adhesion formation between the glomerular tufts and capsule. Advanced glomerular lesions are characterised by increased size and number of parietal cells of Bowman's capsule, dilatation of Bowman's space and small sclerotic glomerular tufts.

As the disease state progresses the amount of damage to the tubules and glomeruli renders more of them non-functional until they can be considered "end stage" kidneys. Secondary hyperparathyroidism and mineralisation of multiple tissues, such as, kidneys, lung, gastrointestinal tract and the media of large arteries occurs. In general, the distribution and amount of mineralisation present in multiple tissues is more severe in rats found dead when compared to those removed in a moribund condition. Due to the alterations in electrolyte levels and compromised acid-base balance, fibrous osteodystrophy is frequently seen in the latter stages of the disease.

The exposure-related kidney effects detected microscopically in the 80 ppm group were interpreted to be identical to those described above which occur normally in aged rats, and were observed at a low incidence in control and lower exposed rats in this study. The incidence and severity of this disease process were exacerbated by exposure in the 80 ppm group, without a comparable effect in the lower exposed groups. The sulfuryl fluoride induced renal toxicity did not result in kidney tumours.

Interestingly, Sprague-Dawley rats exposed to 150 ppm sulfuryl fluoride in a two-generation reproduction study did not have any histopathologic evidence of kidney toxicity, even though changes in the lung and teeth were comparable to those seen in Fischer 344 rats in the chronic study (*Breslin et al., 1992* – IIA 5.6.1a/01, **F01**). Similar strain differences in Fischer 344 rat susceptibility to developing nephrotoxicity following exposure to fluorinated compounds has been reported *(Hook and Goldstein, Toxicology of the Kidney, Second Ed., Raven Press, Ltd., New York, NY, 1993*). Previous subchronic studies on sulfuryl fluoride exposed New Zealand White rabbits (Eisenbrandt and Nitschke, 1989 – IIA 5.3.3.2e/01, **D07** and publ.), CD-1 mice (Nitschke and Quast, 1993 – IIA 5.3.3.2d/01, **D05**), and Beagle dogs (Nitschke *et al.*, 1992 – IIA 5.3.3.2b/01, **D06**), at concentrations higher than those used in the Fischer 344 chronic rat study, failed to produce renal toxicity. Furthermore, the 18 month CD-l mouse oncogenicity study conducted simultaneously with the rat chronic study also failed to produce kidney toxicity (Quast *et al.*, 1993 – IIA 5.5c/01, **I04**). In a chronic Beagle dog study, exposure to 200 ppm sulfuryl fluoride, the highest concentration, did not alter clinical chemistry parameters or result in gross necropsy findings suggestive of renal toxicity (Quast *et al.*, 1993 – IIA 5.3.3.2c/01c, **I01**).

It is likely that Fischer 344 rats may be uniquely sensitive to developing renal toxicity following repetitive exposure to this fluorinated molecule. Based upon the lack of renal toxicity in Sprague-Dawley rats, CD-l mice, New Zealand White rabbits and beagle dogs following sulfuryl fluoride exposure, it would seem inappropriate to use the Fischer rat kidney toxicity data alone for human safety assessment.

Lungs: The lungs of rats from the 24-month study showed comparable microscopic changes to those observed at 12 months. In general, aggregates of alveolar macrophages were classified as moderate in most of the 80 ppm exposed rats at study termination, in contrast to a slight degree at 12 months. There were also a few controls and lower exposed rats with a greater degree of aggregates of alveolar macrophages at the end of the study. These focal or multifocal changes, although slightly more prevalent at the end of the study, only involved a very small amount of the alveolar surface area in the five sections of lung examined. A significant contribution to the slightly increased severity of aggregated macrophages observed during the second year, was likely due to secondary effects of renal failure. Increased severity of multifocal mineralisation of large pulmonary vessels and significant mineralisation of the interalveolar septae were associated with renal

disease in the 80 ppm group. The lungs of some rats with renal failure were extensively mineralised with few of the interalveolar septae unaffected. In addition, chronic passive congestive lung changes, secondary to cardiac atrial thrombosis due to renal failure, were also observed in several rats.

During necropsy of the 20 ppm group of male rats, the lungs of 8/50 were observed to have multifocal pale foci. The gross observations in this group were not supported by histopathological changes. Based upon histopathological evaluation of the lungs, the exposure-related effects were confined to the 80 ppm groups at 12 and 24 months. The exposure-related effects present in this target tissue did not result in an increased incidence of pulmonary tumours.

