Section A6.3.3/04 **Repeated Dose Toxicity (Inhalation, 28-days)**
Annex Point IIA, VI.6.3

Rabbit (IIA 5.3.3.1d/01, D02)

Report:	Eisenbrandt, D.L., Nitschke, K.D., Streeter, C.M., Wolfe, E.L. (1985) Sulfuryl Fluoride (Vikane Gas Fumigant): 2-Week Inhalation Toxicity Probe with Rats and Rabbits Mammalian and Environmental Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan, USA Report K-16399-022, dated 2/4/85; study started 27/11/83. Also published in: Fundam. Appl. Toxicol., 12: 540 (1989).
Guidelines:	None cited. This study was conducted as a 'probe' for a 13-week study. Deviations from EC guideline Method B.8. Repeated Dose (28 Days) Toxicity (Inhalation): This was a 2-week study. The rabbit groups were only 3/sex/dose, otherwise it meets the guideline.
GLP:	Yes
Methodology:	Test material: Vikane gas fumigant (Lot #TWP 830919-408; 99.8% sulfuryl fluoride). Rabbits were exposed to 0, 100, 300, or 600 ppm sulfuryl fluoride (Vikane gas fumigant) for six hours/day, five days/week for nine exposures. Exposure groups consisted of three New Zealand White rabbits of each sex. Animals were observed daily and body weights were recorded several times throughout the study prior to 1st, 5th, 6th and 9th day of exposure (rabbits not weighed by mistake on 9th day). Prior to the 9th exposure, blood samples were collected from rats and rabbits for haematology (PCV, Hgb, RBC, WBC & differential count, MCV, MCH, MCHC and platelets). Serum samples were collected for clinical chemistry (UN, SGPT, SGOT, AP, glucose, total protein, albumin and globulins) and organs (brain, heart, liver, kidneys and testes) were weighed at the terminal sacrifice. A complete necropsy was performed on all animals and extensive histopathology was completed on controls and high dose groups. Otherwise, examination of tissues in intermediate dose groups was confined to target organs and several other tissues.
Findings:	
Mortality:	Two 600 ppm dose female rabbits died during the study: one had a fractured tibia subsequent to convulsions after the fifth exposure and the other had a fractured vertebrum after the 6th exposure, but convulsions were not observed.
Clinical signs:	One top dose rabbit had convulsions after the 5th exposure. Most rabbits exposed to 300 or 600 ppm had moderate nasal exudate, probably indicative of chronic inflammation due to exposure. Male and female rabbits that survived exposure to 600 ppm were slightly hyperactive compared to control animals.
Body weight:	There were no statistically identified effects on body weight.
Food consumption:	Not conducted.
Ophthalmology:	Not conducted.
Haematology:	No effects attributed to treatment other than an elevated WBC in one high dose male rabbit that had pneumonia, which might have been secondary to treatment.
Clinical chemistry:	A slight, non-significant effect on serum albumin in top dose was related to altered nutritional status.

Urinalysis: Not conducted.

Organ weights: Organ weight data are given in Table 5.3.3.1d/01-1. Terminal body weights were slightly decreased at 300 and 600 ppm. Some of these rabbits also had decreased liver weights at termination. The heart weight of the surviving female at 600 ppm appeared increased.

Table 5.3.3.1d/01-1: Summary of Organ Weight Data

Dose (ppm)	No.	Terminal Body Wt. (g)	Brain		Heart		Liver	
			g	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
Males								
0	**3**	3250.2	9.743	0.300	6.692	0.206	114.511	3.506
100	**3**	3212.3	9.269	0.289	6.971	0.217	117.894	3.679
300	**3**	3061.7	9.902	0.324	5.815	0.190	91.662	2.991
600	**3**	2917.8*	9.043*	0.310	5.861	0.201	84.153	2.889
Females								
0	**3**	3503.1	9.286	0.266	6.582	0.189	96.690	2.770
100	**3**	3593.7	9.872	0.276	6.704	0.187	137.213*	3.825*
300	**3**	3384.4	9.688	0.288	6.364	0.188	97.684	2.857
600	**1**	3315.7	10.292	0.310	8.470*	0.255*	88.949	2.683

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

Gross pathology: Other than the fractures observed for the premature decedent rabbits, there were no significant gross pathological findings.

Histopathology: Histopathology findings are summarised in Table 5.3.3.1d/01-2. Histopathology revealed treatment-related, focal malacia (necrosis) in the cerebrum of all rabbits exposed to 600 ppm sulfuryl fluoride as well as one male and one female exposed to 300 ppm. Also, the same part of the cerebrum was vacuolated in all rabbits that were exposed to 300 or 600 ppm. Most rabbits exposed to 300 or 600 ppm sulfuryl fluoride 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 of some rabbits. Alterations included lymphoid hyperplasia in the mediastinal lymph nodes and spleen.

Table 5.3.3.1d/01-2: Summary of Histopathology Findings

Sex	Males				Females			
Dose (ppm)	**0**	**100**	**300**	**600**	**0**	**100**	**300**	**600**
Number of Rabbits Examined	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3 (2)**
Brain								
Number of Tissues Examined	3	3	3	3	3	3	3	3 (2)
Within Normal Limits	3	3	0	0	2	3	0	0 (0)
Malacia, cerebrum, bilateral, focal: - slight	0	0	0	0	0	0	1	2 (2)
- moderate	0	0	1	3	0	0	0	1 (0)
Vacuolation, cerebrum, bilateral, focal:								

Sex	Males				Females			
Dose (ppm)	0	100	300	600	0	100	300	600
Number of Rabbits Examined	3	3	3	3	3	3	3	3 (2)
- very slight	0	0	0	0	0	0	0	2 (2)
- slight	0	0	3	3	0	0	3	1 (0)
Degeneration - individual nerve fibre(s), medulla, focal:	0	0	0	0	1	0	0	0 (0)
Gliosis, cerebrum, focal:	0	0	0	0	1	0	0	0 (0)
Inflammatory changes consistent with encephalitizoonosis, multifocal: - slight	0	0	1	0	0	0	0	1 (1)
Liver								
Number of Tissues Examined	3	3	3	3	3	3	3	3 (2)
Aggregate(s) of mononuclear (predominately lymphoid) cells, periportal:	1	0	2	1	1	0	1	1 (1)
Altered cytoplasmic homogeneity, hepatocellular:	0	0	2	3	0	0	1	3 (2)
Spleen								
Number of Tissues Examined	3	3	3	3	3	3	3	3 (2)
Within normal limits:	3	3	1	0	3	3	2	3 (2)
Extramedullary myelopoiesis: -slight	0	0	0	1	0	0	0	0 (0)
Hyperplasia – reactive, lymphoid, follicles (white pulp):	0	0	2	3	0	0	1	0 (0)
Mediastinal Lymph Node								
Number of Tissues Examined	3	0	0	3	3	0	0	3 (2)
Within Normal Limits	3	-	-	1	3	-	-	2 (2)
Hyperplasia – reactive, lymphoid:	0	-	-	2	0	-	-	1 (0)
Lungs								
Number of Tissues Examined:	3	3	3	3	3	3	3	3 (2)
Within normal limits:	0	1	1	1	0	1	1	1 (1)
Bronchopneumonia-subacute, focal: - moderate	0	0	0	1	0	0	0	0 (0)
Inflammation - acute, bronchi and bronchioles, multifocal: - slight	0	0	0	0	0	0	0	1 (0)
Inflammation - subacute, bronchi, focal: - very slight	0	0	0	0	0	1	0	0 (0)
Inflammation - subacute to chronic, interstitium, multifocal: - very slight	0	0	0	0	3	1	0	2 (1)
- slight	1	2	1	0	0	1	1	0 (0)
- moderate	2	0	1	1	0	0	1	0 (0)
Trachea								
Number of Tissues Examined	3	3	3	3	3	3	3	3 (2)
Within Normal Limits:	2	3	3	1	1	3	3	1 (1)
Inflammation - acute, mucosa, diffuse: - very slight	0	0	0	0	2	0	0	0 (0)
- slight	0	0	0	2	0	0	0	2 (1)

Sex	Males				Females			
Dose (ppm)	**0**	**100**	**300**	**600**	**0**	**100**	**300**	**600**
Number of Rabbits Examined	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3 (2)**
Inflammation - acute, mucosa, focal: - slight	1	0	0	0	0	0	0	0 (0)
Degeneration/regeneration, respiratory epithelium, focal:	0	0	0	0	0	0	0	1 (0)
Nasal Tissues								
Number of Tissues Examined	3	3	3	3	3	3	3	3 (2)
Within Normal Limits:	1	1	0	0	0	0	0	0 (0)
Erosion(s), stratified squamous epithelium, focal:	0	1	0	0	0	0	0	0 (0)
Inflammation – subacute, mucosa, focal: - very slight	0	0	0	0	0	0	1	0 (0)
Inflammation – subacute to chronic, mucosa, multifocal: - slight	2	1	0	0	3	3	1	0 (0)
- moderate	0	0	3	3	0	0	1	3 (2)
Larynx								
Number of Tissues Examined	3	3	3	3	3	3	3	3 (2)
Within normal limits:	3	2	3	0	1	2	1	1 (1)
Inflammation - acute, mucosa: - very slight	0	0	0	0	1	0	0	0 (0)
- slight	0	0	0	2	1	1	1	1 (0)
- moderate	0	1	0	1	0	0	0	1 (1)
Inflammation - subacute, blood vessels, focal: - slight	0	0	0	0	0	0	1	0 (0)
Ulcer, focal	0	0	0	0	0	0	0	1 (1)

Conclusions: **In this 14-day inhalation study in rabbits the NOEL was 100 ppm based on focal malacia (necrosis) and vacuolation in the cerebrum and inflammation of the respiratory tract at 300 ppm.**

Section A6.3.3/04 **Annex Point IIA, VI.6.3**	**Evaluation by Competent Authorities**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2004
Materials and methods	The applicant's version is adopted with the amendment that the exposure was whole-body in chambers.
Results and discussion	The applicant's version is adopted.
Conclusion	The applicant's version is adopted with the revision that NOEL/NOAEL was 100 ppm.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	This study is acceptable.
Remarks	The rat part of this study is presented in section III-A6.3.3/01.

