were not due to sulfuryl fluoride exposure, but due to the spurious values of this mouse with the liver abscess and the associated secondary effects.

Histopathology: There were a number of microscopic observations in mice from the various groups, which were primarily due to the three mice which died during the study, and the one mouse with the liver abscess. These observations were not considered exposure-related. However, a target organ effect was observed in the brain and thyroid gland of 100 ppm exposed mice, as shown in Table 5.3.3.2d/01-5.

Brain: In the section of brain taken from the cerebrum, microvacuolation in all 10 female mice and 9 of 10 male mice was observed in the external capsule and the caudate putamen. The lesion was bilaterally symmetrical and was very slight or slight in severity. There were no recognisable inflammatory or degenerative changes associated with the microvacuoles. The single male mouse from the 100 ppm exposed group without microvacuoles in the brain was the mouse which died during the course of the study. The section of brain taken from the region of the thalamus/hypothalamus also contained microvacuoles of a very slight degree involving the external capsule. In this region of the brain, the microvacuoles usually extended from the external capsule and involved the adjacent amygdaloid region. As in the cerebral section, there were no inflammatory or degenerative changes from the region of the thalamus/hypothalamus, containing the microvacuoles. There were no special stains utilised on these brain sections since they were previously extensively evaluated in the rat, with identical microvacuoles, without obtaining any additional useful information (Eisenbrandt and Nitschke, Fund. Appl. Toxicol., 12: 540-557, 1989).

Thyroid: The microscopic changes in the thyroid gland were characterised by hypertrophy of the follicular epithelial cells associated with a decrease in the amount of colloid present that stained less intensely. The changes in the thyroid gland were very slight in degree and affected slightly more males than females. There were no degenerative or inflammatory changes present in the thyroid gland of these mice.

Salivary Gland: Microscopic changes in the salivary gland of 100 ppm male mice were characterised by a very slight decrease in secretory content. They were not considered a target organ effect, but rather reflective of the decreased weight gain. These minimal effects were not present in the female mice of this group.

Nervous System/Special Process (see Table 5.3.3.2d/01-6): Tissues were examined from the nervous systems of the mice which were whole-body perfused for neuropathology assessment in accordance with the EPA (1991) test guidelines for neurotoxicity screening. The sections from various regions of the brain were the same as for the mice in the general toxicity portion of the study, except that the olfactory lobe was not evaluated in the neurotoxicity study, since it was not affected in previous studies and was located in the nasal turbinate section which was not decalcified and prepared for examination. The initial evaluation of the brain sections consisted of three sections from each of the various regions (see Methods). To maximise the detection of possible changes in the perfusion fixed tissue, in contrast to the immersion fixed tissue, a total of nine sections of brain were prepared from each mouse from regions 3, 4 and 7 (Figure not shown in this summary; see below). Despite the additional evaluation of extra brain sections, the microvacuoles in the various affected regions appeared less prevalent in the perfusion fixed than in the immersion fixed tissue. Whether this was an artifact or due to fewer mice being evaluated was undetermined. There were no other nervous system tissues affected in the perfusion fixed mice, and these findings were consistent with the absence of observed effects in other parts of the nervous system following immersion fixation.

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, V nerve, pyramidal tract, nucleus trapezoid body

Table 5.3.3.2d/01-5: Histopathologic Examinations (Brain, Thyroid, Salivary Gland-main study)

Sex	Males				Females			
Exposure Concentration (ppm)	**0**	**10**	**30**	**100**	**0**	**10**	**30**	**100**
Number of Mice Examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Brain - Cerebellum (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	10	10	10	10	10	10	10	10
Brain - Cerebrum (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	10	10	10	1	10	10	10	0
Vacuolation, caudate putamen, bilateral, focal:								
- very slight	0	0	0	7	0	0	0	3
- slight	0	0	0	2	0	0	0	5
Vacuolation, external capsule, bilateral, focal:								
- very slight	0	0	0	7	0	0	0	6
- slight	0	0	0	2	0	0	0	4
Brain - Medulla oblongata (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	10	10	10	10	10	10	10	10
Brain - Olfactory Lobe (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	10	10	10	10	10	10	10	10
Brain - Thalamus/hypothalamus (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	10	10	10	1	10	10	10	0
Vacuolation, external capsule, bilateral, focal:								
- very slight	0	0	0	9	0	0	0	10
Salivary Glands (# of tissues examined)	10	10	10	10	10	9	10	10
Missing:	0	0	0	0	0	1	0	0
Within normal limits:	9	10	9	0	9	9	10	10
Decreased secretory material: - very slight	1	0	1	10	1	0	0	0
Thyroid Gland (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	8	10	10	1	10	10	10	4
Cystic dilatation, follicles(s), focal:	1	0	0	0	0	0	0	0
Degeneration - fibrinoid, blood vessels, focal:	1	0	0	0	0	0	0	0
Hypertrophy, follicle(s): - very slight	0	0	0	9	0	0	0	6

Table 5.3.3.2d/01-6: Histopathologic Examinations (Brain-auxiliary study)

Sex	Males				Females			
Exposure Concentration (ppm)	**0**	**10**	**30**	**100**	**0**	**10**	**30**	**100**
Number of Mice 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

Sex	Males				Females			
Exposure Concentration (ppm)	0	10	30	100	0	10	30	100
Number of Mice Examined	4	4	4	4	4	4	4	4
Brain - Cerebrum (# of Tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	0	4	4	4	0
Vacuolation, caudate putamen, bilateral, focal: - very slight	0	0	0	2	0	0	0	2
- slight	0	0	0	1	0	0	0	1
Vacuolation, external capsule, bilateral, focal: - very slight	0	0	0	3	0	0	0	3
- slight	0	0	0	1	0	0	0	1
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 - Thalamus/hypothalamus (# of tissues examined)	4	4	4	4	4	4	4	4
Within normal limits:	4	4	4	0	4	4	4	0
Vacuolation, external capsule, bilateral, focal: - very slight	0	0	0	3	0	0	0	3
- slight	0	0	0	1	0	0	0	1
Dorsal Root Ganglia with Roots-Cervical (# of tissues examined)	4	0	0	4	4	0	0	4
Within normal limits:	4	0	0	4	4	0	0	4
Dorsal Root Ganglia with Roots-Lumbar (# of tissues examined)	4	0	0	4	4	0	0	4
Within normal limits:	4	0	0	4	4	0	0	4
Eyes (# of tissues examined)	4	0	0	4	4	0	0	4
Within normal limits:	4	--	--	3	4	--	--	4
Mineralisation, cornea, unilateral, focal: - very slight	0	--	--	1	0	--	--	0
Peripheral Nerve - Sciatic (# of tissues examined)	4	0	0	4	4	0	0	4
Within normal limits:	4	--	--	4	4	--	--	4
Peripheral Nerve - Sural (# of tissues examined)	4	0	0	4	4	0	0	4
Within normal limits:	4	--	--	4	4	--	--	4
Peripheral Nerve - Tibial (# of tissues examined)	4	0	0	4	4	0	0	4
Within normal limits:	4	--	--	4	4	--	--	4
Spinal Cord - Cervical (# of tissues examined)	4	0	0	4	4	0	0	4
Within normal limits:	4	--	--	4	4	--	--	4
Spinal Cord - Lumbar (# of tissues examined)	4	0	0	4	4	0	0	4
Within normal limits:	4	--	--	4	4	--	--	4
Trigeminal Ganglia (# of tissues examined)	4	0	0	4	4	0	0	4
Within normal limits:	4	--	--	4	4	--	--	4

Conclusions: **In 13-week inhalation study in CD-1 mice the NOAEL was 30 ppm primarily based on reduced body weight gain (ca. 10%) and changes in the brain at 100 ppm. These comprised very slight to slight vacuolation in the cerebrum and thalamus/hypothalamus of males and females.**

Section A6.4.3/04 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 minor amendments. The exposure was whole-body in chambers. Some minor deviations from the guideline, except the ones already described by the applicant, occurred. No food consumption or water consumption was measured. Ophtalmology should have been conducted at the end of the exposure.
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 A6.4.3/05 **Subchronic Inhalation Toxicity**
Annex Point IIA, VI.6.4

Rabbits (IIA 5.3.3.2e/04, D07)