Teeth: Dental fluorosis was present in the incisor teeth of all 80 ppm exposed male and female rats and was statistically increased in the middle dose group of males. These minimal microscopic changes were comparable to those observed at 12 months and were without any apparent biological effect on dentition. Similar gross or microscopic changes were not observed in the incisor teeth of CD-1 mice in an 18-month oncogenicity study concurrently conducted with these rats (Quast *et al.*, 1993 – IIA 5.5c/01, **I04**). Furthermore, in a subchronic rabbit study (IIA 5.3.3.2e/01, **D07**) simultaneously exposed with rats (IIA 5.3.3.2a/01, **D04**) there were no gross or microscopic dental changes in rabbits, while rats were affected (*Eisenbrandt and Nitschke, Fund. Appl. Toxicol., 12, 540-557, 1989*). Dogs subchronically exposed to sulfuryl fluoride also failed to exhibit dental changes following gross and microscopic examination (Nitschke *et al.*, 1992 - IIA 5.3.3.2b/01, **D06**). In a chronic 1-year dog study, exposures of 200 ppm at the highest concentration, did not result in dental fluorosis recognised during in-life examinations nor at necropsy (Quast *et al.*, 1993 - IIA 5.3.3.2c/01, **I01**). Therefore, the toxicological significance of dental fluorosis in rat incisors which erupt continuously was interpreted to be equivocal, and was only considered a good biomarker of fluoride exposure.

Additional oral tissue histopathology, not considered due to sulfuryl fluoride exposure, occurred and reflected the normal spectrum of inflammatory, degenerative and tumour formations involving teeth, gingiva, hard palate, lip and muzzle. The male rats were generally afflicted with more of these conditions than females. Fractured teeth were infrequent in females in contrast to males. Interestingly, nearly all of the broken teeth were also inflamed and involved the molars. There were many rats in all exposed groups and controls with acute or chronic active inflammatory reactions involving the gingiva and teeth. These conditions were again more prevalent in males than females and were almost always associated with feed embedded in the inflammatory foci. Not infrequently, some rats with lesions in the oral tissues had aspirated feed particles deposited in the lungs which caused pulmonary granulomas. The reason for the higher incidence of lesions in the oral tissues of males is unknown. These observations were not adversely affected by exposure to sulfuryl fluoride in any group, including the rats with dental fluorosis.

Lesions Secondary to Renal Disease: Due to end-stage kidney disease in the high-exposure group, there were numerous other organs with statistically-identified changes. These observations were not considered primary effects of exposure, rather they were expected changes associated with renal failure and early death, and were infrequently observed in the lower exposure groups and controls. (See list in Table 5.5a/01-12 for those identified grossly).

Many tissues in the 80 ppm exposed rats were mineralised secondarily to chronic renal disease and frequently were involved with other microscopic changes, which were also due to renal failure, and were not considered a direct effect of exposure. Frequently, these increased or decreased observations were statistically identified.

(**Brain**: The optic nerve and lacrymal glands were affected by orbital sinus puncture

blood drawing. Also, there were several statistically-identified differences in the nervous system of 80 ppm rats. The observations were decreases from controls and some due to early mortality but none were of toxicological significance.)

Table 5.5a/01-13: Histopathologic Observations--12-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	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Kidneys (# of tissues examined)	10	10	10	10	10	10	10	10
Adhesions, capsule, focal:	0	0	0	0	1	0	0	0
Aggregate(s) of mononuclear (predominately lymphoid) cells, interstitium:	10	10	9	10	9	9	9	10
Chronic progressive glomerulonephropathy, bilateral: - very slight	9	10	10	0	3	1	1	9
- slight	0	0	0	10	0	0	0	1
- any severity (combined)	9	10	10	10	3	1	1	10
Infarct, cortex, unilateral, focal: - very slight	1	0	0	0	0	0	0	0
Mineralisation, tubule(s): - very slight	10	10	10	10	10	10	10	10
Lungs (# of tissues examined)	10	10	10	10	10	10	10	10
Alveolar histiocytosis, multifocal: - very slight	10	10	10	0	10	10	10	0
Hyperplasia, alveolar cell, focal:	0	0	1	0	0	0	0	0
Mineralisation, blood vessels, multifocal: - very slight	10	10	10	10	9	8	8	7
Aggregates of alveolar macrophages, multifocal: - very slight	0	0	0	2	0	0	0	0
- slight	0	0	0	8	0	0	0	10
- any severity (combined)	0	0	0	10	0	0	0	10
Oral tissues (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	6	8	4	0	10	10	10	1
Fracture - old, tooth, unilateral, focal:	0	0	1	1	0	0	0	0
Inflammation - acute, tooth, unilateral, focal:	0	1	0	1	0	0	0	0
Inflammation - chronic active, gingiva, unilateral, focal:	0	0	1	1	0	0	0	0
Inflammation - chronic active, tooth, unilateral, focal:	0	0	2	0	0	0	0	0
Foreign body reaction, gingiva, unilateral, focal:	3	1	3	1	0	0	0	0
Foreign body reaction, gingiva, bilateral, focal:	1	0	0	0	0	0	0	0
Foreign body reaction, gingiva, any symmetry, focal: (combined)	4	1	3	1	0	0	0	0
Fluorosis - dental, upper incisors, bilateral: - very slight	0	0	3	3	0	0	0	0
- slight	0	0	0	7	0	0	0	0
- any severity (combined)	0	0	3	10	0	0	0	9

