuryl fluoride in Florida was performed in 1991–1992. The fumigation workers were compared with a group of friends or neighbours who had never been employed as pesticide applicators. The fumigators using sulfuryl fluoride for > 80% of the fumigations made up the group exposed to sulfuryl fluoride (n = 91; duration of use, 0.1–20 years, median, 2.8 years). Because of the limited use of methyl bromide, the criterion for this group was set at 50% use of methyl bromide and it is likely that many of the workers in the group exposed to methyl bromide (n =28) would also use sulfuryl fluoride. The study investigated general medical condition/history, nerve conduction velocity, vibration sensitivity, coordination, balance, reactions, memory, learning, visual function, balance, smell, dexterity, and gross neurological abnormalities. The numerical data presented in the paper were only for fumigators versus controls and did not differentiate between groups ewpoxed to sulfuryl fluoride or methyl bromide. Some statistical comparisons were performed with duration of individual fumigant use. The fumigators and controls were relatively well matched and corrections were performed for a number of parameters. Significant effects associated with use of sulfuryl fluoride were noted in tests on forearm nerve conduction, dexterity, pattern memory and odour recognition. The results for the forearm nerve and for dexterity were considered to be related to physical activity involved with part of the fumigation process. It was also considered unlikely that an effect on one nerve would be related to chemical exposures—the fumigators using sulfuryl fluoride performed better in 8 out of 13 tests of nerve conduction and vibration. Pattern memory (p = 0.05) was the only computer-administered neurobehavioural test in which fumigators performed significantly less well than controls, so it is unclear whether this was a real or chance finding. The odour recognition finding (p = 0.03) was considered to be of uncertain etiology—irritation, olfactory receptor damage or odour recognition. In the tests for odour recognition and pattern memory, the group using methyl bromide (which probably contained several workers using sulfuryl fluoride) performed better than did the controls (Calvert et al., 1998).

Comments

Biochemical aspects

In rats exposed to [^{35}S]-labelled sulfuryl fluoride at 30 or 300 ppm by inhalation, the radiolabel was rapidly absorbed, achieving maximum concentrations in both plasma and erythrocytes near the end of the 4-h exposure period. Once absorbed, the radiolabel was rapidly excreted, primarily via the urine. The radiolabel was rapidly cleared from the plasma and erythrocytes with initial half-lives of approximately 2.5 h after exposure at 30 ppm and 1–2.5 h after exposure at 300 ppm, but the terminal half-life of radioactivity was approximately 2.5-fold longer in erythrocytes than in plasma. The identification of fluorosulfate and sulfate in blood and urine suggests that sulfuryl fluoride is rapidly hydrolysed to fluorosulfate, with the release of fluoride, followed by further hydrolysis to sulfate and release of the remaining fluoride. This is supported by the observation of increases in fluoride in blood and urine after exposure of rats to sulfuryl fluoride. Seven days after exposure, radioactivity was widely distributed with significant concentrations remaining in tissues at the site of first exposure to the gas.

Toxicological data

The primary concern of the Meeting was the risk assessment for dietary exposures to sulfuryl fluoride. Sulfuryl fluoride is a gas and routine tests for toxicity via the oral and dermal routes are difficult to perform. All the critical studies involved exposures by inhalation (for about 6 h/day, 5 days/week) and it was necessary to convert these to systemic doses in order to derive health-based guidance values. To convert from concentrations in air to a systemic dose in mg/kg bw per day, account was taken of the respiratory rates and volumes of the animals[3], the duration

[3] Twenty-four h respiratory volumes for test species: rats, 0.96 m^3/kg bw; rabbits, 0.54 m^3/kg bw; mice, 1.8 m^3/kg bw;

of exposure (hours/day and days/week) and the proportion (10%) of the inspired dose that was absorbed based on a toxicokinetic study.