Report:	[redacted] (1987) Sulfuryl Fluoride (Vikane* Gas Fumigant): 13-Week Inhalation Toxicity Study with Rabbits [redacted] Report-K-016399-025B, dated 16/11/87; study began 11/9/84.
Guidelines:	US EPA 82-4 Deviations from EC guideline: There is no guideline for a Non-Rodent 90-Day Inhalation Study.
GLP:	Yes
Methodology:	Test material: Vikane gas fumigant (Lot # TWP 830919-408) which was 99.8% sulfuryl fluoride. Groups of 7 rabbits/sex were scheduled for exposure to 0, 30, 100 or 600 ppm sulfuryl fluoride for 6 hours/day, 5 days/week for 13 weeks. After 9 exposures to 600 ppm, rabbits from the highest exposure group were exposed to 300 ppm for the remainder of the study. All exposures were whole-body exposures conducted in 4.1 m^3 chambers. Parameters examined were: daily clinical observations; weekly body weights; haematology (HCT, Hgb, RBC, MCV, MCH, MCHC, WBC/differential and platelets) at the end of the study; clinical chemistry (Ca, P, creatinine, UN, ALT, AST, AP, glucose, total protein, albumin, globulins and fluoride) at the end of the study; gross pathology of all unfasted rabbits; weights of brain, heart, liver, kidneys and testes; complete histopathological examination of control and top dose animal tissues; brain, kidneys, liver, nasal tissues, oral tissues, trachea and lungs of low and middle dose rabbits; and special staining techniques and light microscopy of sections of brain from one female rabbit exposed to 100 ppm and 3 males and 4 females from the top dose. Chamber atmospheres were determined analytically using a Miran 1A IR.
Findings:	Chamber concentrations were very close to target concentrations. 30 ppm → 29.8 ppm 100 ppm → 100 ppm 300 ppm → 337 ppm (including initial 9 exposures at 600 ppm)
Mortality:	One female was euthanised following the 8th exposure to 600 ppm due to not having the use of its hind limbs.
Clinical signs:	Convulsions were observed in 1 male and 1 female rabbit following the ninth exposure to 600 ppm sulfuryl fluoride. In the male, head bobbing was observed prior to and after a tonic convulsion; the female rabbit exhibited deep breathing, salivation, rapid eye blinking accompanied by convulsions and head bobbing. A second female rabbit did not have use of hind limbs following the eighth exposure to 600 ppm sulfuryl fluoride and was euthanised. Due to these effects in male and female rabbits exposed to 600 ppm sulfuryl fluoride, these animals were exposed to 300 ppm for the remainder of the study. There were no clinically visible effects in rabbits exposed to 30 or 100 ppm for 13 weeks or 300 ppm sulfuryl fluoride for 11

weeks.

Approximately the same time as the concentration was reduced from 600 to 300 ppm sulfuryl fluoride, the (non-measured) food consumption of male and female rabbits at this concentration was decreased and remained decreased for approximately 4 weeks. The decrease in food consumption was based on visual inspection and was most likely due to previous exposure to 600 ppm sulfuryl fluoride.

Body weight: The body weights of male rabbits exposed to 300 ppm sulfuryl fluoride were statistically significantly decreased from control values from days 11 to 60 and remained slightly decreased until termination; the body weights of females were slightly decreased from control values also (Table 5.3.3.2e/01-1). Body weights of male and female rabbits exposed to100 ppm sulfuryl fluoride were slightly decreased from control values at the end of the 13-week exposure period. Body weights of rabbits exposed to 30 ppm sulfuryl fluoride were comparable to control values throughout the 13-week exposure period.

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

Conc (ppm)		Males				Females			
		0	30	100	600/300	0	30	100	600/300
Days on Test	-1	3318	3329	3322	3334	3255	3273	3301	3333
	4	3338	3358	3329	3346	3275	3283	3351	3363
	11	3481	3499	3408	3254*	3368	3408	3476	3378
	25	3712	3714	3674	3323*	3646	3662	3699	3555
	88	4139	4109	3963	3823	4564	4591	4383	4301

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

Food consumption: Not conducted.

Ophthalmology: Not conducted.

Haematology: The white blood cell counts for male rabbits exposed to 300 ppm sulfuryl fluoride were statistically significantly increased from control values (Table 5.3.3.2e/01-2. The differential counts for these animals were normal. All other haematologic parameters were unaffected in rabbits exposed to sulfuryl fluoride.

Table 5.3.3.2e/01-2: White Blood Cell Counts (Males)

Conc. (ppm)	0	30	100	300
WBC x 10^3/cu. mm	7.6	7.8	7.5	9.9*

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

Clinical chemistry: Several clinical chemistry values from rabbits exposed to 30, 100 or 300 ppm sulfuryl fluoride were statistically significantly different from control values (Table 5.3.3.2e/01-3). The statistically significant decrease in total protein, albumin and globulin observed in males exposed to 300 ppm is most likely due to the decreased nutritional status. None of the remaining statistically significant parameters occurred in a concentration-related manner and/or were observed in only one sex. Thus, these were considered to be due to normal variation and not a result of exposure to sulfuryl fluoride. No other clinical chemistry parameters were affected in rats exposed to sulfuryl fluoride.

Serum fluoride levels of male and female rabbits exposed to 30, 100 or 300 ppm sulfuryl fluoride were significantly increased from controls (Table 5.3.3.2e/01-4).

Table 5.3.3.2e/01-3: Clinical Chemistry

Conc. (ppm)	UN (females)	Glucose (males)	Total Protein (males)	Albumin (males)	Globulin (males)	Ca (males)	P (males)
	mg/dl	mg/dl	g/dl	g/dl	g/dl	mg/dl	mg/dl
0	20	176	63	4.4	1.9	15.3	6.2
30	21	194	5.7*	4.1*	1.6*	14.5*	6.1
100	21	219*	6.4	4.4	2.0	14.8	7.2*
300	24*	199	5.7*	4.1*	1.5*	14.7	6.1

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

Table 5.3.3.2e/01-4: Fluoride Concentration (µg/ml)

Conc. (ppm)	0	30	100	300
Males	0.065	0.170$	0.408$	0.621$
Females	0.560	0.697*	0.829*	1.003*

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

Urinalysis: Not conducted.

Organ weights: The final body weights of male and female rabbits exposed to 100 or 300 ppm sulfuryl fluoride were slightly decreased from control values (Table 5.3.3.2e/01-5). Absolute liver weights of male rabbits exposed to 100 ppm and absolute and relative liver weights of female rabbits exposed to 300 ppm sulfuryl fluoride were statistically significantly decreased from control values. The absolute liver weight of male rabbits exposed to 300 ppm sulfuryl fluoride was also slightly decreased from control values. Due to the lack of a dose-response relationship in male rabbit liver weights following exposure to 100 or 300 ppm, these values are probably related to decreased food consumption. Although the absolute and relative liver weights of female rabbits exposed to 300 ppm sulfuryl fluoride were either close to or within the range of historical control values (Table 5.3.3.2e/01-6), the decrease in liver weight values was considered to be exposure-related and most likely secondary to decreased food consumption. All other absolute and relative organ weight values for rabbits exposed to 30, 100 or 300 ppm sulfuryl fluoride were comparable to control values.

Table 5.3.3.2e/01-5: Organ Weights

Conc. (ppm)	Final Weight	Liver	
	(g)	(g)	(g/100g)
Males			
0	4132.4	130.269	3.147
30	4128.9	129.289	3.128
100	3827.9	99.430*	2.598
300	3858.8	110.924	2.871

Females			
0	4549.3	143.366	3.153
30	4597.3	137.743	3.000
100	4396.2	113.613	2.565
300	4314.4	103.587*	2.399*

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

Table 5.3.3.2e/01-6: Historical Control Liver Weights

Parameter	# of Groups	Total # of Animals	Minimum Control Group Mean	Maximum Control Group Mean	Mean of Means	S.D. of Means
Males						
Liver wt (g)	4	26	93.8	130.3	106.7	16.4
Liver wt (g/100g)	4	26	2.31	3.15	2.59	0.38
Females						
Liver wt (g)	4	26	105.7	143.4	118.2	17.6
Liver wt (g/100g)	4	26	2.32	3.15	2.58	0.39

Gross pathology: One female rabbit exposed to 600 ppm was euthanised after the 8th exposure. This animal had lost use of its hind limbs and had a fractured vertebra.

The few grossly observed lesions at the end of the 13-week study were considered to be due to normal variation and not a result of exposure to sulfuryl fluoride.

Histopathology: Microscopic changes were observed in the nasal tissues of most male and female rabbits exposed to 300 ppm and one male exposed to 100 ppm sulfuryl fluoride (Table 5.3.3.2e/01-7). The changes consisted of varying degrees of purulent nasal exudate, olfactory epithelial degeneration and hyperplasia and hypertrophy of the respiratory epithelium. The most prominent features were the luminal exudate and hyperplasia and hypertrophy of the respiratory epithelium. The affected respiratory epithelium was primarily located on the nasal turbinates and consisted of goblet cells and pseudostratified epithelial cells.

Microscopic changes were observed in the brain of most male and female rabbits exposed to 300 ppm and 1 female rabbit exposed to 100 ppm sulfuryl fluoride. The microscopic changes ranged from vacuolation of the white matter following exposure to 100 ppm, to malacia of the internal and external capsules, putamen and globus pallidus following exposure to 300 ppm. In addition, some animals exposed to 300 ppm had gliosis and/or hypertrophy of vascular endothelial cells in the same location. Special stains of the brain with LFB-PAS or Sevier Munger stain did not reveal any additional effects.

All other histopathologic changes observed were considered to be spontaneous changes typical of rabbits of this age and strain and unrelated to exposure.