Section A6.4.1 Annex Point IIA, VI.6.4	Subchronic Oral Toxicity Test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [x]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	Sulfuryl fluoride is a gas so exposure by ingestion cannot happen in practice. No subchronic oral toxicity test has been therefore conducted. Route of exposure is via inhalation, therefore please refer to the Subchronic inhalation toxicity tests under III A6.4.3 (**PPP DOC. M-II 5.3.3.2**)	
Undertaking of intended data submission [ ]	No studies are planned.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	June 2004	
Evaluation of applicant's justification	Agree with applicant. According to TNsG on Data requirements "Ann IIA, VI. 6.4.3: In cases where inhalation exposure is significant, an inhalation study is required instead of the oral study". Sulfuryl fluoride is a substance in gas form. Exposure via oral ingestion does not normally occur. Inhalation is the most significant exposure route. Subchronic inhalation toxicity study has been conducted (Ann IIA, VI. 6.4.3).	
Conclusion	Applicant's justification is acceptable.	
Remarks	No remarks.	

Section A6.4.2 Annex Point IIA,VI.6.4	Subchronic Dermal Toxicity Test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	Not relevant – sulfuryl fluoride is a gas. Since the active ingredient is a gas, therefore main route of exposure is inhalation. Exposure through skin will be negligible if not zero. To conduct a dermal Subchronic toxicity study would constitute unjustified use of animals. Sulfuryl fluoride is a gas therefore the primary route of exposure to humans is via the lungs. All regulatory toxicity studies have therefore been conducted by the inhalation route. However, all inhalation studies were conducted by whole-body exposure so dermal (percutaneous) contact was an integral part of the study design.	
Undertaking of intended data submission [ ]	No studies are planned.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	June 2004	
Evaluation of applicant's justification	The applicant's justification is acceptable.	
Conclusion	Dermal exposure is not a relevant pathway for sulfuryl fluoride. In several inhalation studies, dermal exposure was included due to the whole body exposure. There were no dermal changes observed. Overall, there are no indications that sulfuryl fluoride acts as a dermal toxicant.	
Remarks	No remarks.	

Section A6.4.3/01 **Subchronic Inhalation Toxicity**
Annex Point IIA, VI.6.4

Rat (IIA 5.3.3.2a/01, D04)

Report:	Nitschke, K.D., Dittenber, D.A., Eisenbrandt, D.L. (1987) Sulfuryl Fluoride (Vikane Gas Fumigant): 13-Week Inhalation Toxicity Study with Rats Mammalian and Environmental Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan, USA Report K-016399-025R, dated 16/11/87; study began 11/9/84. Also published in: *Fundam. Appl. Toxicol., 12: 540, 1989.*
Guidelines:	US EPA 82-4 (19). Deviations from EC guideline Method B.29. Sub-Chronic Inhalation Toxicity Study: 90-Day Repeated Inhalation Dose Study Using Rodent Species: There was no ophthalmologic examination. All other aspects of the guideline have been met.
GLP:	Yes
Methodology:	Test material: Vikane gas fumigant (Lot #TWP 830919-408; consisting of 99.8% sulfuryl fluoride). Groups of 10 rats/sex were exposed to 0, 30, 100 or 300 ppm sulfuryl fluoride for 6 hours/day, 5 days/week for 13 weeks. Observations included daily clinical observations; weekly body weights; haematology (PCV, Hgb, RBC, MCV, MCH, MCHC, WBC/differential count, platelets) on the day of necropsy; clinical chemistry (Ca, P, creatinine, UN, ALT, AST, AP, glucose, total protein, albumin, globulins, F) at terminal sacrifice; urinalysis (bilirubin, glucose, ketones, blood, pH, protein and urobilinogen, specific gravity) after 11 weeks of exposure; organ weights (brain, heart, liver, kidneys, thymus, testes) for all animals; gross pathology for all animals; complete histopathologic examination of a wide range of tissues in control and top dose rats; histopathological examination of brains, kidneys, nasal tissues, trachea and lungs of rats in the 30 and 100 ppm groups; special staining of brain sections from 3 rats/sex from the top dose; electron microscopy of top dose rat brain sections attempted but failed due to numerous artifacts (tissues previously fixed for light microscopy). Exposure chambers were monitored for concentrations of SO_2F_2 by a Miran 1A IR.
Findings:	Chamber concentrations were very close to the targets: 30 = 29.8; 100 = 100; 300 = 297 ppm The test chambers were run within guideline requirements.
Mortality:	None
Clinical signs:	There were no clinically observed effects which were considered to be exposure-related.
Body weight:	Body weights of males and females in the 300 ppm groups were statistically significantly decreased from control values after 45 and 24 days, respectively, as shown in Table 5.3.3.2a/01-1. The body weights of rats exposed to 30 or 100 ppm sulfuryl fluoride were comparable to control values throughout the 13-week exposure period.

Table 5.3.3.2a/01-1: Mean Body Weights (g)

Conc. (ppm)		Male				Female			
		0	30	100	300	0	30	100	300
Days on Test	-1	164.3	160.3	164.1	165.2	118.8	121.6	119.5	119.8
	3	178.5	174.4	179.1	177.8	127.6	130.0	128.0	127.4
	10	195.9	192.2	199.4	197.4	135.6	138.6	134.7	134.1
	17	211.8	208.0	215.8	212.0	141.2	142.7	140.4	137.4
	24	225.0	222.1	230.0	225.9	149.0	148.7	147.1	142.0*
	31	237.8	236.2	245.2	236.8	152.3	153.0	152.8	144.8*
	38	251.0	248.4	251.8	239.8	157.0	154.4	151.5	141.8*
	45	260.5	257.6	265.6	240.8*	161.2	158.0	157.3	142.6*
	52	269.0	271.5	275.5	238.8*	164.8	162.9	160.4	145.7*
	59	280.0	282.8	285.7	236.8*	168.0	167.5	165.9	144.1*
	66	285.0	292.8	294.6	234.6*	172.1	171.2	169.4	145.5*
	73	295.3	296.5	299.8	243.2*	173.4	169.7	170.8	149.9*
	80	302.1	305.4	307.7	251.7*	177.2	173.2	172.8	155.7*
	87	307.9	308.7	313.9	256.1*	177.9	173.4	174.1	151.7*

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

Food consumption: Not conducted.

Ophthalmology: Not conducted.

Haematology: The red blood cell counts for male rats exposed to 300 ppm sulfuryl fluoride were statistically significantly decreased from control values; the red blood cell counts of female rats exposed to the same concentration of sulfuryl fluoride were slightly decreased from control values, as shown in Table 5.3.3.2a/01-2. Platelet counts for male rats exposed to 300 ppm sulfuryl fluoride were statistically significantly increased from control values. Since the red blood cell and platelet counts were within the range of historical control values, these differences were not considered to be toxicologically significant (Table 5.3.3.2a/01-3). The white blood cell counts of rats exposed to concentrations as high as 300 ppm sulfuryl fluoride were not significantly elevated from control values. However, a slight increase in the percentage of segmented neutrophils and a slight decrease in the percentage of lymphocytes was observed in male rats and to a lesser extent in female rats exposed to 300 ppm. No other haematologic parameters were affected in rats exposed to sulfuryl fluoride.

Table 5.3.3.2a/01-2: Haematology (RBC/Platelets)

Conc. (ppm)	Males		Females	
	RBC x 10^6/cu. mm	Plat x 10^3/cu.mm	RBC x 10^6/cu. mm	Plat x 10^3/cu.mm
0	9.07	502	8.63	582
30	9/05	516	8.73	565
100	9.08	548	8.74	599
300	8.64*	565*	8.28	619

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

Table 5.3.3.2a/01-3: Historical Control Values for RBC and Platelet Counts in Male Rats from 13-Week Studies as of December 30, 1985.

	# of Groups	# of Rats	Minimum Mean	Maximum Mean	Mean of Means	S.D. of Means
RBC Counts	10	99	8.51	9.41	8.97	0.30
Platelet Counts	10	99	502	1381	846	207

Clinical chemistry:

Several clinical chemistry values from rats exposed to 100 or 300 ppm sulfuryl fluoride were statistically significantly different from control values, as shown in Table 5.3.3.2a/01-4 and 5.3.3.2a/01-6. These differences included elevated globulin and alkaline phosphatase activity in male rats exposed to 300 ppm and decreased serum alanine aminotransulfuryl fluorideerase activity in male rats exposed to 100 or 300 ppm. In females exposed to 300 ppm sulfuryl fluoride, increases in urea nitrogen and alkaline phosphatase activity and decreases in total protein, albumin and calcium were observed. The slight but statistically significant increase in urea nitrogen and decrease in calcium levels in female rats exposed to sulfuryl fluoride, while possibly due to the test material, were not considered to be toxicologically significant. The observed increases in AP activity and globulin values and decreases in ALT activity, total protein and albumin were either within or very close to the range of historical control values (Table 5.3.3.2a/01-5 and 5.3.3.2a/01-7) and were not considered to be toxicologically significant. No other clinical chemistry parameters were affected in rats exposed to sulfuryl fluoride.

Serum fluoride levels were unaffected in male rats exposed to 300 ppm or male and female rats exposed to 30 or 100 ppm. Serum fluoride levels of female rats exposed to 300 ppm sulfuryl fluoride were slightly increased from control values (Table 5.3.3.2a/01-8).

Table 5.3.3.2a/01-4: Clinical Chemistry (Males)--(ALT, AP, Globulin)

Conc. (ppm)	ALT mU/ml	AP mU/ml	Glob g/dl
0	65	65	2.5
30	62	67	2.5
100	52$	72	2.7
300	62$	85*	2.9*

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

Table 5.3.3.2a/01-5: Historical Control Values for ALT, AP and Globulin in Male Rats from 13-Week Inhalation Studies as of December 30, 1985.

	# of Groups	# of Animals	Minimum Mean	Maximum Mean	Mean of Means	S.D. of Mean
ALT	8	79	42	92	57	15
AP	18	184	62	94	72	8
Globulin	9	94	2.2	2.7	2.4	0.1

Table 5.3.3.2a/01-6: Clinical Chemistry (Females)--(UN, AP, TP, ALB, Ca)

Conc. (ppm)	UN mg/dl	AP mU/ml	TP g/dl	ALB g/dl	Ca mg/dl
0	16	48	6.0	3.7	11.0
30	16	51	5.9	3.6	10.8
100	17	53	5.9	3.6	10.8
300	21$	72*	5.6*	3.4*	10.7*

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

Table 5.3.3.2a/01-7: Historical Control Values for UN, AP, TP and ALB in Female Rats from 13-Week Inhalation Studies as of December 30, 1985

	# of Groups	# of Animals	Minimum Mean	Maximum Mean	Mean of Means	S.D. of Mean
UN (mg/dl)	16	160	14	21	17	2.0
AP (mU/ml)	16	160	32	69	53	10
TP (g/dl)	8	80	5.4	6.2	5.8	0.3
ALB (g/dl)	8	80	3.2	4.0	3.5	0.2

Table 5.3.3.2a/01-8: Serum Fluoride

Conc. (ppm)	Male Fluoride (µg/ml)	Female Fluoride (µg/ml)
0	0.996	0.607
30	0.715	0.738
100	0.881	0.575
300	1.154	1.366

Urinalysis: The specific gravity of urine from male rats exposed to 300 ppm sulfuryl fluoride was statistically significantly decreased from control values, as shown in Table 5.3.3.2a/01-9; a slight decrease in urinary specific gravity values of female rats exposed to 300 ppm was also observed.