In assessing the effects of sulfuryl fluoride, the Meeting focused on effects related to systemic exposures rather than local effects linked with sulfuryl fluoride gas. In foodstuffs exposed to sulfuryl fluoride the predominant residue is fluoride ion, although some residues of sulfuryl fluoride have been detected in certain fumigated products. The data indicated that some toxic effects observed after exposure to sulfuryl fluoride (e.g. renal toxicity) were consistent with the toxicity of fluoride. The Meeting concluded that the "slight" dental fluorosis seen in some studies was not an adverse finding. Although no studies on fluoride were submitted, the Meeting was aware of a number of recent expert evaluations of exposure to and toxicity attributable to fluoride.

Sulfuryl fluoride was found to be moderately acutely toxic when administered by the oral route (LD_{50} of approximately 100 mg/kg bw; sulfuryl fluoride bubbled into corn oil), but the Meeting noted that the results of this study were difficult to interpret owing to the very high volume of corn oil administered (40 ml/kg bw). A standard study of dermal toxicity could not be performed, but whole-body (excluding head) exposure did not indicate any significant toxicity after exposure via the dermal route. Sulfuryl fluoride gas administered via inhalation has been extensively investigated in several studies of acute toxicity in rats and mice and was found to have low to moderate toxicity. All studies in rats and one of two studies in mice reported 4-h LC_{50} values of > 2 mg/l (about 500 ppm). Exposure of humans to sulfuryl fluoride gas at high concentrations within enclosed structural fumigation areas has resulted in death. No tests for skin and eye irritation or studies of skin sensitization have been conducted. However, whole-body exposures and experience in humans over a period of 40 years of use indicate that sulfuryl fluoride is not a significant irritant, nor a skin sensitizer. Mechanistic studies on "time to acute incapacitation" have revealed an approximately linear relationship between concentration and duration of exposure.

In a study carried out in 1959 in which rats were fed for 66 days with diets previously exposed to sulfuryl fluoride, the NOAEL was 2.5 mg of total fluoride/kg bw per day on the basis of reduced body-weight gain and evidence of fluorosis, but the details reported were limited and relatively few end-points were investigated. Sulfuryl fluoride has been studied in short-term studies of toxicity in rats, dogs, mice and rabbits exposed by inhalation; in most experiments, the exposure period was 6 h/day, 5 days/week. In 14-day studies of exposure by inhalation, the lowest NOAEC was 30 ppm (approximately equivalent to systemic exposure at 4.1 mg/kg bw per day) in mice on the basis of brain vacuolation, while the NOAEC in dogs was 100 ppm (approximately equivalent to systemic exposure at 2.9 mg/kg bw per day) on the basis of tremors and tetany, but no evidence of brain lesions. The NOAEC was also 30 ppm in 90-day studies in mice (approximately equivalent to systemic exposure at 4.1 mg/kg bw per day), and in rabbits (approximately equivalent to systemic exposure at 1.4 mg/kg bw per day). In these studies the LOAEC was 100 ppm on the basis of vacuolation in the brain. Local effects on the respiratory tract were seen in many of the studies of administration via inhalation, but the Meeting considered that these were not relevant to dietary intakes. In a 1-year study in dogs exposed by inhalation, the NOAEC was 80 ppm (approximately equivalent to systemic exposure at 2.3 mg/kg bw per day) on the basis of deaths and general toxicity (including brain vacuolation) at 150 ppm. A higher concentration of 200 ppm was not tolerated by the dogs beyond approximately 9 months, when primarily respiratory effects were associated with a terminal decline in health status. Slight dental fluorosis was the most sensitive effect in the 13-week study in rats and the 1-year study in dogs, but the Meeting concluded that this was not an adverse finding. Although no specific investigations were performed on other end-points associated with excess exposure to fluoride, e.g. bone density, the Meeting concluded that the NOAELs used for risk assessment provided adequate protection for the bone effects of fluoride, as such effects are considered to be at least threefold less sensitive than dental fluorosis, on the basis of human observations.

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and dogs, 0.39 m^3/kg bw.