Table 5.3.3.2e/01-7: Histopathology (Brain and Nasal Tissues)

Sex	**Males**				**Females**			
Exposure Concentration (ppm)	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Number of Rabbits Examined	**7**	**7**	**7**	**7**	**7**	**7**	**7**	**7**
Brain (# of tissues examined)	7	7	7	7	7	7	7	7
Within normal limits:	7	7	7	1	7	7	6	1
Gliosis, cerebrum, focal: - slight	0	0	0	0	0	0	0	2

Sex	Males				Females			
Exposure Concentration (ppm)	0	30	100	300	0	30	100	300
Number of Rabbits Examined	7	7	7	7	7	7	7	7
Hypertrophy, endothelium, focal: - very slight	0	0	0	0	0	0	0	2
Inflammatory changes consistent with encephalitizoonosis, multifocal: - slight	0	0	0	0	0	0	0	1
Malacia, cerebrum, focal: - severe	0	0	0	3	0	0	0	1
Vacuolation, cerebrum, focal: - very slight	0	0	0	3	0	0	0	3
Vacuolation, cerebrum, focal: - slight	0	0	0	0	0	0	0	2
Vacuolation, cerebrum, focal: - moderate	0	0	0	0	0	0	1	0
Nasal Tissues (# of tissues examined)	7	7	7	7	7	7	7	7
Within normal limits:	2	1	1	0	1	0	2	0
Degeneration, olfactory epithelium, multifocal: - slight	0	0	0	1	0	0	0	2
Degeneration, olfactory epithelium, diffuse: - slight	0	0	0	0	0	0	0	1
- moderate	0	0	0	1	0	0	0	0
Dilated, blood vessels, diffuse: - severe	0	0	0	0	0	0	0	1
Inflammation – chronic, submucosa, focal: - very slight	0	1	0	0	1	0	2	1
Inflammation – chronic, submucosa, multifocal: - very slight	4	5	3	2	4	5	3	3
- slight	1	0	1	0	1	2	0	1
Inflammation – chronic, submucosa, diffuse: - severe	0	0	0	2	0	0	0	0
Inflammation – chronic acitve, submucosa, multifocal: - very slight	0	0	1	0	0	0	0	1
- slight	0	0	0	0	0	0	0	1
- moderate	0	0	0	2	0	0	0	0
Inflammation - suppurative, submucosa, focal: - very slight	0	0	1	0	0	0	0	0
- moderate	1	0	0	0	0	0	0	0
Inflammation - suppurative, submucosa, multifocal: - very slight	0	0	1	0	0	0	0	0
Inflammation - suppurative, submucosa, diffuse: - slight	0	0	0	1	0	0	0	0
Necrosis, respiratory epithelium, multifocal: - very slight	0	0	0	0	0	0	0	1
Exudate, lumen, diffuse: - very slight	0	0	1	1	2	1	0	1
- slight	1	0	0	3	0	0	0	2
- moderate	0	0	1	1	0	0	0	1
- severe	0	0	0	2	0	0	0	2
Hyperplasia and hypertrophy, respiratory epithelium, diffuse: - very slight	0	0	0	1	0	0	0	0
- slight	0	0	0	2	0	0	0	3
- moderate	0	0	0	2	0	0	0	0
- severe	0	0	0	2	0	0	0	3

Conclusions: **In this 13-week inhalation study in NZW rabbits the NOAEL was 30 ppm based on changes in the upper respiratory tract and brain, comprising moderate vacuolation in the cerebrum, in one animal at 100 ppm. At 300 ppm (partially 600 ppm) all rabbits had respiratory tract and brain changes, comprising vacuolation to malacia in the putamen and globus pallidus.**

Section A6.4.3/05 **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 the following amendments. The rabbits were of the strain New Zealand White. After 9 exposures the rabbits in the 600 ppm group were exposed to 300 ppm for the remainder of the study.
Results and discussion	The applicant's version is adopted with one revision. The figure of total protein in control males (0 ppm) should be 6.3 according to original report (DOC IV) in Table 5.3.3.2e/01-3.
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 NOEL was <30 ppm based on the statistically significant increase in serum fluoride.

Section A6.5/01 **Chronic Toxicity (Combined with Carcinogenicity A6.7)**
Annex Point AII, VI. 6.5

Long term toxicity and carcinogenicity (Rat, 5.5a/01, I03)

Report: [redacted] (1993)
Sulfuryl Fluoride: 2-Year Inhalation Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats
[redacted]
Report DECO-HET-K-016399-040, dated 18/8/93; study began 17/7/90.

Guidelines: US EPA 83-5
OECD 453
87/302/EEC: Combined Chronic Toxicity/Oncogenicity Test
MAFF Guideline: Combined Chronic Toxicity/Oncogenicity Test
Deviations from EC test guideline Method B.33. Combined Chronic Toxicity/Carcinogenicity Test: the satellite groups had 15 rats instead of 20; food consumption was not conducted as dosing was by inhalation; the top dose males reached 50% mortality at about 20 months, females of the top dose at slightly over 20 months and both sexes reached 100% mortality in the top dose at about 23 months (due to toxicity).

GLP: Yes

Methodology: Test Material: Several smaller quantities (lot #'s WP 880329-752, WP 901011- 907, WP 910321-918, WP 910826-929 and WP 920131-940) of sulfuryl fluoride were obtained from DowElanco, Pittsburg, CA during the course of the 2-year study, due to safety and storage considerations. 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 of each lot were analysed by 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 the purity of the lots ranged from 93.6% to 99.7% sulfuryl fluoride. Three minor impurity peaks were observed and identified as air, water and thionyl fluoride by GC/MS.

Groups of 50 rats/sex were exposed to targeted concentrations of 0, 5, 20 or 80 ppm sulfuryl fluoride for 6 hours/day, 5 days/week for up to 2 years. Fifteen additional rats/sex/exposure level were randomly designated at the beginning of the study as a satellite group. The satellite group of rats were necropsied after approximately 12 months of exposure to evaluate chronic general toxicity and chronic neurotoxicity of sulfuryl fluoride, prior to the onset of confounding geriatric changes. The methods and results of the neurotoxicity portion of this study were the subject of a separate report (IIA 5.8.2a/03, **G06**).

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 for assessment of exposure-related effects. Animals 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 haematologic (HCT, Hgb, RBC, WBC/diff, platelets) and clinical chemistry (AP, ALT, creatine kinase, UN, creatinine, total protein, albumin, globulin, glucose, cholesterol, triglycerides, total bilirubin, Na, K, P, Cl and Ca) determinations from 10 animals/sex/exposure level at approximately 6, 12, 19 and 21 months, and from 20 animals/sex/exposure level at the 24-month necropsy. Urinalyses (pH, bilirubin, glucose, proteins, ketones, occult blood, urobilinogen, colour, appearance and specific gravity) were evaluated from the same animals at approximately the same time intervals as for haematology and clinical chemistry.

At scheduled necropsies a full range of tissues was collected and major organs weighed. All tissues from control and 80 ppm exposed rats were examined histopathologically from the 12-month necropsy. Target tissues and grossly visible lesions were also examined from intermediate exposure levels. Because all of the 80 ppm exposed rats in the 2-year study were removed moribund or dead prior to study termination, a complete set of tissues from all groups was prepared and examined microscopically, per agreement with California Department of Food and Agriculture (CDFA) toxicologists.

Animals were exposed in 14.5 m^3 stainless steel and glass chambers under dynamic airflow conditions. Sulfuryl fluoride concentrations were generated using the J-tube method and analysed using a Miran 1A IR.

Appropriate statistical methods were employed.

Findings: The chambers ran at target measurements. Analytical concentrations were:

5.1, 20.2 and 79.6 ppm.

Mortality: Cumulative mortality data are given in Table 5.5a/01-1. Satellite Group: A single male control rat from the 12-month interim scheduled necropsy was found dead on day 198. He may have died of choking on food.

Oncogenicity Group: Only a small number of male rats were removed from study prior to day 519 in any of the exposure groups; however, increased morbidity and mortality was readily apparent thereafter in the 80 ppm exposed males. By day 665 only a single male rat was alive in this group and was removed from study by day 707. The mortality rates in the 5 and 20 ppm male exposure groups were not statistically identified as different from the control group.

A slight increased mortality rate in female rats of the 0 and 5 ppm groups was apparent by day 519, in contrast to the lower rate in the 20 and 80 ppm exposed groups.

It was not until day 560 when the mortality rate in the 80 ppm group was equal to the controls; however, during the remainder of the study, excessive morbidity and mortality increased rapidly in the high exposure group in contrast to any other group of females. Similar to 80 ppm exposed male rats, only a single female was alive by day 665, and was removed from study by day 701. The mortality rates of the 5 and 20 ppm exposed groups of females were lower than the controls and statistically identified.

Cause of Death/Moribund Condition. The expected disease conditions routinely observed in chronic toxicity/oncogenicity studies using Fischer 344 rats were noted in this study. However, 80 ppm exposed male and female rats were removed from study primarily due to advanced chronic renal disease as a result of sulfuryl fluoride exposure. There were also 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 were also observed in rats that were removed from study. In the 5 and 20 ppm exposed rats, there were no significant exposure-related effects on morbidity or mortality due to renal disease.