Urinary specific gravity was not affected in rats exposed to 30 or 100 ppm sulfuryl fluoride. No other urinary parameters were affected in rats exposed to sulfuryl fluoride.

Table 5.3.3.2a/01-9: Urinary Specific Gravity

Conc. (ppm)	Males	Females
0	1.063	1.049
30	1.061	1.049
100	1.062	1.050
300	1.050*	1.040

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

Organ weights: The final body weights of male and female rats exposed to 300 ppm sulfuryl fluoride were statistically significantly decreased from control values at the

scheduled necropsy. Many of the absolute and relative organ weights of rats exposed to 300 ppm sulfuryl fluoride were statistically significantly different from the respective control values, as shown in Table 5.3.3.2a/01-10 and 5.3.3.2a/01-11. These changes were considered to be a secondary reflection of the decreased body weights. Similar organ weight changes have been observed in rats on a food restricted diet (Oishi *et al.*, Toxicol. Appl. Pharmacol. 47: 15-22,1979).

The final body weights of rats exposed to lower concentrations of sulfuryl fluoride were comparable to control values. The absolute brain weight of female rats exposed to 100 ppm sulfuryl fluoride was statistically significantly decreased from control values. Since the relative brain weight was comparable to control values, this decrease in absolute weight was considered to be due to the slight difference in body weight and of no toxicologic significance. All other absolute and relative organ weights of rats exposed to 30 or 100 ppm sulfuryl fluoride were comparable to control values.

Table 5.3.3.2a/01-10: Organ and Organ/Body Weights (Males)

Conc. (ppm)	Final Body Weight (g)	Brain		Heart		Kidneys	
		(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	285.0	1.900	0.668	0.864	0.303	2.051	0.720
30	289.3	1.924	0.666	0.908	0.314	2.125	0.735
100	292.6	1.932	0.661	0.899	0.307	2.103	0.718
300	238.1*	1.830	0.773$	0.782	0.329*	1.849	0.779*

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

Table 5.3.3.2a/01-10: Organ and Organ/Body Weights (Males)--Continued

Conc. (ppm)	Liver		Testes		Thymus	
	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	7.474	2.622	3.048	1.071	0.218	0.076
30	7.394	2.554	3.111	1.077	0.216	0.075
100	7.338	2.506	3.063	1.048	0.230	0.078
300	6.029*	2.538	2.982	1.258*	0.165*	0.070

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

Table 5.3.3.2a/01-11: Organ and Organ/Body Weights (Females)

Conc. (ppm)	Final Body Weight (g)	Brain		Heart		Kidneys	
		(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	163.5	1.815	1.112	0.593	0.363	1.313	0.803
30	159.3	1.784	1.122	0.555	0.348	1.307	0.821
100	157.4	1.749*	1.114	0.573	0.364	1.313	0.834
300	141.9*	1.722*	1.218*	0.517	0.365	1.246	0.880*

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

Table 5.3.3.2a/01-11: Organ and Organ/Body Weights (Females) -Continued

Conc. (ppm)	Liver		Thymus	
	(g)	(g/100g)	(g)	(g/100g)
0	4.557	2.789	0.207	0.126
30	4.339	2.724	0.213	0.133
100	4.335	2.754	0.208	0.132
300	3.690*	2.603*	0.165*	0.117

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

Gross pathology: At necropsy, gross examination of rats exposed to 100 or 300 ppm sulfuryl fluoride revealed mottled upper and lower incisors. In addition pale foci were observed on the lung surface of most rats exposed to 300 ppm sulfuryl fluoride. All other grossly visible lesions were considered normal background variation commonly observed in Fischer 344 rats.

Histopathology: A summary of histopathological findings is presented inTable 5.3.3.2a/01-12. Subacute inflammation was detected histopathologically in the nasal tissues of all male and female rats exposed to 300 ppm sulfuryl fluoride. 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 also observed in the lungs of rats exposed to 300 ppm sulfuryl fluoride.

Microscopic vacuolation was observed in the brains of all rats exposed to 300 ppm sulfuryl fluoride. 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.

A slight decrease in protein droplet formation was observed in the renal cortical tubules of male rats exposed to 300 ppm and is considered to be secondary to the decreased body weight. Most female rats exposed to 300 ppm sulfuryl fluoride 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 histopathologic correlate to the mottled teeth which were observed at necropsy. All other histopathologic changes observed were considered to be spontaneous changes typical of Fischer 344 rats and unrelated to exposure to sulfuryl fluoride.

Table 5.3.3.2a/01-12: Histopathology (Brain, Kidneys, Lungs, Nasal Tissues)

Sex	Males				Females			
Exposure Concentration (ppm)	0	30	100	300	0	30	100	300
Number of Rats Examined	10	10	10	10	10	10	10	10
Brain (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	10	10	10	0	10	10	10	0
Vacuolation, cerebrum, bilateral, focal: - slight	0	0	0	10	0	0	0	10
Kidneys (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	0	3	2	0	1	7	8	1
Aggregate(s) of reticuloendothelial cells:	0	0	0	0	1	1	0	1
Atrophy, individual nephron(s): - very slight	9	7	8	3	4	0	1	2
Cyst, medulla, focal:	0	0	1	0	0	0	0	0
Hyperplasia, collecting ducts: - very slight	0	0	0	0	0	0	0	9
Mineralisation, 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
Dilated with proteinaceous casts, tubule(s), focal:	0	0	0	0	2	2	0	0
Lungs (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	10	9	10	0	8	9	8	0
Alveolar histiocytosis, subpleural, focal: - very slight	0	1	0	0	2	1	2	0
Alveolar histiocytosis, subpleural, multifocal: - slight	0	0	0	10	0	0	0	10
Nasal Turbinates (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	10	8	9	0	6	6	4	0
Cyst, focal:	0	0	0	0	0	0	1	0
Inflammation - subacute, mucosa, diffuse: - very slight	0	0	0	4	0	1	0	9
- slight	0	0	0	3	0	0	0	1
- moderate	0	0	0	1	0	0	0	0
- severe	0	0	0	2	0	0	0	0
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

Conclusions: **In this 13-week inhalation study in F344 rats the NOEL was 30 ppm based on dental fluorosis (mottled teeth) at 100 ppm (and 300 ppm). Therefore, the NOAEL was arguably 100 ppm, primarily based on changes in the brain, respiratory tract and kidneys at 300 ppm. These changes comprised slight vacuolation of the cerebrum, inflammation of nasal turbinates and alveolar histiocytosis, and very slight hyperplastic changes observed in the renal collecting ducts of female rats.**

Section A6.4.3/01 Annex Point IIA, VI.6.4	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2004
Materials and methods	The applicant's version is adopted with some amendments. The rats used were of the strain F344. The exposure was whole-body in chambers.There were some minor deviations from the guideline EC Method B.29 without significant impact on the outcome of the study.
Results and discussion	The applicant's version is adopted.
Conclusion	The applicant's version is adopted. The mottled teeth can be considered an adverse effect. However, in this case it is not considered relevant.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	The study is acceptable.
Remarks	No remarks

Section A6.4.3/02 **Subchronic Inhalation Toxicity**
Annex Point IIA, VI.6.4

Dog (IIA 5.3.3.2b/01, D06)

Report:	[redacted] (1992) Sulfuryl Fluoride: 13-Week Inhalation Toxicity Study in Beagle Dogs [redacted] Report K-016399-041 and –041A, dated 24/2/92; study began 10/9/90.
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 Deviations from EC guideline: There is no guideline for a Non-Rodent 90-Day Inhalation Study.
GLP:	Yes
Methodology:	Test material: Vikane gas fumigant (Lots WP880329 752 and WP901011 907 with minimum purity of 96.26%). Groups of 4 male Beagle dogs/sex were exposed to 0, 30, 100 or 200 ppm sulfuryl fluoride for 6 hours/day, 5 days/week for 13 weeks. Whole-body exposures occurred under dynamic airflow conditions. Parameters measured included daily clinical observations; weekly body weights; haematology (HCT, Hgb, RBC, WBC/differential, platelets) on 3 occasions: prior to exposure/midway/week 12; clinical chemistry (AP, AST, ALT, CK, UN creatinine, total protein, albumin, globulin, glucose, cholesterol, triglycerides, total bilirubin, Na, K. P, Cl and Ca) on 3 occasions: prior to exposure/midway/week 12; ophthalmology prior to start of study and within 1 week of scheduled necropsy; urinalysis (pH, bilirubin, glucose, proteins, ketones, occult blood and urobilinogen, colour, appearance, specific gravity) at time of necropsy; gross pathology of all animals; weights of 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 to demonstrate glial fibrillary acidic protein. Chambers were 14.5 m^3 in size; sulfuryl fluoride was generated using a J-tube method for mixing. Chamber concentration was determined using a Miran 1A IR.
Findings:	Chamber concentrations were determined analytically to be: 30 ppm → 29.9 ppm 100 ppm → 99.0 ppm 200 ppm → 197.6 ppm Nominal concentrations were lower than expected due to incorrect calibration of the manometers which was corrected 1 month into the study.
Mortality:	All animals survived to the end of the study.
Clinical signs:	One male (90A6510) exhibited lateral recumbency, tetany, tremors, salivation and incoordination 75 minutes after exposure to 200 ppm SO_2F_2 on day 19. An hour later, during the weekly clinical examination, the activity of this animal was

decreased relative to controls but was otherwise normal. All other clinical effects noted were considered to be unrelated to exposure to SO_2F_2.