In rats, the principal effects of long-term exposure by inhalation were reduced survival, brain vacuolation, chronic progressive glomerular nephrosis and associated lesions such as fibrous osteodystrophy in both sexes exposed at 80 ppm (approximately equivalent to 5.6 mg/kg bw per day). These latter findings are consistent with toxicity attributable to fluoride ions. In mice, the principal effects were reduced survival and slight vacuolation in the cerebrum. In rats, the NOAEC was 20 ppm (approximately equivalent to 1.4 mg/kg bw per day). In mice, the NOAEC was 20 ppm (approximately equivalent to 3.0 mg/kg bw per day). Sulfuryl fluoride was not tumourigenic or carcinogenic in rats or mice at concentrations of up to 80 ppm, the highest concentration tested (approximately equivalent to 5.6 and 12 mg/kg bw per day, respectively).

Sulfuryl fluoride showed no genotoxic potential in tests in vitro for bacterial cell mutation or unscheduled DNA synthesis in mammalian cells. The results of tests for mutagenicity and clastogenicity in mammalian cells in vitro (mouse lymphoma $Tk^{+/-}$ and rat lymphocytes) were positive, consistent with the database on genotoxicity of the fluoride ion. A test for micronucleus formation in vivo gave negative results. The Meeting noted that sulfuryl fluoride is a highly reactive compound and dietary exposures would be predominantly to fluoride ion. It is generally recognized that fluoride does not represent a genotoxic risk to humans in vivo.

The Meeting concluded that consumption of foodstuffs treated with sulfuryl fluoride would not present a genotoxic risk to humans.

In view of the negative results obtained in studies of genotoxicity in vivo and the absence of carcinogenicity in mice and rats, the Meeting concluded that sulfuryl fluoride is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproduction, no effect on reproductive parameters was observed in rats exposed by inhalation to sulfuryl fluoride at concentrations of up to 150 ppm, the highest concentration tested (approximately equivalent to 11 mg/kg bw per day). At 150 ppm, parental toxicity comprised reduced body weights and brain vacuolation; the NOAEC was 20 ppm. Reduced body-weight gain in F_1 and F_2 pups during the lactation period was noted at 150 ppm and was the only effect in offspring. The NOAEC in offspring was 20 ppm (approximately equivalent to 1.4 mg/kg bw per day). Sulfuryl fluoride has been tested for developmental effects in both rats and rabbits and found not to be teratogenic in either species. Pregnant rabbits were somewhat more sensitive to sulfuryl fluoride than were pregnant rats. In rats, there were no adverse effects on dams or offspring exposed to sulfuryl fluoride at concentrations of up to 225 ppm, the highest concentration tested (approximately equivalent to 16 mg/kg bw per day). In rabbits, however, there was slight toxicity to dams and offspring at 225 ppm, which was manifested as reduced body weights and lower fetal weights. The lowest relevant NOAEC for developmental toxicity was 75 ppm (approximately equivalent to 4.3 mg/kg bw per day) in rabbits.

Three studies specifically investigated the neurotoxicity of sulfuryl fluoride: a study of acute toxicity in rats exposed via inhalation, a 13-week study in rats exposed via inhalation and a 1-year study in rats exposed via inhalation (a satellite group of the long-term/carcinogenicity study). The 13-week study was conducted first and comprised comprehensive electrophysiological tests, a functional observational battery (FOB) and histological examination of the peripheral and central nervous system. It demonstrated that the most sensitive indicator of effects on the nervous system after 13 weeks was a change in evoked potentials (visual, auditory and somatosensory). At a dietary concentration of 100 ppm and greater, visual and somatosensory evoked potentials were significantly slower in exposed female rats and auditory brainstem responses were possibly slower in exposed males relative to controls. Only at 300 ppm were histological effects evident, in the form of mild vacuolation in the brain (specifically, white fibre tracts of the caudate putamen). The NOAEC in the 13-week study was 30 ppm (approximately equivalent to 2.2 mg/kg bw per day) on the basis of alterations in evoked potentials at 100 ppm in females.