Table 5.5.a/01-1:Summary of Percentage Cumulative Mortality--24-Month Study

Conc. (ppm)	Males				Females			
Test Days	**0**	**5**	**20**	**80**	**0**	**5**	**20**	**80**
1-63	0	0	0	0	0	0	0	0
64-70	2	0	0	0	0	0	0	0
204-210	2	0	0	2	0	0	0	0
337-343	2	0	0	2	2	0	0	0
351-357	2	2	0	2	2	0	0	0
414-420	2	2	0	2	2	2	0	0
421-427	2	2	0	4	2	2	0	0
435-441	2	2	0	4	2	2	2	0
449-455	2	2	0	4	2	4	2	0
456-462	2	2	2	4	2	4	2	0
477-483	2	2	2	4	2	6	2	0
484-490	4	2	2	4	2	6	2	0
498-504	4	2	4	4	6	6	2	2
512-518	4	2	4	6	8	6	2	2
519-525	4	2	4	10	10	8	2	2
526-532	4	2	4	10	10	8	4	4
533-539	4	4	6	12	10	8	4	6
540-546	4	4	6	12	12	8	4	8
547-553	4	6	6	22	12	8	4	10
554-560	10	6	6	22	12	8	4	12
561-567	10	8	6	30	12	8	4	12
568-574	10	10	6	36	12	8	4	14
575-581	10	12	6	38	12	8	6	18
582-588	10	14	6	46	16	8	6	26
589-595	10	16	8	52	18	8	6	28
596-602	12	16	8	62	18	8	6	38
603-609	14	16	8	66	22	10	6	48
610-616	14	16	8	66	22	10	10	54
617-623	14	16	8	72	22	10	10	54
624-630	14	16	8	76	28	10	10	58
631-637	16	16	12	86	28	12	10	70
638-644	18	18	14	86	28	12	12	82
645-651	20	20	14	90	30	14	12	90
652-658	24	20	14	92	30	14	12	90
659-665	24	22	16	94	34	14	14	94
666-672	26	24	18	98	34	14	14	98
673-679	28	26	20	98	36	14	14	98
680-686	32	26	22	98	40	16	18	98
687-693	32	26	24	98	42	18	20	98

Conc. (ppm)	Males				Females			
Test Days	**0**	**5**	**20**	**80**	**0**	**5**	**20**	**80**
694-700	34	30	28	98	44	18	20	98
701-707	36	34	28	100	46	20	22	100
708-714	38	34	28	100	46	22	24	100
715-721	38	34	30	100	46	24	24	100
722-728	40	34	42	100	46	26	24	100
729-735	42	34	44	100	50	26	24	100
736-737M/738F	42	36	44	100*	50	26	24	100*

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

Clinical signs: After day 374, the number of rats for which observations were made in the various groups were fewer in number because 15 rats/sex/exposure group were removed for the 12-month necropsy. A number of rats in each group had external mass/nodule observations in various locations of the skin and subcutaneous tissue including the mammary region. Preputial and clitoral gland swellings were frequently observed which upon subsequent examination were no longer present. In all likelihood these swellings were, for the most part, abscesses that drained to the exterior and rapidly healed. In an occasional rat, vaginal bleeding was observed and was usually due to a uterine endometrial stromal polyp.

Although nervous system effects were previously observed clinically in rats exposed to higher concentrations of sulfuryl fluoride, none were seen in rats necropsied for the 12 month neurotoxicity study nor in those from the 12- and 24-month toxicity/oncogenicity study.

In general, the clinical observations were those commonly seen as age-related observations in chronic studies with Fischer 344 rats and not due to sulfuryl fluoride exposure.

Palpable Masses: Part of the in-life examinations consisted of detecting palpable masses that could suggest an early onset of an oncogenic effect. The location and date of initial appearance of clinically observed masses, and their final outcome based upon gross and histopathologic findings, were summarised in the report. In male rats, various tumours of the skin, mammary gland and preputial gland were observed. A number of these grossly observed mass/nodules were found microscopically to be inflammatory reactions, abscesses and epidermal inclusion cysts, and not neoplasms. In female rats, the majority of the mass/nodules were microscopically found to be mammary gland tumours, galactoceles and cystic dilatations of mammary gland acini and/or ducts.

FOB: See study summary III-A6.9/03, **G06** for FOB, motor activity and neuropathology data on 12-month rats.

Body weight: Data are given in Table 5.5a/01-2.

Males: Although the body weights of all groups on day -5 were comparable, they were slightly lower in the 5, 20 and 80 ppm groups compared to controls on the day prior to study initiation. Sporadic differences were observed throughout the first year of exposure and were occasionally statistically identified. Because of the lower pre-exposure body weights, the changes during the first year were not considered exposure related; however, after one year a statistically-identified progressively lower body weight was seen in the rats of the 80 ppm exposed group. The male rat body weights in the 5 and 20 ppm groups were unaffected by exposure. As a

reflection of normal age-related changes, the mean weights of all groups of surviving male rats at the end of the study were less than at approximately 12 and 18 months.

Females: The body weights of the 80 ppm group were minimally lower than the other exposed groups and controls on the day prior to study initiation. Sporadic, statistically-identified decreases in body weight were seen in the 5 and 20 ppm groups which were not considered exposure related. However, in the 80 ppm exposed group the decreased body weights were consistent throughout the study, and as in the males, became progressively more severe after one year. In contrast to the age-related decreased body weight of male controls and lower exposure groups during the last 6 months of study, the females in these groups continued to maintain weight during this time.

The body weights of female rats, as for males, in the 5 and 20 ppm groups were interpreted to be unaffected by sulfuryl fluoride exposure.

Table 5.5a/01-2: Summary of Body Weights

Conc. (ppm)		Male				Female			
		0	**5**	**20**	**80**	**0**	**5**	**20**	**80**
Days on Test	**-1**	98.1	97.7	95.8	96.1	84.4	84.9	84.7	83.9
	5	121.8	120.7	119.3	120.4	100.5	100.4	99.3	98.5*
	12	161.3	158.8	155.9*	156.9	120.6	120.5	119.3	115.4*
	26	215.9	211.8	209.9*	211.1	140.8	140.8	138.8	134.4$
	89	314.6	307.9	307.3*	307.4*	180.3	180.3	177.6	171.7$
	173	359.7	361.5	359.9	358.4	200.2	199.7	198.7	192.8*
	369	414.2	414.2	416.7	407.0	222.8	220.0	220.8	216.5*
	453	432.1	435.5	434.3	414.4*	237.0	231.8	232.3	225.3*
	537	432.4	429.1	429.4	384.9*	254.4	245.9	245.3*	219.9*
	621	435.9	430.4	427.1	347.5*	266.2	263.2	262.6	214.4*
	649	424.7	422.8	421.0	333.1$	260.7	262.1	260.6	207.6*
	677	424.4	417.8	413.4	374.6	262.2	264.5	260.2	247.9
	706	411.8	407.5	400.1	--	257.4	265.7	259.2	--
	734	401.7	388.7	378.7	--	255.9	265.4	258.4	--

*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, as this is an inhalation study.

Ophthalmology: There were no pre-study eye abnormalities in any rats assigned to study. In addition, there were no exposure-related ophthalmologic effects noted grossly or microscopically, except for some secondary changes due to kidney failure in the 80 ppm exposed group.

Haematology: Males: The 6- and 12-month samples were collected from the same rats that were pre-designated for the 12-month necropsy. At each time-point, there were 10 rats/group, except for the control males with 9 at 12 months, due to a death prior to this bleed. Collection of blood samples from the same rats at 6 and 12 months allowed for comparison of time-related changes and correlation with other toxicologic and pathologic findings at the one-year necropsy. The blood samples for the 19 and 21 month intervals were collected from the first 10 surviving rats of each

group, except for only 8 in the 80 ppm group at 19 months. At the 24-month termination of the study, the first 20 surviving male rats of the 0, 5 and 20 ppm groups were used for sample collection.

There were occasional statistically identified increased and decreased haematologic values observed at various times that were not considered to be exposure related. Most noteworthy of these were the apparent decreased erythroid parameters at 19 and 21 months in the 80 ppm male group. These were not considered exposure-related because of high control male values at this time, when compared to their values at 6, 12 and 24 months.

Females: Prior to terminal sacrifice there were 10 rats/sex/group bled at each time-point except for 9 (all survivors) in the 80 ppm group at 21 months. When the study was terminated at 24 months, the first 20 surviving females in the 0, 5 and 20 ppm groups were bled for haematology.

As seen in male rats, some of the parameters in females were increased or decreased relative to controls and were occasionally statistically identified. None of these parameters showed a consistent pattern for an exposure-related effect and were not interpreted to be biologically significant. Interestingly, the high control values for the erythroid parameters of males at 19 and 21 months were not seen in the female controls. Therefore, at these time intervals the erythroid values in the 80 ppm females were not identified as statistically different from controls, as they were in the males.

Evaluation of individual rat differential blood counts and morphology of blood smears from the various time periods failed to detect an effect other than the expected changes associated with degenerative and inflammatory conditions, blood loss, or most commonly, changes due to Fischer rat leukaemia syndrome. Furthermore, histopathologic evaluation of bone marrow sections did not reveal an exposure-related effect.

Based upon the results of repeated evaluations of haematologic parameters throughout the study, it was concluded that no adverse effects were observed in any exposed group.

Data for RBC, haemaglobin and haematocrit are presented inTable 5.5.a/01-3.

Table 5.5a/01-3: Red Blood Cell Parameters (19, 21 Months for dosed rats and all time periods for controls)

Conc. (ppm) and Group	RBC x10^6/cu mm	HGB g/dl	HCT %
Males			
6 Month, 0 ppm	8.51	15.9	53.8
12-Month, 0 ppm	9.20	14.4	58.2
19-Month			
0	10.10	16.1	59.8
5	9.44	15.4	56.1
20	9.67	15.7	57.6
80	8.92$^{\$}$	14.7	54.2$^{\$}$
21-Month			
0	10.19	17.2	61.7
5	9.25	15.5	55.9
20	9.09	15.3	55.0
80	9.14	15.4$^{\$}$	55.0$^{\$}$

24-Month, 0 ppm	8.16	14.1	53.6
5	7.99	13.6	52.1
20	8.07	14.0	53.0
Females			
6-Month, 0 ppm	7.67	15.6	52.7
12-Month, 0 ppm	8.30	14.5	57.5
19-Month			
0	8.16	14.1	52.0
5	9.09	15.4	57.0
20	9.43$	15.9$	58.8$
80	8.21	14.2	51.8
21-Months			
0	8.44	15.4	52.1
5	9.50	16.9	57.1
20	9.97$	17.7$	60.0$
80	7.53	13.4	44.9
24-Month, 0 ppm	7.78	14.0	53.3
5	8.09	14.4	55.1
20	8.40$	14.8	56.7

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

Clinical chemistry:

Data are presented in Table 5.5a/01-4 and 5.5a/01-5 for males and females, respectively. A number of data points that lacked consistency in response with time, sex, and exposure concentration were identified as statistically significant, but were not considered relevant to sulfuryl fluoride exposure. However, the following parameters in the 80 ppm group were affected by exposure: urea nitrogen, total protein, albumin, cholesterol, triglycerides, creatinine, phosphorus and chloride. The clinical chemistry parameters of kidney function that were statistically identified were consistent with the gross and histopathologic renal toxicity observed.