Body weight: Pre-exposure (average of day -4 and 1 values) mean body weight values of male and female dogs exposed to 200 ppm SO_2F_2 were 94 and 99% of control values, respectively. Since there were no statistical time-sex-dose interactions, body weight values for male and female dogs within a group were combined to increase statistical power. Thus, mean body weight values of male and female dogs exposed to 200 ppm SO_2F_2 were statistically significantly decreased from control values, as shown in Table 5.3.3.2b/01-1. By the end of the study, mean body weight values of male and female dogs exposed to 200 ppm SO_2F_2 were 88 and 96%, respectively, of control values. Male dogs appeared to be slightly more affected than female dogs. Mean body weight values for male and female dogs exposed to lower concentrations of SO_2F_2 were comparable to control values.

Table 5.3.3.2b/01-1: Summary of Body Weights (g)

Conc. (ppm)		Males				Females			
		0	**30**	**100**	**200***	**0**	**30**	**100**	**200***
Days on Test	-4	10714	10686	10245	10166	9835	9530	9633	9558
	5	10912	10825	10325	10208	9737	9323	9689	9370
	12	11320	11320	10680	10569	10018	9663	10102	9563
	26	11766	11718	11087	10862	10023	10085	10675	9817
	94	12680	13522	12584	12095	11186	11882	11621	10757

*Time-dose interaction statistically significant for combined sex, alpha = 0.05

Food consumption: Not conducted

Ophthalmology: No effects were reported.

Haematology: There were no exposure-related effects noted.

Clinical chemistry: Aspartate aminotransulfuryl fluorideerase activity and albumin levels in male and female dogs exposed to 200 ppm SO_2F_2 were statistically significantly decreased from control values (Table 5.3.3.2b/01-2). However, the decrease in aspartate aminotransulfuryl fluorideerase activity and albumin levels were very slight and were considered to be of no toxicological significance. All remaining clinical chemistry values for male and female dogs exposed to sulfuryl fluoride were comparable to control values.

Table 5.3.3.2b/01-2: Clinical Chemistry (Aspartate aminotransulfuryl fluorideerase and Albumin)

Conc (ppm)	Pre-Exposure #1	Pre-Exposure #2	Pre-Exposure #3	6-Weeks	13-Weeks
Males-AST					
0	28	24	33	24	27
30	27	24	33	25	26
100	26	22	26	22	23
200*	28	26	30	22	22
Females-AST					
0	21	26	22	22	23
30	25	23	26	26	25
100	21	22	21	20	21
200*	24	24	29	20	20
Males-Albumin					
0	3.0	2.9	3.1	3.2	3.7
30	3.0	2.9	3.1	3.1	3.6
100	3.0	2.9	3.0	3.2	3.6
200*	3.0	2.9	3.1	3.1	3.5
Females-Albumin					
0	2.8	2.9	3.1	3.2	3.6
30	2.9	3.0	3.2	3.0	3.6
100	3.0	3.0	3.2	3.2	3.8
200*	3.0	3.0	3.2	3.0	3.6

*Time-dose interaction statistically significant, alpha = 0.05

Urinalysis: Urinalysis values were comparable to control values.

Organ weights: Although there was a slight decrease in mean terminal body weights in dogs (not statistically significant exposed to 200 ppm, mean absolute and relative organ weights were comparable to control values at all exposure levels.

Gross pathology: There were no grossly visible exposure-related lesions associated with exposure to concentrations as high as 200 ppm SO_2F_2. All gross lesions were considered to be incidental findings unrelated to exposure to the test material.

Histopathology: Exposure-related histopathological effects were observed in the midbrain region of one male and one female dog exposed to 200 ppm SO_2F_2, as shown in Table 5.3.3.2b/01-3. In these two dogs (male-90A6510 and female-90A6529), there was a single, small bilaterally symmetrical focal microscopic change noted in the putamen of the midbrain. This focal change was characterised microscopically by vacuolation, gliosis (microglial gitter cells), perivascular cuffing, hypertrophy of endothelial cells and individual cells showed nuclear pyknosis and karyorrhexis. The size of the lesion was extremely small and barely recognisable microscopically. The focal reaction was slightly more prominent in the affected male dog when compared to 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 haematoxylin and eosin sections, with the exception of the microglial gitter cell reaction. All other microscopic observations noted in several other tissues were considered to be incidental findings unrelated to exposure to sulfuryl fluoride.

Table 5.3.3.2b/01-3 Histopathology (Brain)

Sex	Males				Females			
Exposure Concentration (ppm)	0	30	100	200	0	30	100	200
Number of Dogs Examined	4	4	4	4	4	4	4	4
Brain - Cerebellum (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	4	4	4	4	4
Brain - Cerebrum (# of Tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	3	4	4	4	4	4	4	4
Mineralisation, meninges, focal:	1	0	0	0	0	0	0	0
Brain - Forebrain (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	4	4	4	4	4
Brain - Medulla Oblongata (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	4	4	4	4	4
Brain - midbrain (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	3	4	4	4	3
Gliosis, midbrain, bilateral, focal: - very slight	0	0	0	1	0	0	0	1
Vacuolation, midbrain, bilateral, focal: - very slight	0	0	0	1	0	0	0	1
Brain - Pons (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	4	4	4	4	4

Conclusions: **In this 13-week inhalation study in Beagle dogs the NOAEL was 100 ppm based on slightly reduced body weights in males and females and changes in the brain. These comprised very slight, small, bilateral, focal vacuolation and gliosis in the putamen region of two of eight dogs at 200 ppm (the male dog exhibited transient tremors and tetany shortly after exposure ended on day 19 but appeared normal at all other times).**

Section A6.4.3/02 **Annex Point IIA, VI.6.4**	**Evaluation by Competent Authorities**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2004
Materials and methods	The applicant's version is adopted with some amendments. 4 male and 4 female dogs/dose were used.
Results and discussion	The applicant's version is adopted with the following amendment: The clinical effect mentioned in the study report is reddened areas of the skin of the ear.
Conclusion	The applicant's version is adopted.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	The study is acceptable.
Remarks	The purity of the test substance was 96.2% which is lower than the minimum purity stated in section III-A2.7 (99.4%). However, the purity was still high and the impurities were identified.

Section A6.4.3/03 **Subchronic Inhalation Toxicity**
Annex Point IIA, VI.6.4

Dog (IIA 5.3.3.2c/01, I01)

Report: [redacted] (1993)
Sulfuryl Fluoride: One-Year Inhalation Toxicity Study in Beagle Dogs
[redacted]
Report DECO-HET K-016399-044, dated 21/10, 93; study began 13/1/92.

Guidelines: US EPA 83-1
OECD 452
87/302/EEC: Chronic Toxicity Test
MAFF: Chronic Toxicity Test Guidelines
Deviations from EC test guideline: None

GLP: Yes

Methodology: Test material: Several smaller quantities (lot #'s WP 910826-929, WP 920131- 940 and WP 920619-953) of sulfuryl fluoride were obtained from DowElanco, Pittsburg, CA, during the course of the study, due to safety and storage considerations at The Toxicology Research Laboratory. All cylinders of sulfuryl fluoride had a stated purity of 99.8%. Each cylinder was analysed for purity, both prior to and after use in the study, using a Hewlett Packard gas chromatograph equipped with a thermal conductivity detector. In addition, samples were taken from each lot of test material for gas chromatography/mass spectrometry (GC/MS) to verify test material composition. Infrared spectroscopy was also performed on test samples for compositional analysis by Analytical Sciences, 1897 Building, Michigan Division, The Dow Chemical Company. Results of the analyses indicated that the lots ranged from 95.1% to 98.8% sulfuryl fluoride. Three minor impurity peaks were identified as air, water and thionyl fluoride by GC/MS.

The initial study design consisted of groups of four male and four female Beagle dogs which were to be exposed to 0, 20, 80 or 200 ppm sulfuryl fluoride for 6 hours/day, 5 days/week for one year. However, due to excessive morbidity and mortality by approximately 9 months, the 200 ppm exposed group of dogs was removed from study and necropsied.

Since sulfuryl fluoride is a gas at room temperature and pressure, the study was conducted using whole-body exposures under dynamic airflow conditions. Animals were observed daily and weighed prior to 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 prior to initiation of the study, at 3, 6 and 9 months, and once during the last 2 weeks of exposure. Haematology consisted of HCT, Hgb, RBC, WBC/diff., and platelets. Clinical chemistry consisted of AP, AST, ALT, creatine kinase, UN, creatinine, total protein, albumin, globulin, glucose, cholesterol, triglycerides, total bilirubin, Na, K, P, Cl and Ca. Urinalysis was performed at 6 months and at 12 months, and it included pH, bilirubin, glucose, proteins, ketones, occult blood, urobilinogen, colour, appearance, specific gravity and microscopic examination of sediment. An

ophthalmologic examination took place prior to the start of the study and again shortly before the scheduled 1-yr necropsy. One-half of the lower jaw with teeth and one femur from each dog were collected and frozen at necropsy for fluoride analysis, if necessary, for interpretation of study results. However, fluoride analyses were not conducted on these samples.

Animals were necropsied on the day after the last exposure 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 200 ppm group which was terminated early due to excessive pulmonary toxicity (not weighed due to lack of controls). A complete set of tissues collected at necropsy from all animals was examined by light microscopy for evaluation of exposure-related effects.

Exposure Concentrations: The exposure levels selected for this study were based upon findings in the highest exposure concentration used in the subchronic dog study which resulted in clinical and pathologic effects in the nervous system of an occasional dog (III-A 6.4.3/02, **D06**). The intermediate (80 ppm) and lowest (20 ppm) exposure concentrations were selected to parallel those used in chronic rodent studies (III-A6.5/01, **I03** and III-A6.5/02, **I04**). In addition, the lowest exposure level of 20 ppm was anticipated to be a NOEL in this study.

Chambers were 14.5 m^3 stainless steel and glass with pyramidal top and operated under dynamic airflow. Concentrations of the test material were generated using the glass J-tube method. Chambers were monitored analytically at 30 minute intervals using a Miran 1A IR.

Standard statistical analyses were performed.

Findings: Chamber concentrations were within 10% of target (0, 21, 79 and 198 ppm). Chambers operated otherwise within the target ranges.

Mortality: Due to excessive morbidity and mortality, the 200 ppm group was taken off test at 9 months and necropsied. There were no other deaths.

Clinical signs: Cageside: There were no apparent exposure-related effects noted in the 20 and 80 ppm exposed dogs throughout the study.