On the basis of the findings in the 13-week study of neurotoxicity, a study of acute neurotoxicity (two 6 h exposures in 30 h) in female rats was performed. This included extensive neurophysiological and behavioural investigations, including evoked potentials, but there were no

investigations of brain histopathology. The Meeting considered that the absence of investigations of brain histopathology was not crucial as the brain lesions did not appear to be an acute effect, being absent in dogs or rats after 2 weeks, but were present at lower exposures in the 13-week studies. No adverse effects were produced at 300 ppm (approximately equivalent to 31 mg/kg bw per day), the highest concentration tested. The 1-year study of neurotoxicity in male and female F344 rats included a FOB, motor activity tests, fore- and hindlimb grip strength, hindlimb landing foot splay and neurohistopathology with perfusion fixation. These animals were a satellite group of the long-term/carcinogenicity study and no general histopathological examinations were performed as these were covered by other segments of the study. There were no effects on the nervous system at 80 ppm (approximately equivalent to 5.6 mg/kg bw per day), the highest concentration tested.

Sulfuryl fluoride has been used as a structural fumigant for more than 40 years. Health surveillance examinations in manufacturing plants have revealed no significant sulfuryl fluoride-related health problems among employees. Thirteen deaths have been reported in humans who gained access to buildings during fumigation, but the lethal concentration has not been determined. More than 300 incidents of non-lethal adverse effects associated with exposure to sulfuryl fluoride have been reported in the USA. Symptoms included irritation of eyes and respiratory tract, headache, nausea, fever and diarrhoea; some of these might be attributable to exposure to chloropicrin used as a sensory marker. Two epidemiological investigations of sulfuryl fluoride and methyl bromide fumigators have reported a small number of findings in the cohorts exposed to sulfuryl fluoride. Some of these findings appear to be related to physical activities associated with the fumigation process. Others present no clear pattern that can be attributed to the use of sulfuryl fluoride. In neither study was there any biomonitoring to assess exposure.

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

Toxicological evaluation

The Meeting established an ADI for sulfuryl fluoride of 0–0.01 mg/kg bw based on a NOAEC of 20 ppm (approximately equivalent to systemic exposure at 1.4 mg/kg bw per day) in both a 24-month study in rats exposed to sulfuryl fluoride by inhalation, on the basis of effects on the kidney, brain, bone and survival at 80 ppm, and the two-generation study of reproductive toxicity in rats exposed to sulfuryl fluoride by inhalation, on the basis of effects on the brain and body weight at 150 ppm, with a 100-fold safety factor**.** The Meeting noted that some of the end-points in the long-term study in rats, such as kidney toxicity, were consistent with the data on fluoride toxicity. The Meeting considered that the slight dental fluorosis seen at the toxicological NOAEC was not an adverse effect.

The Meeting noted that the residue resulting from sulfuryl fluoride fumigation of foodstuffs was primarily fluoride. The critical studies of toxicity with sulfuryl fluoride used inhalation exposures and while this would result in a significant systemic dose of fluoride, it was impossible to separate reliably the effects attributable to systemic exposure to fluoride with those attributable to gaseous sulfuryl fluoride. The Meeting did not receive any studies on fluoride that would enable it to derive reference values for fluoride. The Meeting concluded that the dietary intake of fluoride associated with the use of sulfuryl fluoride as a fumigant should be included in an overall assessment of fluoride from all sources. Upper levels for fluoride intakes have been proposed by a number of organizations[4].

The Meeting established an ARfD of 0.3 mg/kg bw for sulfuryl fluoride based on a NOAEC of 300 ppm (approximately equivalent systemic exposure at 31 mg/kg bw per day) the highest concentration tested in a study of acute neurotoxicity in rats exposed to sulfuryl fluoride by inhalation, and a 100-fold safety factor. The Meeting noted that there was no clear evidence for

[4] For example, see: http://www.efsa.eu.int/science/nda/nda_opinions/851_en.html or http://www.nap.edu/books/0309063507/html/288.html