Urea Nitrogen: There was a slight but statistically significant increase in urea nitrogen in both sexes of the 80 ppm group at 12 months. Although these values were statistically identified as increased, they were well within the normal accepted range. The slight microscopically detected increase over the normal background renal disease at this time would not be expected to result in a biologically significant increase (generally considered 2x) in serum urea nitrogen. However, by 19 and 21 months, the abnormally elevated levels of urea nitrogen were reflective of the increasing severity of renal toxicity in this group. For rats in the 5 and 20 ppm groups, no toxicologically significant alterations in urea nitrogen were observed, although occasional values were statistically significant.

Total Protein: Only male rat values were slightly decreased and statistically identified at 12 months in the high exposure group. These minimal physiological changes in the males were associated with slightly more severe histopathological effects in this sex when compared to females. The decreased values in both sexes at 19 and 21 months were correlated with the abnormally elevated urea nitrogen and were consistent with chronic renal disease and protein loss. The serum total protein levels were unaffected throughout the study in the 5 and 20 ppm groups. The decreases in total protein were the result of decreased serum levels of albumin.

Albumin: Decreased serum albumin was present only in the high exposed group of males by 12 months. During subsequent samplings albumin levels were decreased

in both sexes of the high exposed group resulting in the decreased total protein levels. A statistically-identified increased albumin level in the 5 ppm group of females at study termination was inconsistent with the exposure-related decreases observed in the 80 ppm group, and therefore, was not considered toxicologically significant nor due to exposure.

Cholesterol: A slight increased serum cholesterol level was present in male rats by 12 months; however, levels in both sexes were abnormally elevated at 19 and 21 months. The serum cholesterol levels were unaffected in the lower exposed groups.

Triglycerides: The increased triglyceride values in the high exposed group of males and females at 19 and 21 months were considered to be biologically affected by sulfuryl fluoride exposure. The absence of statistical significance in the female group was due to a single female control rat (90A5395) which had extreme outlier values for albumin, cholesterol, triglycerides, total bilirubin, creatinine and phosphorus at these time intervals. This rat was in a moribund condition and necropsied on day 707 with advanced chronic renal disease, a complex adrenal gland pheochromocytoma, a pituitary gland adenoma and a thyroid gland parafollicular cell carcinoma which metastasised. Therefore, the triglyceride values in the 80 ppm group were not statistically identified as increased because of this extremely abnormal control rat which was not representative of the group. The triglyceride values in the lower exposed groups were considered to be unaffected by sulfuryl fluoride exposure, although a statistically identified value was observed in the 5 ppm exposed male rats at 12 months.

Creatinine: Although statistically-identified increased values were present in male groups during the first year, and decreased values in both surviving female groups at the end of the study, none of these were considered toxicologically significant. These slight physiologically increased and decreased values were well within the range of accepted normal values and were likely due to variation in hydration status rather than a meaningful toxicological event. The abnormally increased creatinine values at 19 and 21 months parallel those of urea nitrogen. Serum urea nitrogen and creatinine are both measures of glomerular filtration and would be expected to be abnormally elevated in advanced chronic renal disease. In general, markedly abnormal increased values for urea nitrogen and creatinine, as observed at 19 and 21 months, require approximately 70 to 75% or more of the nephrons in both kidneys to be nonfunctional. The abnormal clinical chemistry results at these times were consistent with the gross and histopathologic observations of renal failure in the moribund and/or dead rats during the second year. There were no toxicologically significant changes in creatinine in the 5 and 20 ppm exposed groups of rats.

Phosphorus: The hyperphosphataemia observed in 80 ppm exposed rats at 19 and 21 months was consistent with an interpretation of insufficient renal function required to normally excrete this anion. The serum phosphorus values were interpreted to be normal throughout the study in lower exposed groups; however, at 6 months the value for the female 20 ppm group was statistically identified as elevated.

Chloride: The chloride ion is normally passively absorbed by the kidneys and serum levels are generally decreased in chronic renal failure, in order to maintain anionic equilibrium associated with the hyperphosphataemia. The serum chloride levels in the lower exposure groups were unaffected throughout the study.

AST/CK: The results for serum aspartate aminotransulfuryl fluorideerase (AST) and creatine kinase (CK) were occasionally identified as statistically different from the controls; however, they were not considered a toxicologically significant effect due to sulfuryl fluoride exposure. The values for AST in male rats were identified as statistically increased in the 5 ppm group at 12 months and decreased in the high exposure group at 19 and 21 months; whereas, in the females the values were

statistically significantly increased at 12 and 19 months. All groups showed considerable variability in their values without a consistent pattern over time, between the sexes or exposure concentration. This enzyme is found in many tissues and is considered a marker of soft tissue damage but lacks organ specificity. The absence of a correlation of these statistically significant values with other findings of toxicity suggests they may be due to normal individual animal variability or a result of blood collection procedures.

CK values also demonstrate large variability in all exposure groups and controls at various sampling times. Although CK is found in many cell types, its highest specific activity is found in skeletal muscle with serum levels generally increased in degenerative muscle conditions. The large variations seen in this study have also been observed historically in control rats and those receiving test materials. This, in all probability, was associated with blood sample collection procedures from the posterior orbital sinus used during the course of the study and at terminal necropsy. This method of sample collection may result in significant tissue damage and contamination of the serum by this enzyme. A decrease in muscle mass could also be a possible explanation for the decreased values; however, this interpretation may be appropriate for the 80 ppm exposed rats, but was not for the lower exposed groups, since their body weights were normal and there were no histopathologic effects in muscle. Another possible explanation for the decrease could be inhibition of the enzyme by sulfuryl fluoride and/or metabolites during the assay. This would appear to be unlikely since the results lack a good dose-response in each sex at various sampling times.

In any event, significant known disease conditions are associated with increased levels of this enzyme and not decreases as were seen here. Therefore, the statistically identified values were not considered to be a toxicologically significant effect of sulfuryl fluoride exposure.

Nearly all of the clinical chemistry measures of renal function were abnormal at 19 and 21 months in the 80 ppm group. These changes were consistent with progressive renal damage which was the primary cause of morbidity and mortality in this group prior to study termination. None of the other measured clinical chemistry parameters were considered to be indicative of significant toxicity or organ damage in tissues other than kidneys. There were no toxicologically significant clinical chemistry changes observed in the lower exposure groups throughout the study.

Table 5.5a/01-4: Clinical Chemistry (UN, AST, TP, ALB, Cholesterol, Triglycerides, CK, Creatinine, P, Cl) - Males

Conc. (ppm)	UN mg/dl	AST mU/ml	TP g/dl	ALB g/dl	CHOL mg/dl	TRIG mg/dl	CK mU/ml	CREA mg/dl	P mg/dl	CL mmol/L
6-Mth										
0	17	112	9.0	4.2	67	65	121	0.7	7.4	118
5	16	118	8.8	4.1	67	61	67*	0.7	7.6	120
20	18	136	8.9	4.2	70	63	79	0.8*	7.3	118
80	18	113	8.9	4.1	65	63	73*	0/8	10.9	118
12-Mth										
0	13	109	7.1	3.1	76	57	131	0.6	6.6	107
5	14	153*	7.1	3.1	78	72$	168	0.6	6.5	107
20	14	97	7.1	3.1	75	52	152	0.7	6.5	107
80	17*	105	6.8*	2.8*	102*	99$	88	0.7*	7.0	105

Conc. (ppm)	UN mg/dl	AST mU/ml	TP g/dl	ALB g/dl	CHOL mg/dl	TRIG mg/dl	CK mU/ml	CREA mg/dl	P mg/dl	CL mmol/L
19-Mth										
0	18	67	8.0	3.5	139	131	143	0.8	5.7	114
5	20	93	7.4	3.3	125	164	91*	0.8	5.8	114
20	20	62	7.9	3.4	152	157	63*	0.8	5.9	115
80	62$	45$	7.4$	2.6*	319*	396$	47*	2.4$	9.6$	108
21-Mth										
0	16	62	7.8	3.4	143	125	101	0.7	5.7	117
5	18	93	7.9	3.4	153	148	75	0.7	5.6	118
20	20	111	7.7	3.1	159	198	59*	0.8	5.9	117
80	78$	51$	7.1*	2.6$	349*	432$	53*	2.8$	11.8$	111*
24-Mth										
0	22	100	7.8	3.1	206	169	204	0.8	6.3	113
5	23	102	7.7	3.2	221	192	187	0.8	6.6	114
20	26	148	7.5	3.1	218	224	130	0.9	6.3	114
80	No animals remaining									