Weekly Clinical Examinations: There were no exposure-related observations noted in the 0, 20 and 80 ppm exposed groups. At approximately 9 months into the study, several male and female dogs from the 200 ppm exposure level exhibited clinical signs of toxicity. The observations included, but were not limited to, laboured breathing, shallow rapid respiration, and pale or blue mucous membranes. A 200 ppm male dog (91A6413) died on day 267, and another (91A6410) became moribund and was necropsied on day 271. One of the remaining two male dogs (91A6411) from the 200 ppm exposure group exhibited minor respiratory changes prior to removal from study on day 282. This dog had lost 782 g of body weight during the previous month. The heaviest male dog (91A6412) in the 200 ppm exposed group was clinically normal and was also necropsied on day 282, when the exposures of this group were stopped due to excessive toxicity.

Three female dogs from the 200 ppm exposure group were sacrificed in a moribund condition on days 278 (91A6429) and 281 (91A6426, 91A6427). Although the remaining female dog (91A6428) in this group did not show clinical signs of altered respiration, she had lost 647 g of body weight during the previous month. This lone surviving 200 ppm exposed female dog was also necropsied on day 282.

All of the dogs which became ill during the study exhibited a number of clinical signs indicative of impaired respiratory function. These changes were either visually observed as laboured respiration, or noted as altered respiratory sounds upon auscultation of the thoracic cavity. Occasional discolouration of the mucous membranes was observed and was consistent with the compromised respiratory

function. Generally, the laboured respiration was associated with an increased respiratory rate. 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 due to cardiac arrhythmia. A few dogs were administered an antibiotic and a diuretic which did not significantly alter the clinical outcome of the disease process. The dogs generally were inappetent by the time the respiratory condition was clinically apparent. In fact, they may have been so for a time prior to onset of the clinical respiratory condition, in view of the fact that a significant decrease in body weight was already noted in these dogs several months earlier. Feed consumption was not measured, therefore, a definitive conclusion regarding feed intake associated with their decreased body weight cannot be made.

Although microbiological cultures were taken from the lungs of several dogs, only one showed an Haemophilus bacterial infection. This bacteria was likely an opportunist infecting this debilitated dog and not a primary infectious agent, since it was not isolated from other dogs in the group (200 ppm) which also had laboured respiration.

Body weight: See Clinical Observations. Body weights are shown in Table 5.3.3.2c/01-1.

Table 5.3.3.2c/01-1: Summary of Mean Body Weights (g)

Conc. (ppm)		Males				Females			
		0	20	80	200	0	20	80	200
Days on Test	1	10082	10623	9937	11174	8217	8703	8512	8256
	26	11305	11611	10833	11622	9169	9461	9530	8588
	89	12488	13265	11919	12373	10294	10065	10673	9429
	180	13159	14462	12651	12665	10920	11303	11052	9622
	208	13003	14681	12655	12860	10981	11850	11056	9671
	236	13430	15299	13102	12608	11239	11745	11386	9242
	278	13266	15177	13162	12636 (N=2)	11392	11578	11300	7730
	368	13521	15505	13292	--	12113	12669	11406	--

Food consumption: Not conducted.

Ophthalmology: There were no treatment-related effects.

Haematology: There were no exposure-related effects in the 20 or 80 ppm exposed dogs throughout the study, and in the 200 ppm exposed dogs through 6 months on test. As the male dogs in the 200 ppm group became progressively ill, a blood sample was collected prior to removal from study and their individual results are presented in Table 5.3.3.2c/01-4. The values for dog (91A6410) in the 200 ppm exposed group (Table 5.3.3.2c/01-4) were altered when compared to the mean values for controls bled on test day 270 (9 months, Table 5.3.3.2c/01-2). The erythroid and platelet values for this dog were generally decreased and the WBC was increased when bled at 9 months. In addition, a neutrophilia was observed in the differential WBC of this dog. On day 271, another blood sample was taken from dog (91A6410) prior to being necropsied due to laboured respiration. The results were similar to those from the previous day (Table 5.3.3.2c/01-4). The erythroid values for dogs (91A6411 and

91A6412) were also slightly decreased with a slight increase in WBC. The marked increase in the erythroid values of dog (91A6413) were likely due to extreme clinical dehydration of this dog prior to its death. The decreased erythroid values and the increased WBC's observed in most of these dogs were interpreted to be reflective of their general debility and pneumonia.

There were no exposure-related haematologic alterations in female dogs exposed to 20 or 80 ppm, or in the 200 ppm exposed females through nine months (Table 5.3.3.2c/01-5). However, subsequent to collection of the nine month blood sample, collected on day 270, three of the four dogs developed signs of laboured breathing. Dogs (91A6426, 91A6427 and 91A6429) exhibited laboured respiration when selected for necropsy. The erythroid parameters were quite variable and likely reflected the moribund condition of these three dogs; however, the WBCs of all four dogs were increased when compared to the mean value of control dogs bled at nine months (Table 5.3.3.2c/01-7). In general, a neutrophilia was observed in these 200 ppm exposed females.

In summary, a decrease in erythroid parameters and an increase in WBCs with a neutrophilia was observed in both sexes of 200 ppm exposed dogs that became ill due to impaired respiratory function. These haematologic changes were considered secondary to the observed histopathologic findings in the lungs of these dogs. There were no haematologic alterations observed in male or female dogs exposed to 20 or 80 ppm of sulfuryl fluoride for one year (Table 5.3.3.2c/01-3 and 5.3.3.2c/01-6).

Table 5.3.3.2c/01-2: Haematology and Differential WBC--Males -- 9 Months

Conc. (ppm)	RBC	Hgb	HCT	Plat	WBC	WBC Differential Count					NRBC /100 WBC
	$x10^6$/ cu mm	g/dl	%	$x10^3$/cu mm	$x10^3$/cu mm	SEG %	BAND %	LYM %	MON O %	EOS %	
0	7.34	17.9	51.6	273	7.5	60	1	35	3	3	0
20	7.56	18.3	52.9	250	8.1	57	0	36	3	5	0
80	7.60	18.1	53.3	274	7.5	66	0	26	4	5	0
200	6.91	16.6	48.7	328	14.6	68	0	26	3	3	0

Table 5.3.3.2c/01-3: Haematology and Differential WBC--Males -- 12 Months

Conc. (ppm)	RBC	Hgb	HCT	Plat	WBC	WBC Differential Count					NRBC /100 WBC
	$x10^6$/cu mm	g/dl	%	$x10^3$/cu mm	$x10^3$/cu mm	SEG %	BAND %	LYM %	MONO %	EOS %	
0	7.86	18.2	52.4	284	7.9	61	0	35	2	2	1
20	7.82	18.0	51.1	297	9.7	61	1	32	3	4	0
80	8.07	18.6	53.3	294	9.0	60	0	32	4	4	0

Table 5.3.3.2c/01-4: Haematology and Differential WBC -- Males -- Removed from Study

Conc. (ppm)	Days on Test	Animal Number	RBC	Hgb	HCT	Plat	WBC	WBC Differential Count				
			$x10^6$/ cu mm	g/dl	%	$x10^3$/ cu mm	$x10^3$/ cu mm	SEG %	BAND %	LYM %	MON O %	EOS %
0	270	Mean*	7.34	17.8	51.6	273	7.5	60	1	35	3	3

200	270	91A6410	6.32	15.0	44.4	220	27.9	91	0	7	2	0
	271	91A6410	6.41	15.2	45.2	233	36.2	75	3	13	5	4
	278	91A6411	6.57	15.0	45.9	557	12.9	60	2	31	6	1
	282	91A6411	7.09	16.1	49.8	569	13.1	62	1	30	5	2
	282	91A6412	6.76	15.7	49.2	215	8.9	71	0	25	3	1
	267	91A6413	9.99	23.6	66.0	408	46.1	80	8	7	5	0

*Control mean from dogs bled at 9 months for comparison.

Table 5.3.3.2c/01-4: Haematology and Differential WBC (continued) - Males -Removed from Study

Conc. (ppm)	Days on Test	Animal Number	NRBC /100 WBC	Morphology Erythrocyte/Leukocyte/Platelet
0	270	Mean*	0	
200	270	91A6410	0	Normal
	271	91A6410	0	Normal
	278	91A6411	0	Normal
	282	91A6411	0	Normal
	282	91A6412	0	Normal
	267	91A6413	1	Normal

Table 5.3.3.2c/01-5: Haematology and Differential WBC -Females - 9 Months

Conc. (ppm)	RBC	Hgb	HCT	Plat	WBC	WBC Differential Count					NRBC /100 WBC
	$x10^6$/ cu mm	g/dl	%	$x10^3$/cu mm	$x10^3$/cu mm	SEG %	BAND %	LYM %	MONO %	EOS %	
0	7.32	17.3	51.2	288	8.6	62	0	32	3	3	0
20	7.40	17.7	52.0	306	7.7	65	0	29	2	4	0
80	7.25	17.2	50.1	302	8.4	59	0	34	3	4	0
200	7.61	18.0	53.2	469	10.4	63	0	31	3	4	0

Table 5.3.3.2c/01-6: Haematology and Differential WBC -Females - 12 Months

Conc. (ppm)	RBC	Hgb	HCT	Plat	WBC	WBC Differential Count					NRBC /100 WBC
	$x10^6$/ cu mm	g/dl	%	$x10^3$/cu mm	$x10^3$/cu mm	SEG %	BAND %	LYM %	MONO %	EOS %	
0	7.35	17.1	49.2	388	8.6	55	0	39	3	4	0
20	7.59	17.6	50.6	339	8.6	59	1	32	4	4	0
80	7.14	16.4	46.4	363	9.1	61	1	32	2	5	0

Table 5.3.3.2c/01-7: Haematology and Differential WBC - Females - Removed from Study

Conc. (ppm)	Days on Test	Animal Number	RBC $x10^6$/ cu mm	Hgb g/dl	HCT %	Plat $x10^3$/ cu mm	WBC $x10^3$/ cu mm	WBC Differential Count				
								SEG %	BAND %	LYM %	MONO %	EOS %
0	270	Mean*	7.32	17.3	51.2	288	8.6	62	0	32	3	3
200	281	91A6426	8.39	18.7	58.9	541	23.7	79	1	17	2	1
	281	91A6427	7.87	17.3	55.5	459	25.3	80	0	17	2	1
	282	91A6428	6.75	14.9	47.5	621	20.5	78	0	18	3	1
	278	91A6429	6.53	15.8	47.5	425	11.7	67	0	23	4	6

*Control mean from dogs bled at 9 months for comparison.