acute systemic toxicity associated with sulfuryl fluoride. However, as the acute oral LD_{50} was reported to be about 100 mg/kg bw, the Meeting agreed on the need to derive an ARfD. The Meeting concluded that the only appropriate study for deriving the ARfD was the study of acute neurotoxicity, although this was likely to result in a conservative assessment and was probably not relevant to intakes of fluoride ion as such from sulfuryl fluoride-treated commodities. The Meeting considered that the critical end-point of brain vacuolation, which had not been evaluated in this study, was not an acute effect based on its absence in the 2-week studies in dogs and rats.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month (6 h/day 5 days per week); whole-body exposure	Toxicity	20 ppm (3.0 mg/kg bw per day)	80 ppm (12 mg/kg bw per day)
		Carcinogenicity	80 ppm[a] (12 mg/kg bw per day)	—
Rat	Study of acute neurotoxicity (2 × 6 h in 30 h); whole-body exposure	Toxicity	300 ppm[a] (31 mg/kg bw per day)	—
	Two-year (6 h/day, 5 days per week); whole-body exposure	Toxicity	20 ppm (1.4 mg/kg bw per day)	80 ppm (5.6 mg/kg bw per day)
		Carcinogenicity	80 ppm[a] (5.6 mg/kg bw per day)	—
	Two-generation study of reproductive toxicity (6 h/day, 5 days per week); whole-body exposure	Reproduction	150 ppm[a] (11 mg/kg bw per day)	—
		Offspring	20 ppm (1.4 mg/kg bw per day)	80 ppm (5.6 mg/kg bw per day)
		Parental	20 ppm (1.4 mg/kg bw per day)	8 ppm (5.6 mg/kg bw per day)
	Developmental toxicity (6 h/day, 5 days per week) whole-body exposure	Maternal	225 ppm[a] (16 mg/kg bw per day)	—
		Developmental	225 ppm[a] (16 mg/kg bw per day)	—
Rabbit	Ninety-day (6 h/day, 5 days per week); whole-body exposure	Toxicity	30 ppm (1.4 mg/kg bw per day)	100 ppm (4.1 mg/kg bw per day)
	Study of developmental toxicity (6 h/day, 5 days per week); whole-body exposure	Maternal	75 ppm (4.3 mg/kg bw per day)	225 ppm (13 mg/kg bw per day)
		Developmental	75 ppm (4.3 mg/kg bw per day)	225 ppm (13 mg/kg bw per day)
Dog	One-year (6 h/day, 5 days per week); whole-body exposure	Toxicity and mortality	80 ppm (2.3 mg/kg bw per day)	200 ppm (5.8 mg/kg bw per day)

[a] Highest concentration tested

Estimate of acceptable daily intake for humans

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

Studies with sulfuryl fluoride administered orally

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

Critical end-points for setting guidance values for exposure to sulfuryl fluoride

Absorption, distribution, excretion and metabolism in mammals (studies with ^{35}S-labelled sulfuryl fluoride; fluoride was not investigated specifically)	
Rate and extent of absorption	Rapidly absorbed after exposure via inhalation (nose only); maximum concentrations attained near the end of 4-h exposure). Absorbed dose (radioactivity in urine, faeces and tissues) estimated to be 14% at 30 ppm and 11% of the dose entering lungs at 300 ppm.
Distribution	Seven days after exposure, ^{35}S was widely distributed among the tissues. Significant concentrations of radioactivity remained in tissues at the site of first exposure to the gas. Increased concentrations of fluoride were detected in blood and tissues.
Potential for accumulation	Increased intake of fluoride may lead to fluorosis (i.e. accumulation of fluoride in bones and teeth).
Rate and extent of excretion	Rapidly excreted, primarily via the urine. Radioactivity (^{35}S) was rapidly cleared from plasma and erythrocytes with initial half-lives of approximately 2.5 h after exposure at 30 ppm and 1–2.5 h after exposure at 300 ppm. The terminal half-life of radioactivity was about 2.5-fold longer in erythrocytes than in plasma.
Metabolism in animals	Initially hydrolysed to fluorosulfate, with release of fluoride, followed by further hydrolysis to sulfate and release of the remaining fluoride.
Toxicologically significant compounds (animals, plants and environment)	Sulfuryl fluoride and fluoride ion
Acute toxicity	
Rat LD_{50} oral	Approximately 100 mg/kg bw (bubbled into corn oil, dosed at 40 ml/kg bw)
Rat LD_{50} dermal	No adverse effects at 40.3 mg/l (4 h exposure, whole body except head)
Rat LC_{50} inhalation	4.7–5.8 mg/l (4 h exposure) (1000–1122 ppm)
Skin sensitization (test method used)	No data submitted, but repeated whole-body exposures and experience of use by humans have identified no indications of sensitization.
Short-term studies of toxicity	
Target/critical effects after inhalation	Local effect on respiratory tract (after inhalation): inflammation (rats, dogs, rabbits) and alveolar histiocytosis (rats), aggregates of macrophages in alveoli (dogs) Brain: vacuolation (rats/dogs/mice/rabbits) Kidney: mild hyperplasia, tubular degeneration (rats) Overt dental fluorosis (rats)
Target/critical effects after oral administration	Reduced body-weight gain, overt dental fluorosis, renal lesions
Lowest relevant oral NOAEL	Total fluoride, 2.5 mg/kg bw per day
Lowest relevant dermal NOAEL	None (no data)
Lowest relevant inhalation NOAEC	100 ppm (rats, 7.0 mg/kg bw per day) 80 ppm (dogs, 2.3 mg/kg bw per day)