*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 5.5a/01-5 Clinical Chemistry (UN, AST, TP, ALB, Cholesterol, Triglycerides, CK, Creatinine, P, Cl) - Females

Conc. (ppm)	UN mg/dl	AST mU/ml	TP g/dl	ALB g/dl	CHOL mg/dl	TRIG mg/dl	CK mU/ml	CREA mg/dl	P mg/dl	CL mmol/L
6-Mth										
0	21	78	8.5	4.3	101	42	158	0.7	6.2	118
5	20	75	8.7	4.2	102	46	114	0.8	6.6	120
20	24*	76	8.7	4.3	99	44	122	0.7	6.9*	119
80	20	83	8.9	4.3	105	46	109	0.7	6.5	119
12-Mth										
0	15	65	7.4	3.6	107	47	124	0.6	6.3	108
5	14	66	7.4	3.5	99	48	141	0.6	6.1	107
20	14	69	7.4	3.6	105	44	111	0.6	6.1	107
80	17*	87$	7.6	3.6	114	46	98	0.6	6.3	107
19-Mth										
0	24	90	9.1	3.9	202	199	161	0.9	5.3	115
5	23	66	8.9	4.1	165	117	102$	0.8	4.9	116
20	29$	69	8.4	3.9	151	127	77$	0.8	5.6	116
80	69$	91$	7.8*	2.9$	362$	428	117$	1.9$	9.2$	109$

Conc. (ppm)	UN mg/dl	AST mU/ml	TP g/dl	ALB g/dl	CHOL mg/dl	TRIG mg/dl	CK mU/ml	CREA mg/dl	P mg/dl	CL mmol/L
21-Mth										
0	20	75	7.9	3.6	193	134	68	0.7	5.2	109
5	20	58	8.1	3.8	156	80	61	0.7	5.0	109
20	23	70	8.0	3.8	148	74	54	0.7	5.1	110
80	111$	67	6.7*	2.6$	310$	267	149	2.6$	12.0$	102$
24-Mth										
0	22	108	8.2	3.7	185	117	198	0.7	5.9	109
5	20	95	8.4	3.9$	168	132	133$	0.6*	5.6	110
20	21	86	8.0	3.8	166	110	101$	0.6*	5.8	110
80	No animals remaining									

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

Urinalysis:

Data are presented in Table 5.5a/01-6. The 6-month repeat urinalysis was performed approximately 2 weeks after the initial 6-month sample because of the statistically significantly increased specific gravity observed in females in the 20 and 80 ppm groups, in contrast to the absence of a similar response in males. Evaluation of the data following the 6-month repeat urinalysis for all groups showed the specific gravity response in the 20 and 80 ppm groups to be statistically identified as decreased in the males and unaffected in females as compared to the initial 6-month samples. Therefore, it was concluded that the observed statistically-identified specific gravity changes were a reflection of normal biological variability.

The number of female rats with a +1 protein in their urine was also increased compared to the control group at 6 months; however, it was not observed in the repeat sampling. The reason for the observation of protein in the middle and high exposure groups from the first 6-month urinalysis was due to their higher specific gravity and not due to sulfuryl fluoride exposure. As noted in the repeat sample, the number of rats with a +1 protein was comparable in all of these rats and they were similar to the initial results in the 20 and 80 ppm groups, due to their higher specific gravity. The method for determining urinary protein is normally influenced by the urine specific gravity, and the observed values reflected this normal variation.

In males, there were no significant observations at the 12-month interval. There was an increase in urinary protein, when compared to the 6-month samples, which is an age-related phenomenon normally more prevalent in males than females. The female urine specific gravity was again increased in the 80 ppm group with the expected concurrent increase in urinary protein as a reflection of their greater specific gravity.

The results of repeated urinalyses and microscopic examination of urinary sediment during the first year did not detect an exposure-related effect even though early renal histopathologic changes were seen in these rats.

In contrast to the absence of an effect on urinalyses during the first year, the specific gravity findings in male and female rats at 19 and 21 months clearly showed significant biological effects which were identified as statistically significant. The decreased urine specific gravity in males and females at each time interval in the 80 ppm exposed group was interpreted to be due to a loss of functional nephrons associated with progressive chronic renal disease. The findings of a slight increase in urinary blood may have been secondary to the renal histopathologic changes.

At the termination sampling, the statistically-identified increased urinary specific

gravity in the 20 ppm group of males was considered a reflection of normal variability as previously seen at the 6-month samplings. The expected response, if exposure related, would be a decrease and not an increase in specific gravity. Similar changes were not observed in the female rats, nor were there any other toxicologically significant effects observed in urinalyses of either males or females at this time.

Table 5.5.2/01-7 Urinalysis (Specific Gravity, Protein and Blood at specified intervals)

	Male			**Female**		
Conc. (ppm)	**Specific Gravity**	**Protein**	**Blood**	**Specific Gravity**	**Protein**	**Blood**
6-Mth						
0	1.059	+ (1) +++ (9)	Neg (10)	1.022	Neg (6) TRC (3) + (1)	Neg (10)
5	1.052	+ (2) ++ (5) +++ (3)	Neg (10)	1.029	Neg (5) TRC (2) + (3)	Neg (10)
20	1.051	+ (1) ++ (6) +++ (3)	Neg (10)	1.052*	TRC (2) + (8)	Neg (10)
80	1.048	+ (1) ++ (3) +++ (6)	Neg (10)	1.046*	TRC (3) + (7)	Neg (10)
6-Mth Repeat						
0	1.055	++ (6) +++ (3)	Neg (8) ++(1)	1.043	Neg (2) + (8)	Neg (9) +(1)
5	1.051	+ (1) ++ (7) +++ (2)	Neg (10)	1.038	Neg (1) TRC (1) + (8)	Neg (10)
20	1.035*	+ (4) ++ (3) +++ (3)	Neg (10)	1.048	Neg (2) + (8)	Neg (10)
80	1.038*	+ (1) ++ (2) +++ (7)	Neg (10)	1.041	TRC (3) + (6) ++ (1)	Neg (10)

	Male			Female		
Conc. (ppm)	**Specific Gravity**	**Protein**	**Blood**	**Specific Gravity**	**Protein**	**Blood**
12-Mth						
0	1.054	+++ (9)	Neg (8) +(1)	1.041	TRC (1) + (2) ++ (3) +++ (4)	Neg (10)
5	1.062	+++ (10)	Neg (10)	1.036	Neg (1) TRC (1) + (4) ++ (3) +++ (1)	Neg (10)
20	1.050	+++ (10)	Neg (10)	1.043	+ (6) ++ (2) +++ (2)	Neg (9) +(1)
80	1.051	+++ (10)	Neg (10)	1.057*	+++ (10)	Neg (10)
19-Mth						
0	1.047	+++ (10)	Neg (10)	1.044	+++ (10)	Neg (10)
5	1.042	+ (1) +++ (9)	Neg (8) + (1) ++ (1)	1.045	++ (3) +++ (7)	Neg (10)
20	1.042	+++ (10)	Neg (10)	1.045	++ (1) +++ (9)	Neg (9) +++ (1)
80	1.027*	+++ (10)	Neg (7) + (3)	1.025*	+++ (10)	Neg (5) + (3) ++ (2)
21-Mth						
0	1.044	+++ (10)	Neg (10)	1.046	++ (1) +++ (9)	Neg (8) + (1) +++ (1)
5	1.044	++ (1) +++ (9)	Neg (10)	1.046	+ (2) +++ (8)	Neg (10)
20	1.050	+ (1) +++ (9)	Neg (10)	1.048	+++ (10)	Neg (9) +++ (1)
80	1.028*	+++ (10)	Neg (3) + (6) +++ (1)	1.025*	+++ (9)	Neg (3) + (3) ++ (2) +++ (1)

	Male			Female		
Conc. (ppm)	**Specific Gravity**	**Protein**	**Blood**	**Specific Gravity**	**Protein**	**Blood**
24-Mth						
0	1.037	+++ (20)	Neg (13) + (7)	1.042	++ (5) +++ (15)	Neg (20)
5	1.039	+++ (20)	Neg (19) + (1)	1.040	++ (2) +++ (18)	Neg (19) ++ (1)
20	1.044*	++ (1) +++ (19)	Neg (16) + (3) ++ (1)	1.038	++ (2) +++ (18)	Neg (17) + (1) ++ (1) +++ (1)
80	No animals remaining					

(N) indicates number of animals with the stated value.
Specific gravity is the mean for the test group.
TRC: Trace; Neg: negative
*Statistically different from control mean by Dunnett's test, alpha = 0.05.

Organ weights:

12-Month Study: There were no statistically-identified differences in fasted body weights of males or females even though the non-fasted mean body weights of the 65 female rats in the 80 ppm exposure group on day 369 were decreased. None of the absolute or relative organ weights of sulfuryl fluoride exposed females were statistically identified as different from the controls, as shown in Table 5.5a/01-8. The relative kidney and liver weights of 80 ppm exposed male rats were higher than for controls and were statistically identified. There were slight histopathological changes in the kidneys, but not in the liver of these rats. The slightly decreased fasted body weight in the 80 ppm exposed males likely contributed, as would be expected, to the observed increased relative organ weights.