Table 5.3.3.2c/01-7 Haematology and Differential WBC (continued) - Females - Removed from Study

Conc. (ppm)	Days on Test	Animal Number	NRBC /100 WBC	Morphology Erythrocyte/Leukocyte/Platelet
0	270	Mean*	0	
200	281	91A6426	0	Normal
	281	91a6427	0	Normal
	282	91a6428	1	Normal
	278	91a6429	0	Normal

*Control mean from dogs bled at 9 months for comparison.

Clinical chemistry:

A decreased alkaline phosphatase value in the 20 ppm exposed males was statistically identified (Table 5.3.3.2c/01-8). The likely reason for this identified difference may be due to a control dog (91A6398) having an elevated value in both pre-study samples. Therefore, a higher mean value and a large standard deviation for alkaline phosphatase was observed in the control male dogs at each bleeding prior to study start and likely resulted in the statistically identified decrease in the 20 ppm exposed group upon repeated measures analysis.

The clinical chemistry findings for male dogs in the 200 ppm exposed group which became ill at approximately 9 months and were removed from study are presented in Table 5.3.3.2c/01-9 below. The mean values for the control dogs bled at 9 months are also presented for comparison. Dog (91A6413) was extremely dehydrated clinically with a 66% haematocrit, and also had a number of clinical chemistry and electrolyte values that were altered when compared to the control group. The clinical chemistry changes in this dog were considered to be reflective of his dehydration and moribund state. The values for the other dogs within the group were generally quite similar to the controls. There were no clinical chemistry alterations in male dogs exposed to any concentration of sulfuryl fluoride which were indicative of a primary target organ effect.

The repeated measures statistical analyses identified a decreased alkaline phosphatase value in the 20 ppm exposed females (Table 5.3.3.2c/01-10). The likely reason for the identified difference in alkaline phosphatase was due to the control female dog (91A6415) having an elevated value at both times when bled prior to study initiation. Therefore, a higher mean value and a large standard deviation for alkaline phosphatase was observed in the control female dogs at each bleeding prior to study start and likely resulted in the statistically identified decrease

in the 20 ppm exposed group.

The clinical chemistry findings for female dogs in the 200 ppm exposed group which became moribund at approximately 9 months and were removed from study are presented in Table 5.3.3.2c/01-11 below. The mean values for the control dogs bled at 9 months are also presented for comparison. The decreases and/or increases in UN, AP, GLUC, CHOL, TRIG, and CK, as well as the electrolyte values, of some dogs were interpreted to be secondary to their poor clinical condition, as was reflected by their marked weight loss and respiratory alterations. There were no clinical chemistry alterations in the female dogs exposed to any concentration of sulfuryl fluoride which were indicative of a primary target organ effect.

In conclusion, there were no alterations in clinical chemistry which were indicative of a primary target organ effect in male or female dogs at any exposure concentration. However, minor secondary clinical chemistry changes were observed in some of the 200 ppm exposed dogs which were ill and removed from study due to impaired respiratory function.

Table 5.3.3.2c/01-8: Alkaline Phosphatase (mU/ml)--Males (Means)

Conc. (ppm)	Pre-study bleed 1	Pre-study bleed 2	3 Months	6 Months	9 Months	12 Months
0	305	270	66	45	38	37
20	209	192	50	36	30	32
80	261	233	66	46	42	48
200	257	256	60	45	65	--

Table 5.3.3.2c/01-9: Clinical Chemistry--Males--Removed from Study

Conc. (ppm)	Day on Test	Animal Number	UN mg/dl	ALT mU/ml	AP mU/ml	AST mU/ml	GLUC mg /dl	TP g/dl	ALB g/dl	Glob g/dl	Chol mg/dl
0	270	Mean*	16	26	38	23	95	6.0	3.4	2.6	176
200	278	91A6411	18	5	56	22	85	5.8	2.3	3.5	188
	282	91A6411	11	5	59	22	97	6.0	2.5	3.5	193
	282	91A6412	14	9	33	18	94	5.9	3.3	2.6	149
	267	91A6413	68	8	68	29	111	4.4	1.7	2.7	393

***Control mean from the group bled at 9 months--for comparison.**

Table 5.3.3.2c/01-9: Clinical Chemistry--Males--Removed from Study (Cont.)

Conc. (ppm)	Day on Test	Animal Number	TRIG mg/dl	TBIL I mg/ dl	CK mU/ ml	CREA mg/dl	Ca mg/ dl	P mg/ dl	Na mmol /l	K mmol /l	Cl mmol /l
0	270	Mean*	37	0.1	70	1.0	10.1	4.2	147	5.0	115
200	278	91A6411	62	0.1	79	0.7	9.3	5.6	147	4.9	114
	282	91A6411	59	0.1	63	0.8	9.5	5.1	145	4.8	115
	282	91A6412	38	0.1	38	1.0	10.4	4.9	146	4.4	114
	267	91A6413	169	0.2	318	1.1	8.8	7.7	139	4.9	104

***Control mean from the group bled at 9 months--for comparison.**

Table 5.3.3.2c/01-10: Alkaline Phosphatase (mU/ml)--Females (Means)

Conc. (ppm)	Pre-study bleed 1	Pre-study bleed 2	3 Months	6 Months	9 Months	12 Months
0	289	300	52	46	43	35
20	201	198	49	39	33	33
80	221	226	52	41	41	35
200	181	186	48	42	52	--

Table 5.3.3.2c/01-11: Clinical Chemistry--Females--Removed from Study

Conc. (ppm)	Day on Test	Animal Number	UN m g/dl	ALT mU/ml	AP mU/ml	AST mU/ml	GLUC mg/dl	TP g/dl	ALB g/dl	Glob g/dl	Chol mg/dl
0	270	Mean*	15	18	43	19	96	5.7	3.4	2.3	175
200	281	91A6426	11	25	96	33	45	6.1	2.3	3.8	230
	281	91A6427	11	25	90	34	40	6.2	2.2	4.0	227
	282	91A6428	14	8	149	17	93	5.7	2.9	2.8	309
	278	91A6429	9	6	49	18	87	5.7	2.6	3.1	148

*Control mean from the group bled at 9 months--for comparison.

Table 5.3.3.2c/01-11: Clinical Chemistry--Females--Removed from Study (Cont.)

Conc. (ppm)	Day on Test	Animal Number	TRIG mg/dl	TBILI mg/dl	CK mU/ml	CREA mg/dl	Ca mg/dl	P mg/dl	Na mmol/l	K mmol/l	Cl mmol/l
0	270	Mean*	43	0.2	58	0.9	10.1	3.8	149	4.6	114
200	281	91A6426	94	0.1	245	0.7	9.2	5.4	146	5.1	111
	281	91A6427	91	0.1	252	0.6	9.4	5.4	146	5.1	111
	282	91A6428	50	0.1	47	0.9	9.4	4.4	144	4.5	114
	278	91A6429	57	0.1	96	0.7	9.3	4.6	145	5.0	115

*Control mean from the group bled at 9 months--for comparison.

Urinalysis: There were no alterations in any values at either time interval with the exception of a slight statistically identified increased specific gravity in the 20 ppm exposed dogs at 6 months. This urine specific gravity in the 20 ppm group was within the accepted range of normal, and was also identical to the value observed in the control males at 12 months.

There were no differences found in any exposed group of females at either time interval.

Organ weights: The relative heart weights of male and female dogs in the 20 ppm exposed group were decreased and statistically identified as significant (Table 5.3.3.2c/01-12 and 5.3.3.2c/01-13). Mean body weights of the 20 ppm exposed dogs were also heavier than the controls and 80 ppm groups, although not statistically identified. Most of the relative organ weights of the 20 ppm exposed male and female dogs were decreased as a reflection of their heavier mean body weights. There was no toxicological significance associated with the minor decreased relative heart weight difference in the 20 ppm exposed males and females, since it was not related to

exposure concentration, and there were no histopathologic changes detected. There were no other absolute or relative organ weight differences statistically identified for either male or female dogs.

Table 5.3.3.2c/01-12: Terminal Body Weights and Organ Weights - Males

Conc. (ppm)	Final Body Wt.	Adrenals		Brain		Heart		Kidneys	
	(g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	13521	1.437	0.0109	79.518	0.602	97.32	0.725	61.320	0.459
20	15505	1.607	0.0103	81.689	0.529	95.600	0.620*	68.608	0.438
80	13292	1.434	0.0110	82.977	0.638	90.326	0.688	63.593	0.482

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

Table 5.3.3.2c/01-12: Terminal Body Weights and Organ Weights - Males (Cont.)

Conc. (ppm)	Liver		Lungs		Pituitary		Testes		Thyroid	
	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	334.4	2.465	91.788	0.685	0.071	0.0005	19.574	0.147	0.999	0.0075
20	339.1	2.203	90.520	0.590	0.077	0.0005	17.424	0.112	1.280	0.0083
80	325.4	2.451	96.829	0.737	0.071	0.0005	16.204	0.125	1.145	0.0087

Table 5.3.3.2c/01-13: Terminal Body Weights and Organ Weights - Females

Conc. (ppm)	Final Body Wt.	Adrenals		Brain		Heart		Kidneys	
	(g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	11699	1.293	0.0111	74.638	0.659	75.277	0.647	42.635	0.364
20	12407	1.598	0.0129	77.382	0.626	71.366	0.577*	43.086	0.347
80	11076	1.243	0.0113	75.427	0.689	67.691	0.615	38.820	0.354

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

Table 5.3.3.2c/01-13: Terminal Body Weights and Organ Weights - Females (Cont.)

Conc. (ppm)	Liver		Lungs		Ovaries		Pituitary		Thyroid	
	g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g
0	277.0	2.384	70.489	0.615	1.787	0.0147	0.063	0.0006	0.783	0.0069
20	295.1	2.357	75.959	0.618	1.389	0.0110	0.074	0.0006	0.890	0.0072
80	240.4	2.181	81.940	0.743	1.187	0.0108	0.070	0.0006	0.756	0.0067

Gross pathology: There were 2 male and 3 female dogs in the 200 ppm exposed group which exhibited laboured respiration for several days prior to removal from study. One of the two males was found dead, while the other male and all three females were euthanised in a moribund condition. The decreased ingesta in the gastrointestinal tract was consistent with the in-life observation of inappetence. One of the male dogs (91A6410) which was moribund had consolidation of the right cardiac lobe of the lung, hydrothorax, fibrinous pleuritis and fibrinous pericarditis suggestive of an acute infectious process. The other male dog (91A6413) had diffuse consolidation of the lungs (Table 5.3.3.2c/01-14). Female dogs (91A6426, 91A6427, and

91A6429) all had a variable degree of pulmonary consolidation. All of these dogs exhibited laboured respiration with grossly consolidated lungs which was consistent with the in-life symptoms indicative of respiratory dysulfuryl fluorideunction.