	30 ppm (mice, 4.1 mg/kg bw per day; rabbits, 1.4 mg/kg bw per day)
Genotoxicity	
	Some positive results in vitro, negative results in vivo. No genotoxic risk to humans from dietary exposure.
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Kidney: renal failure (rats). Reduced survival (mice, rats) Brain: minimal vacuolation of cerebrum (mice, rats)
Lowest relevant NOAEC/NOAEL	20 ppm (mice, 3.0 mg/kg bw per day) 20 ppm (rats, 1.4 mg/kg bw per day)
Carcinogenicity	Not carcinogenic in rats or mice
Reproductive toxicity	
Reproduction target/critical effect ‡	Reproduction: none Parental toxicity: reduced body weight and brain vacuolation Offspring: reduced body weight during lactation
Lowest relevant reproductive NOAEC	Reproduction: 150 ppm (11 mg/kg bw per day)[a] Parental: 20 ppm (1.4 mg/kg bw per day) Offspring: 20 ppm (1.4 mg/kg bw per day)
Developmental target/critical effect	Rabbit: reduced fetal weights. Not teratogenic.
Lowest relevant developmental NOAEC	Maternal: 75 ppm (rabbits, 4.3 mg/kg bw per day) Developmental : 75 ppm (rabbit, 4.3 mg/kg bw per day) Teratogenicity: 225 ppm (rats and rabbits)[a]
Neurotoxicity/delayed neurotoxicity	
Two-day (two 6-h exposures in 30 h) study of acute neurotoxicity in female F344 rats	No effects at 30 ppm (31 mg/kg bw), the highest concentration tested
Thirteen-week (6-h exposures, 5 days per week) study of neurotoxicity in F344 rats	Mild vacuolation of the brain, slowing of visual auditory and somatosensory evoked potentials at 300 ppm. Evoked potentials slower in female rats and auditory brainstem responses possibly slower in males at 100 ppm. The NOAEC was 30 ppm (2.2 mg/kg bw per day). Recovery within 2 months.
Twelve-month (6-h exposures, 5 days per week) study of neurotoxicity in F344 rats	No effects on the nervous system at the highest concentration tested, NOAEC was 80 ppm (5.8 mg/kg bw per day).
Other toxicological studies	
	None submitted.
Medical data	
	In the USA, 335 reports of alleged human health effects associated with sulfuryl fluoride have been made since 1993. Thirteen human deaths, primarily from unauthorized entry into the tented fumigated structures. 60% of non-fatal incidents involved symptoms of irritation possibly related to residual chloropicrin (a sensory marker). The next most common (9%) complaint was flu-like symptoms of nausea, diarrhoea, fever, and headache; about 6% complained of shortness of breath or respiratory distress. No findings in production plant workers. Epidemiology studies of fumigators inconclusive.

Summary

Value	Study	Safety factor

ADI	0–0.01 mg/kg bw	Rat, 24-month study of toxicity and carcinogenicity after inhalation; reproductive toxicity after inhalation.	100
ARfD	0.3 mg/kg bw	Rat, acute neurotoxicity after inhalation	100

[a] Highest concentration tested

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