24-Month Study: A statistically-identified increased relative brain weight and decreased absolute and relative testes weight were observed in the 5 ppm exposure males, as shown in Table 5.5a/01-9. The interpretation of a decreased testes weight was compromised by the presence of tumours, since nearly 100% of Fischer 344 male rats had multiple and variable sized Leydig cell tumours by the end of the 2-year study. Furthermore, the weights of these organs were unaffected at the 12-month necropsy. The relative brain weight in female rats of this group was identified as decreased in contrast to the increase in males. The statistically identified organ weight differences were considered normal variation in aged rats and not exposure related.

Table 5.5a/01-8: Organ Weights--12-Month Study (Liver and Kidney)

	Male					Female		
Conc. (ppm)	**Final Body Wt.**	**Kidneys**		**Liver**		**Final Body Wt.**	**Liver**	
	(g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g)	(g/100g)
0	389.1	2.462	0.634	9.606	2.470	235.8	1.990	0.850
5	391.6	2.419	0.618	9.841	2.510	247.4	1.984	0.806*
20	402.1	2.491	0.619	9.780	2.429	240.0	1.980	0.831
80	385.8	2.627	0.681*	10.164	2.638*	No animals		

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

Table 5.5a/01-9: Organ Weights--24-Month Study/Males (Brain and Testes)

Conc. (ppm)	Final Body Wt.	Brain		Testes	
	(g)	(g)	(g/100g)	(g)	(g/100g)
0	373.0	2.160	0.580	6.807	1.828
5	361.1	2.179	0.609$	5.206*	1.429*
20	356.6	2.158	0.609	6.376	1.792
80	No animals				

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

Gross pathology: **12-Month Study**: A single male control rat (90A5187) was found dead prior to the scheduled necropsy with generalised visceral congestion and self mutilation or cannibalisation of a hindlimb. The microscopic findings in this rat indicated death was due to choking and asphyxiation associated with the distended oesophagus containing feed. All of the other rats survived to the scheduled necropsy. An exposure-related effect was observed in the lungs of both sexes in the 80 ppm group, as shown in Table 5.5a/01-10. The pulmonary changes were characterised by multiple, small pale coloured foci scattered subpleurally and located primarily in the diaphragmatic lobes. After formalin fixation of tissues the labial surface of the incisor teeth of all 80 ppm exposed rats were found to be discoloured with a repetitive pale and slightly darker coloured horizontal line. None of the molar teeth were similarly affected after formalin fixation. The observations on the incisor teeth were not included in the gross pathology table, nor in the individual necropsy pathology sheets, since they were only observed in the fixed tissue.
Exposure-related changes in other organs of the 80 ppm exposed rats were not observed at necropsy or following formalin fixation. In addition, the 5 and 20 ppm exposed groups of rats were unaffected by exposure.

In the liver, kidneys, spleen and lungs, there were adhesions observed in occasional animals due to misplacement or migration of the microchip used for animal identification. Apparently, the microchip was accidentally injected or migrated within the abdominal or thoracic cavity, rather than remaining subcutaneously, and caused the adhesions of visceral organs.

24-Month Study: The most significant gross observations in these rats were related to advanced chronic renal disease in the 80 ppm group, as shown in Table 5.5a/01-11. Due 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. The salient findings of the grossly affected tissues in the high-exposure group are listed in alphabetical order and are shown in Table 5.5a/01-12 below.

The kidneys, lungs and incisor teeth were grossly affected and their identification as target tissues was consistent with the 12-month necropsy observations. The incidences of gross tumours for the most part were decreased in the high-exposure group, while in the lower-exposed groups they were comparable to controls. The numerous gross observations in other (non-target) tissues were considered to be secondarily affected due to kidney failure.

Table 5.5a/01-10: Gross Pathology--12-Month Study (Kidneys and Lungs)

Sex	Males				Females			
Exposure Conc. (ppm)	0	5	20	80	0	5	20	80
Number of Rats Examined	9	10	10	10	10	10	10	10
Kidneys								
Within normal limits:	9	10	10	10	9	10	9	10
Adhesions - fibrous, left:	0	0	0	0	1	0	1	0
Lungs								
Within normal limits:	8	10	10	0	10	10	10	0
Adhesions - fibrous, left diaphragmatic lobe:	1	0	0	1	0	0	0	0
Focus - pale, bilateral, multifocal: - very slight	0	0	0	10	0	0	0	10

Table 5.5a/01-11: Gross Pathology--24-Month Study (Kidneys, Lungs and Teeth/Oral Tissues)

Sex	Males				Females			
Exposure Conc. (ppm)	0	5	20	80	0	5	20	80
Number of Rats Examined	50	50	50	50	50	50	50	50
Kidneys								
Within normal limits:	8	17	8	3	42	49	47	3
Cyst cortex:	0	0	0	0	0	0	0	1
Dilated, pelvis, unilateral:	0	0	0	0	0	0	1	0
Area - pale, unilateral:	0	0	0	0	0	1	0	0
Cyst - clear, cortex, right:	0	2	0	0	0	0	0	0
Cyst - clear, cortex, right, focal:	0	0	0	0	0	0	0	1
Cyst - clear, cortex, unilateral:	0	0	0	0	0	0	1	0
Cyst - dark, unilateral:	0	0	0	1	0	0	0	0
Dark, bilateral:	10	4	7	0	2	0	1	0
Dark, cortex:	0	0	0	0	1	0	0	0
Dark, cortex, bilateral:	0	0	0	0	1	0	0	0
Focus - dark, cortex, focal:	0	0	0	0	0	0	0	1
Focus - depressed, unilateral:	1	0	0	0	0	0	0	0
Focus - pale, cortex, bilateral, multifocal:	0	0	0	1	0	0	0	0
Focus - pale depressed, left:	1	0	0	0	0	0	0	0
Mottled:	0	1	0	0	0	0	0	1
Mottled, bilateral:	2	0	1	0	0	0	0	0
Pale:	0	0	0	1	0	0	0	2
Pale, bilateral:	0	2	3	18	1	0	0	10
Roughened surface:	0	0	0	1	1	0	0	1
Roughened surface, left:	0	0	1	0	0	0	0	0
Roughened surface, unilateral:	0	0	1	0	0	0	0	0
Roughened surface, bilateral:	33	28	31	45	4	0	1	40
Roughened surface, bilateral: - moderate	1	1	2	0	0	0	0	0
Mass/nodule:	0	0	1	0	0	0	0	0
Lungs								

Sex	Males				Females			
Exposure Conc. (ppm)	0	5	20	80	0	5	20	80
Within normal limits:	39	38	30	1	43	44	45	0
Adhesions - fibrous, left diaphragmatic lobe:	2	0	0	1	0	0	1	0
Atelectasis, generalised:	0	1	0	0	1	0	0	0
Atelectasis, right cardiac lobe:	1	0	0	0	0	0	0	1
Congestion:	2	2	0	0	1	0	0	1
Oedema:	2	3	1	0	0	0	0	1
Haemorrhage, generalised:	0	0	0	0	0	1	0	0
Mineralisation:	0	0	0	1	0	0	0	0
Area – dark, generalised, multifocal:	0	0	0	0	0	0	1	0
Area – depressed, left lobe:	0	0	0	1	0	0	0	0
Area – pale, multifocal:	0	1	0	0	0	0	0	0
Area - pale, left diaphragmatic lobe:	0	0	0	0	1	0	0	0
Firm:	0	0	0	2	0	0	0	0
Firm, multifocal:	0	0	0	1	0	0	0	0
Focus - dark:	0	0	0	1	0	1	0	0
Focus - dark, multifocal:	2	1	3	3	2	3	1	14
Focus - dark, generalised, multifocal:	1	0	2	0	0	1	0	0
Focus - dark, left diaphragmatic lobe, focal:	0	0	1	0	0	0	0	0
Focus -dark, left lobe, multifocal:	0	1	0	0	0	0	0	0
Focus - dark, right apical lobe:	0	0	0	0	1	0	0	0
Focus - pale:	0	0	0	1	0	0	0	0
Focus - pale, multifocal:	0	1	8	46	0	0	1	46
Focus - pale, bilateral:	0	0	0	0	0	0	0	1
Focus - pale, generalised, multifocal:	0	0	2	1	0	0	0	2
Focus - pale, left cardiac lobe:	0	1	0	0	0	0	0	0
Focus - pale, right apical lobe:	0	0	1	0	0	0	0	0
Focus - pale, right diaphragmatic lobe:	0	1	0	0	0	0	1	0
Focus - pale, right diaphragmatic lobe, focal:	1	0	0	0	0	0	0	0
Mottled:	1	2	0	3	0	0	0	0
Mottled, generalised:	1	1	0	0	0	0	1	0
Mottled, right apical lobe:	0	0	1	0	0	0	0	0
Mottled, right cardiac lobe:	0	0	1	0	0	0	0	0
Pale:	0	1	0	0	0	0	0	0
Aspirated blood - secondary to decapitation:	0	1	0	0	0	0	0	0
Mass/nodule--probably metastatic tumour:	0	0	0	0	1	0	0	0
Mass/nodule--probably metastatic tumour, multifocal:	0	0	1	0	0	0	0	0
Mass/nodule:	2	0	0	0	0	0	0	0
Oral tissues								
Within normal limits	49	49	48	36	49	48	49	42
Inflammation - chronic active, hard palate:	0	1	0	0	0	0	0	0