One of the two male dogs (91A6411), had laboured breathing prior to necropsy and his lungs were found to be diffusely consolidated. The other male (91A6412) was breathing normally during in-life observations, and he was also found to be normal at necropsy. Although the female dog (91A6428) appeared normal in-life she had a slight degree of pulmonary consolidation at necropsy.

The gross appearance of the lungs of most of the 200 ppm exposed group consisted of scattered darkened areas admixed with pale areas. The dark coloured areas appeared to be consolidated and were firm upon palpation, whereas, the pale areas appeared normal. Lungs from dogs which clinically exhibited laboured respiration were predominantly consolidated and non-functional. There were no other tissues which appeared to be grossly affected in the 200 ppm exposed dogs.

An occasional control and 80 ppm exposed dog had an occasional pale focus in their lungs which are routinely seen in chronic studies. A pale focus in the spleen was also observed in a control and 80 ppm exposed male dog. There were no observations in these exposed groups of dogs which were considered due to sulfuryl fluoride exposure.

In summary, there were no significant exposure-related effects observed in tissues other than the lungs.

Table 5.3.3.2c/01-14: Gross Pathology (Lungs)

Sex	Males				Females			
Concentration (ppm)	**0**	**20**	**80**	**200**	**0**	**20**	**80**	**200**
Spontaneous/Moribund – 12-Month Study								
Number of Dogs Examined	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**2**
Within normal limits:	--	--	--	0	--	--	--	0
Consolidation, diffuse:	--	--	--	1	--	--	--	0
Consolidation, right cardiac lobe:	--	--	--	1	--	--	--	0
Focus - pale elevated, generalised, diffuse:	--	--	--	0	--	--	--	1
Sacrificed - Day 282 - 12-Month Study								
Number of Dogs Examined	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**1**
Within normal limits:	--	--	--	1	--	--	--	0
Consolidation, multifocal: - slight	--	--	--	0	--	--	--	0
Consolidation, diffuse:	--	--	--	1	--	--	--	0
Terminal Sacrifice - 12-Month Study								
Number of Dogs Examined	**4**	**4**	**4**	**0**	**4**	**4**	**4**	**0**
Within normal limits:	3	4	3	--	4	4	3	--
Focus - pale, left diaphragmatic lobe, multifocal:	1	0	1	--	0	0	0	--
Focus - pale, right diaphragmatic lobe, multifocal:	1	0	1	--	0	0	1	--
All Modes of Death -- 12-Month Study								
Number of Dogs Examined	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Within normal limits:	3	4	3	1	4	4	3	0
Consolidation, multifocal: - slight	0	0	0	0	0	0	0	1
Consolidation, diffuse:	0	0	0	2	0	0	0	2
Consolidation, right cardiac lobe:	0	0	0	1	0	0	0	0

Focus - pale, left diaphragmatic lobe, multifocal:	1	0	1	0	0	0	0	0
Focus - pale, right diaphragmatic lobe, multifocal:	1	0	1	0	0	0	1	0
Focus - pale elevated, generalised, diffuse:	0	0	0	0	0	0	0	1

Histopathology: Exposure to sulfuryl fluoride during the chronic study resulted in exposure-related effects in the lungs, brain, thyroid gland and canine teeth (Table 5.3.3.2c/01-15).

Lungs: The histopathologic changes in the lungs of 200 ppm exposed dogs corresponded with the severity of laboured respiration in-life and they were consistent with the consolidation noted at necropsy. The pulmonary changes appeared to primarily involve the peripheral portions of the lung, without recognisable alterations in the major airways. Early changes appeared to be associated with an increase in the number of alveolar macrophages within scattered subpleural alveoli. A variable mixed inflammatory cell infiltrate was usually associated with these foci. In the more advanced stages of the chronic active inflammatory process these foci apparently increased in size and hypertrophied type II pneumocytes were observed. In addition, the epithelial cells lining the respiratory and alveolar ducts in the affected areas were hypertrophied and hyperplastic. The areas of chronic active inflammation were separated by nearly normal areas of pulmonary parenchyma. In the most severe cases, a continuum of these changes were present which ultimately resulted in pulmonary consolidation of a significant portion of all affected lobes. Associated with the 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 due to collagen deposition. Although the peripheral portions of the lungs of 200 ppm exposed dogs were adversely affected, their nasal turbinates, larynx, trachea and major portions of the bronchial tree were unaffected.

One male dog (91A6410) in the 200 ppm exposed group had an acute suppurative inflammatory process within one lobe of lung in which many bacterial colonies were present. The acute inflammatory process in this dog extended into the thoracic cavity and resulted in hydrothorax, fibrinous pleuritis and fibrinous pericarditis. Microbiological culture of this lung lesion isolated Haemophilus species. There were no other dogs in this exposure group with a comparable acute inflammatory reaction in their lungs. The bacteria isolated from the lung of this dog was also not found in two other dogs from which lungs were cultured; therefore, this isolate was considered an opportunist and not a primary etiologic agent causing the lung pathology.

In the 80 ppm exposed dogs, there were three of four males and one of four females with a very slight increase in the aggregates of alveolar macrophages which were multifocal in distribution. The minor microscopic changes suggest a very slight cellular response to an irritant. In this group, a very slight degree of the chronic active inflammatory process noted in the 200 ppm exposed group was observed in two females. The minor microscopic lung changes in the 80 ppm exposed dogs were not associated with clinical symptoms or gross consolidation. An occasional granulomatous foci was observed in the lungs of some female dogs due to apparent aspiration of feed particles or hairshafts. There were no exposure-related microscopic changes in the lungs of 20 ppm exposed dogs.

Brain: Microscopic changes in the brain were present in the head of the caudate nucleus of two of four males and three of four females in the 200 ppm exposure group. 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 due to cells lost at the time of tissue processing, or due to migration from the site prior to the time of necropsy was undetermined. The microscopic features of the lesion would suggest that it was 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 adjacent to the malacic foci were normal in appearance. The character of the microscopic change was suggestive of ischemic tissue damage rather than cytotoxicity to neuronal elements. There were no other recognisable changes in the numerous sections of brain stained with H&E, Luxol Fast Blue Cresyl Violet and Sevier-Munger.

Throughout the study no in-life clinical manifestations of neurotoxicity were observed by study personnel. The additional handling and restraint associated with repeated blood collection for haematology, clinical chemistry, and catheterization for urine collection were also not associated with any recognisable abnormal clinical behaviour suggestive of neurotoxicity. The microscopic effects in the brain were confined to dogs exposed to 200 ppm sulfuryl fluoride.

Thyroid: The effects in the thyroid gland of all male and three female dogs in the 200 ppm exposure group consisted of very slight hypertrophy of the follicular epithelium. The epithelial cells were minimally enlarged, follicles were decreased in size, and their colloid stained less intensely. There were no degenerative or inflammatory changes associated with the very slight hypertrophy. The microscopic thyroid gland effects were confined to dogs in the 200 ppm exposure group.

Teeth: A minor change was observed microscopically in the canine tooth that was evaluated for possible changes suggestive of dental fluorosis. The change consisted of concentric rings which stained slightly darker and corresponded with each day of exposure. The dentin which had formed prior to the first day of exposure lacked these rings. For the first 13 weeks of exposure there were 5 rings present for each exposure day of the week, with a larger space representing the two weekend days of non-exposure, between each group of 5 rings. During week 14 there were only 4 rings present which corresponded to Good Friday, a holiday, when the dogs were not exposed. The subsequent weeks contained the repetitive 5 rings associated with the daily exposure. As the teeth reached their maturity it was more difficult to recognise the presence of the rings. Other than the presence of these growth rings in the dentin, there were no other recognisable changes in the canine tooth. All of the 200 ppm exposed dogs and several in the 80 ppm were affected. The teeth of these dogs were found to be unaffected during in-life examination and at necropsy. There were no comparable effects recognised in the canine tooth of 20 ppm exposed dogs or the controls.

Miscellaneous: Some other tissues (liver and lymphoid tissue atrophy; bone marrow myeloid hyperplasia) had microscopic changes observed more frequently in the 200 ppm exposed group compared to the other groups; however, they were considered secondary to the effects described in the lungs. The other microscopic changes were considered incidental and normally observed in chronic dog studies.

There were no observations in the 20 ppm groups which were considered due to sulfuryl fluoride exposure.

Table 5.3.3.2c/01-15: Histopathologic Observations -- 12-Month Study

Sex	Males				Females			
Concentration (ppm)	0	20	80	200	0	20	80	200
Number of Dogs Examined	4	4	4	4	4	4	4	4
Bone Marrow (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	3	4	4	4	3
Hyperplasia, myeloid: - slight	0	0	0	0	0	0	0	1
- moderate	0	0	0	1	0	0	0	0
- any severity (combined)	0	0	0	1	0	0	0	1
Brain Cerebellum (# of tissues examined)	4	4	4	3	4	4	4	4
Missing:	0	0	0	1	0	0	0	0
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
Perivascular mononuclear (lymphoid) cell cuffing, multifocal: - very slight	0	0	0	0	0	1	0	0
Brain Cerebrum (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	2	4	3	4	1
Malacia, caudate nucleus, bilateral, focal: - very slight	0	0	0	1	0	0	0	0
- slight	0	0	0	1	0	0	0	2
- moderate	0	0	0	0	0	0	0	1
- any severity (combined)	0	0	0	2	0	0	0	3
Perivascular mononuclear (lymphoid) cell cuffing, multifocal: - very slight	0	0	0	0	0	1	0	0
Brain - Medulla Oblongata (# of tissues examined)	4	4	4	3	4	4	4	4
Missing:	0	0	0	1	0	0	0	0
Within normal limits:	4	4	4	3	4	4	4	4
Brain - Olfactory Lobe (# of tissues examined	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	4	4	4	4	4
Brain - thalamus/hypothalamus (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	3	4	4	4	4	4
Perivascular mononuclear (lymphoid) cell cuffing, unilateral, focal: - very slight	0	0	1	0	0	0	0	0
Liver (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	0	0	0	0	0	0	0	0
Aggregate(s) of reticuloendothelial cells:	4	4	4	4	4	4	4	4
Atrophy secondary to inanition, hepatocellular:	0	0	0	2	0	0	0	3
Pigment - haemosiderin, Kupffer cells, multifocal - very slight	0	0	0	0	1	1	4	0
- slight	0	0	0	0	2	0	0	2
- any severity (combined)	0	0	0	0	3	1	4	2
Lungs (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	0	0	0	0	0	0	0	0
Inflammation - chronic active, alveoli, multifocal:	0	0	0	2	0	0	2	1

Sex	Males				Females			
Concentration (ppm)	**0**	**20**	**80**	**200**	**0**	**20**	**80**	**200**
Number of Dogs Examined	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
- very slight								
- moderate	0	0	0	1	0	0	0	0
- severe	0	0	0	1	0	0	0	3
- any severity (combined)	0	0	0	4	0	0	2	4
Inflammation - fibrinous, pleura, multifocal: - moderate	0	0	0	1	0	0	0	0
Inflammation – granulomatous, focal: - very slight	0	0	0	0	1	0	0	0
Inflammation – granulomatous, multifocal: - very slight	0	0	0	0	0	1	2	0
Inflammation – granulomatous, focal or multifocal: - very slight (combined)	0	0	0	0	1	1	2	0
Inflammation – suppurative, lobar, focal: - severe	0	0	0	1	0	0	0	0
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 - Cervical (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	4	4	4	4	4
Lymph Node - Mediastinal (# of tissues examined)	4	3	4	4	4	4	4	4
Missing:	0	1	0	0	0	0	0	0
Within normal limits:	4	3	4	3	4	4	4	2
Atrophy:	0	0	0	1	0	0	0	0
Inflammation - acute, medulla: - very slight	0	0	0	0	0	0	0	1
Plasmacytosis of medullary cords: - very slight	0	0	0	0	0	0	0	1
Lymph Node - Mesenteric (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	2	4	4	4	1
Atrophy:	0	0	0	2	0	0	0	3
Oral Tissues (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	1	0	3	4	3	0
Ulcer, hard palate, focal:	0	0	0	0	1	0	0	0
Fluorosis - dental, canine tooth: - very slight	0	0	2	0	0	0	1	0
- slight	0	0	1	4	0	0	0	4
- any severity (combined)	0	0	3	4	0	0	1	4
Thyroid Gland (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	3	4	0	4	3	4	1
Aggregate(s) of mononuclear (predominately lymphoid) cells, multifocal: - very slight	0	1	0	0	0	0	0	0
Cyst, ducts, unilateral, focal:	0	0	0	0	0	1	0	0
Hypertrophy, epithelial cells: - very slight	0	0	0	4	0	0	0	3

Conclusions: **In this 1-year study inhalation study in Beagle dogs the NOAEL was 20 ppm based on very slight inflammation and aggregates of macrophages in alveoli at 80 ppm.**
The highest concentration of 200 ppm was not tolerated beyond approximately 9 months, when this level was terminated. The primary effects were respiratory in nature (e.g., severe inflammation) although other changes were evident, notably in the brain.

Section A6.4.3/03 **Annex Point IIA, VI.6.4**	**Evaluation by Competent Authorities**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2004
Materials and methods	The applicant's version is adopted.
Results and discussion	The applicant's version is adopted.
Conclusion	The applicant's version is adopted.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	The study is acceptable.
Remarks	No remarks.

Section A6.4.3/04 **Subchronic Inhalation Toxicity**
Annex Point IIA, VI.6.4

Mouse (IIA 5.3.3.2d/01, D05)

Report: [redacted] (1993)
Sulfuryl Fluoride: Thirteen-Week Inhalation Toxicity Study in CD-1 Mice
[redacted]
Report K-016399-032, dated 28/12/93; study began 29/11/88.

Guidelines: US EPA 82-4
OECD 412
84/449/EEC Method B8 [sic]
MAFF Guideline: Subchronic Inhalation Toxicity
Deviations from EC guideline Method B.29. Sub-Chronic Inhalation Toxicity Study: 90-Day Repeated Inhalation Dose Study Using Rodent Species: This study, with its extra groups for evaluation of brain pathology and serum fluoride, exceeds requirements of the guideline. Although the eyes were not examined after exposure using an ophthalmoscope, they were histologically examined in two separate groups using separate methods for fixing tissues.

GLP: Yes

Methodology: Test material: Sulfuryl fluoride (Lot WP 880329 752 MAR/88; consisting of 99.6% sulfuryl fluoride).
Groups of 14 CD-1 mice/sex were exposed to 0, 10, 30 or 100 ppm sulfuryl fluoride for 6 hours/day, 5 days/week for 13 weeks. On the day following the last exposure, subgroups of 10 mice/sex/level were examined for gross pathologic lesions.
Parameters examined were daily clinical observations; weekly detailed clinical examination; functional observational battery (FOB) on all mice after 4, 8 and 12 weeks of exposure; weekly body weights; haematology (HCT, Hgb, RBC, WBC/differential, platelets) of 10 mice/sex/group prior to necropsy; clinical chemistry (ALT, AST, AP, UN, creatinine, total protein, albumin, globulin, glucose, cholesterol, triglycerides, total bilirubin, Ca and P) on 10 mice/sex/level at necropsy; gross pathologic examinations of 10/sex/dose level; histopathologic examination of a wide range of tissues from the control and 100 ppm groups; histologic examination of nasal tissues, trachea, lungs, brain, thyroid, heart, liver, kidneys, salivary glands and testes from mice in the 10 and 30 ppm groups.
The remaining animals, 4/sex/dose level, were used to measure serum fluoride levels. In addition, tissues of these 4 animals/sex/exposure level 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.
Chamber (1000 L glass and stainless steel) concentrations were monitored using a Miran 1A IR.
Ophthalmological examination was conducted prior to the initial exposure and all found to be within normal limits.

Findings: Chamber concentrations determined analytically, were exactly the same as target

concentrations.

Mortality: Three mice died during the study: one control female due to inanition (tongue abscess) and the other two due to accidental handling trauma.

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

FOB: The auditory brainstem response in about half of the CD-1 mice from several different purchase orders had been shown to be poor; thus, the startle response, typically measured in FOBs, was not measured in this study.

Body weight: In general, mean body weights of male and female mice exposed to 100 ppm were statistically significantly decreased from control values from day 25 to the end of the study, as shown in Table 5.3.3.2d/01-1. By the end of the study, the mean body weights for male and female mice exposed to 100 ppm SO_2F_2 were 89 and 91% of control values, respectively. Mean body weights of male and female mice exposed to 10 or 30 ppm SO_2F_2 were comparable to control values.

Table 5.3.3.2d/01-1: Summary of Body Weights (g)

Conc. (ppm)	Males				Females			
	0	10	30	100	0	10	30	100
Days on Test								
1	33.1	32.4	32.4	32.8	24.9	24.2	24.9	24.1
25	35.9	35.5	35.3	33.9	27.5	27.2	28.2	25.5*
93	39.6	38.8	38.9	35.3*	30.3	30.7	31.2	27.6$

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

Food consumption: Not conducted.

Ophthalmology: Only conducted prior to exposure to assure animals were in good health and had good eyes.

Haematology: All parameters comparable with controls.

Clinical chemistry: Serum fluoride levels in male and female mice exposed to 100 ppm and female mice exposed to 30 ppm were statistically significantly elevated above controls in an exposure-related manner. Alkaline phosphatase activity and triglyceride levels in male mice exposed to 100 ppm were slightly but statistically significantly increased from control values. Although the slight increases in alkaline phosphatase activity and triglyceride levels in males appear to be exposure-related, they may be a secondary effect associated with an altered nutritional status and the body weight decrease. Data are given in Table 5.3.3.2d/01-2. In any event, there were no histopathologic changes which corresponded to these clinical chemistry alterations in males. All remaining clinical chemistry and electrolyte parameters for mice exposed to 10, 30 or 100 ppm SO_2F_2 were comparable to control values.

Table 5.3.3.2d/01-2: Clinical Chemistry (AP, Triglycerides in Males and Fluorides in Males and Females)

Conc. (ppm)	AP mu/ml	Trig mg/dl	Male Serum Fluoride, ppm (N=4)	Female Serum Fluoride, ppm (N=4)
0	43	91	0.107	0.090
10	42	83	0.112	0.088

30	47	89	0.156	0.132*
100	57*	143*	0.259*	0.233*

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

Urinalysis: Not conducted in mice.

Organ weights: The mean terminal body weight in male and female mice exposed to 100 ppm was statistically significantly decreased (Table 5.3.3.2d/01-3 and 5.3.3.2d/01-4). Several absolute organ weight values in males and females exposed to 100 ppm were different from control values. These differences included a statistically significant decrease in absolute brain, 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 histopathologic changes and the relative organ weights for these animals were comparable to control values. Thus, the decrease in absolute organ weights was considered to be a secondary reflection of the body weight decrease. Terminal body weights and absolute and relative organ weights of male and female mice exposed to 10 or 30 ppm SO_2F_2 were comparable to control values.

Table 5.3.3.2d/01-3: Organ Weights (Males)

Conc. (ppm)	Final Body Wt.	Brain		Heart		Kidneys		Liver	
	(g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	38.9	0.502	1.294	0.170	0.436	0.625	1.604	2.212	5.682
10	37.5	0.493	1.321	0.166	0.444	0.614	1.645	2.146	5.770
30	37.3	0.479	1.288	0.164	0.439	0.638	1.703	2.163	5.779
100	34.3*	0.471*	1.377	0.147*	0.428	0.490*	1.427	1.861*	5.411

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

Table 5.3.3.2d/01-4: Organ Weights (Females)

Conc. (ppm)	Final Body Wt.	Brain		Heart		Liver	
	(g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	29.7	0.511	1.721	0.134	0.451	1.621	5.460
10	31.1	0.504	1.619	0.141	0.453	1.650	5.300
30	30.6	0.504	1.647	0.139	0.454	1.673	5.463
100	27.5*	0.482*	1.760	0.125*	0.454	1.477*	5.373

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

Gross pathology: There were no exposure-related gross pathologic changes noted in mice exposed to concentrations as high as 100 ppm SO_2F_2. All gross lesions were considered to be incidental findings unrelated to exposure to the test material. A single male mouse (88A8025) in the 10 ppm exposed group contained a large abscess (approximately 3 cm in diameter) in the liver. The body weight of this mouse was low and the liver weight was high compared to other mice in this exposure group or controls. In addition, the red blood cell and haematocrit values for this mouse were decreased with an increase in white blood cell count and associated neutrophilia. In addition, ALT, AST, TP, ALB and GLOB were all altered in this mouse due to the liver abscess. Any alterations in mean values for these parameters in this exposed group