Sex	Males				Females			
Exposure Conc. (ppm)	0	5	20	80	0	5	20	80
Fractured/missing teeth, upper incisors:	0	1	0	0	0	0	0	0
Malocclusion:	0	0	0	0	1	0	0	0
Malocclusion, tooth:	0	0	1	1	0	0	0	0
Mottled, tooth:	0	0	0	12	0	0	0	8
Overgrown incisors:	0	0	0	0	0	1	0	0
Overgrown incisors, lower incisors:	1	1	0	2	0	0	0	0
Overgrown incisors, upper incisors:	1	0	0	0	0	0	0	0
Ingesta present, oral cavity:	0	0	0	0	0	1	0	0
Mass/nodule:	0	0	1	0	0	0	1	0

Table 5.5a/01-12: Gross Pathologic Observations in 24-Month High Dose Group (alphabetical order)

Tissue	Observation
Aorta	Increased incidence of mineralisation and/or firm, both sexes
Digestive tract	Increased incidence of decreased ingesta, both sexes
External and Skin	Decreased incidence of mass/nodules, females
Eyes	Increased incidence of corneal opacity, both sexes
General	Increased incidence of decreased abdominal fat, both sexes
	Decreased incidence of icterus usually seen in Fischer rat leukaemia suggesting fewer leukaemic rats, both sexes
Heart	Increased incidence of pale areas or foci in the muscle, both sexes
Kidneys	Increased incidence of pale-coloured kidneys, both sexes
	Increased incidence of roughened kidney surface, both sexes
Liver	Decreased incidence of increased size of liver probably due to lymphoreticular tumour, both sexes
Lung	Increased incidence of pale foci, multifocal, in both sexes of high-dose and middle-dose males
Oral tissues	Increased incidence of mottled teeth (fluorosis), both sexes
Parathyroid gland	Increased incidence of increased size, both sexes
Pituitary gland	Decreased incidence of mass/nodule, both sexes
Spleen	Decreased incidence of increased size probably due to lymphoreticular tumour, both sexes
Stomach	Increased incidence of mineralisation, both sexes
	Increased incidence of erosions/ulcers, both sexes
Testes	Increased incidence of a decrease in size, males
Uterus	Decreased incidence of mass/nodule, females

Histopathology: **12-Month Study**: Exposure-related effects were observed in kidneys, lungs and incisor teeth of males and females in the 80 ppm group, and in the teeth of several 20 ppm males, as shown in Table 5.5a/01-13.

Kidney: A very slight degree of chronic progressive glomerulonephropathy, normally seen at this age in this strain of rat, was present in nearly all 0, 5 and 20 ppm males, and to a lesser extent in the 0, 5 and 20 ppm females. Male rats in the 80 ppm exposure group were more severely affected with chronic progressive

glomerulonephropathy, than control and lower exposure groups. The exposure-related renal change in 80 ppm females paralleled the response in males, except all females in the 0, 5, 20 and 80 ppm groups generally had less severely affected kidneys than males. Only one female rat from the 80 ppm group was as severely affected as were all of the males in this group.

The minimal exposure-related microscopic renal effects in the 80 ppm group of rats were not associated with toxicologically significant alterations in clinical chemistry parameters, urinalyses values, absolute and relative kidney weights, nor were they grossly detected at necropsy. However, these early microscopic renal effects did increase in severity with continued exposure and resulted in altered clinical pathology parameters, morbidity and mortality due to renal failure during the second year.

There were early changes suggestive of glomerular and tubular epithelial cell damage more prevalent in the 80 ppm exposure group when compared to control and lower exposure groups of rats. It was likely that increased protein loss through the glomerular basement membranes occurred and exerted a secondary effect on the tubular epithelial cells during protein reabsorption. These changes were identical to the age-related changes which occur in control Fischer 344 rats, and to a much greater degree in Sprague-Dawley rats, which generally have a significantly higher incidence and severity of renal disease. In neither of these rat strains is the mechanism understood for the normally occurring age-related glomerulonephropathy. In any event, the background renal disease seen in control rats was minimally exacerbated in the 80 ppm sulfuryl fluoride exposed male and female rats after 12 months.

Lung: The lung changes were microscopically consistent with the gross observations in the high-exposure group. In the 0, 5 and 20 ppm exposure groups of rats, a very slight degree of alveolar histiocytosis was microscopically present in all rats. These lesions consisted of single or multiple foci of individual cells, or aggregates of alveolar macrophages within alveoli of the five sections of lung routinely examined from each rat. The small microscopic foci of alveolar histiocytosis seen in the 0, 5 and 20 ppm groups were generally not of sufficient size to be grossly evident. In our experience, and the experience of others *(Boorman et al., Pathology of the Fischer Rat. Reference and Atlas, Academic Press, Inc., San Diego, CA, 1990; Mohr et al., Pathobiology of the Aging Rat, Volume 1, International Life Sciences Inst., Washington, DC, 1992; Anver and Cohen, Lesions Associated with Aging. In: The Laboratory Rat, Vol 1, Ed. Baker, Lindsey, and Weisbroth, p. 394, Academic Press, 1979; Haschek and Witschi, The Respiratory System. In: Handbook of Toxicologic Pathology, Ed. Haschek and Rousseaux, pp. 761-827, Academic Press, 1991*), these are common incidental lung findings in older rats. The lesions may be more extensive following infection by respiratory pathogens or various experimental regimens (e.g., dietary lipid imbalances or chronic exposure to airborne irritants) which lead to the presence of multiple greyish-white plaque-like foci visible from the pleural surface. The lesion may represent a continuum ranging from focal accumulations of alveolar macrophages (histiocytes), through mixed inflammatory lesions in which large foamy macrophages predominate, to lesions in which fibrosis is a major factor, often in association with cholesterol clefts.

Microscopic changes in the lung of the high-exposed group of rats, diagnosed as aggregates of alveolar macrophages, multifocal, very slight or slight, represent the continuum described above for alveolar histiocytosis, and not simple alveolar histiocytosis as was observed in the controls and lower exposure groups. These minor pulmonary changes have previously been observed in the sub-chronic study using Fischer 344 rats (III-A6.4.3/01, **D04**) and in the reproduction study using Sprague-Dawley rats (III-A6.8/01, **F01**).

The extent of involvement of the lung surface examined in the five sections, even in the most severe case, was very minimal, and was likely not to be associated with significant impaired function. There were no exposure-related pulmonary changes observed in the 5 and 20 ppm exposed rats necropsied at 12 months.

Teeth: Dental fluorosis involving the upper incisor teeth of male rats was microscopically very slight to slight in degree, while in females essentially all were very slight. The incisor and molar teeth were examined as part of the evaluation of the four decalcified sections of nasal turbinates (*Young, Histopathologic Examination of the Rat Nasal Cavity, Fund. Appl. Toxicol., 1: 309-312, 1981*). The most consistent finding was the formation of basophilic lines in dentin and enamel which likely correlated with the formalin fixed tissue observations of alternating pale and darker coloured horizontal lines in the incisor teeth. Significant changes in ameloblasts, odontoblasts and dental pulp were not readily apparent. Furthermore, the microscopic changes in the upper incisors were not detected in the molars. This may be due to the fact that only the incisors erupt continuously and are maintained at a constant length by attrition of the occlusal surfaces. Total renewal of incisors normally occurs approximately every 40 to 50 days. Therefore, during the course of the 24-month study the incisor teeth were renewed approximately 15 to 18 times without significant clinical dental problems in any group. There were several male rats in the 20 ppm group with very slight microscopic change in their teeth. The toxicological significance, if any, of changes in the incisor teeth was equivocal and best considered a biomarker of fluoride exposure in the rat.

There were a number of microscopic diagnoses in many tissues that represent the normal age-related changes in rats of this strain. An occasional neoplasm was present in some of these tissues. None of these tumours were interpreted to be associated with sulfuryl fluoride exposure.

24-Month Study: Since there were numerous statistically-identified differences in many tissue these findings are discussed in the order of their biological significance as they affected the well-being of the rat. The kidneys, lung, and incisor teeth were the only primary target organs affected after 24 months of exposure to sulfuryl fluoride, as they were after 12 months, as shown in Table 5.5a/01-14.

Kidney: The grades of severe and very severe chronic progressive glomerulonephropathy were used to describe the renal effects in 47/50 males and 45/50 females. In contrast, the kidneys of only 5/50 control males and 1/50 control females were comparably affected. Since kidney toxicity caused by exposure to 80 ppm sulfuryl fluoride resulted in morbidity and death of most rats in this group, a brief description of this disease process which normally occurs in aged rats is presented below. The kidney changes in the 80 ppm exposed rats parallel the normal disease process described by *Boorman et al. (1990) and Mohr et al. (1992) (ibid.)*, and only differs in that it was significantly exacerbated.

According to *Boorman et al. (1990) and Mohr et al. (1992)* and the experience of the author of this report, the following sequence of microscopic changes occur in chronic progressive glomerulonephropathy in the rat. The earliest microscopic changes consist of a few scattered foci of tubular cell regeneration. These tubules usually have more cells with an increased intensity to their cytoplasmic staining. Basement membranes surrounding these tubules and their associated glomeruli are slightly thickened. As the disease progresses, the thickened tubular and glomerular basement membranes become more pronounced. Some of the tubules become dilated and contain proteinaceous casts, their epithelial cells accumulate haemosiderin and lipochrome pigments, and interstitial fibrosis and inflammatory cell infiltration occurs. Occasional tubules with thickened basement membranes may be lined by multiple layers of cells while others may have a flattened epithelium. The concurrent changes in the glomerulus are characterised by increased mesangial cell proliferation with basement membrane thickening and