Table 5.5a/01-14: Histopathologic Observations--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 (# of tissues examined)	50	50	50	50	50	50	50	50
Abscess, cortex, focal:	1	0	1	0	0	0	0	0
Abscess, cortex, multifocal:	0	0	1	0	0	0	0	0
Abscess, cortex, focal or multifocal: (combined)	1	0	2	0	0	0	0	0
Chronic progressive glomerulonephropathy, bilateral: - very slight	8	9	7	2	42	46	48*	0*
- slight	13	16	18	0*	5	1	2	3
- moderate	24	20	22	1*	0	0	0	2
- severe	4	2	2	4	0	0	0	5*[T]
- very severe	1	2	1	43*	1	0	0	40*
- any severity (combined)	50	49	50	50	48	47	50	50
Cyst, cortex, focal:	2	2	0	1	0	0	1	2
Dilatation, pelvis, unilateral:	0	0	0	0	0	0	1	0
Hyperplasia, tubule(s), focal:	0	1	1	1	0	0	0	0
Inflammation - acute, pelvis, unilateral:	0	0	1	0	0	0	0	0
bilateral:	0	1	0	0	0	0	0	0
any symmetry: (combined)	0	1	1	0	0	0	0	0
Inflammation - acute, papilla(e), unilateral:	0	0	0	1	0	0	0	0
bilateral:	0	0	0	1	0	0	0	0
any symmetry: (combined)	0	0	0	2	0	0	0	0
Mineralisation, tubule(s): - very slight	48	48	49	5*	49	50	50	12*
- slight	0	0	0	0	0	0	0	1
- any severity (combined)	48	48	49	5*	49	50	50	13*
Pigment, tubule(s), bilateral: - very slight	3	2	3	0	0	1	4	3
- slight	3	3	2	0	0	1	0	0
- moderate	3	1	1	0	2	1	0	0
- severe	0	0	0	0	1	0	0	0
- any severity (combined)	9	6	6	0*[T]	3	3	4	3
Hyperplasia - transitional, pelvis, bilateral:	0	1	0	0	0	0	0	0
Mineralisation--secondary to renal disease:	1	2	1	45*	1	0	0	37*
Adenoma, tubule(s), benign, primary:	0	1	0	0	0	0	0	0
Myxosarcoma, papilla(e), malignant, primary, no metastasis:	0	0	1	0	0	0	0	0
Lung (# of tissues examined)	50	50	50	50	50	50	50	50
Within normal limits:	0	0	0	0	1	2	1	1
Adhesions, pleura, focal:	1	0	1	1	1	1	0	0
Adhesions, pleura, multifocal:	0	0	0	0	0	0	1	0
Adhesions, pleura, focal or multifocal: (combined)	1	0	1	1	1	1	1	0

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
Alveolar histiocytosis, multifocal: - very slight	41	46	43	1*	46	47	45	0*
Alveolar histiocytosis, multifocal: - slight	1	1	3	0	0	0	1	1
- moderate	0	0	0	0	1	0	0	0
- any severity (combined)	42	47	46	1*	47	47	46	1*
Chronic passive congesion: - very slight	0	0	0	1	0	0	0	2
- slight	0	0	0	0	0	0	0	1
- moderate	0	0	0	1	0	0	0	2
- any severity (combined)	0	0	0	2	0	0	0	5*[T]
Granuloma(s) - micro, alveoli/septa - focal:	1	0	2	0	0	2	2	0
- multifocal:	6	2	7	4	2	1	1	0
- focal or multifocal: (combined)	7	2	9	4	2	3	3	0
Heteroptic bone, focal:	1	0	1	0	0	0	0	0
Hyperplasia, bronchial/bronchiolar epiethelium, - focal:	0	0	0	1	0	1	1	0
- multifocal:	0	0	0	0	1	0	0	0
- focal or multifocal: (combined)	0	0	0	1	1	1	1	0
Mineralisation, blood vessels, multifocal: - very slight	50	50	50	28*	42	41	34	38
- slight	0	0	0	22*	2	1	0	9*
- any severity (combined)	50	50	50	50	44	42	34*	47
Thrombus - acute or recent, blood vessels, focal:	0	0	0	1	0	0	0	0
Inflammation - subacute to chronic: - very slight	8	5	9	1	0	3	1	0
- slight	3	2	3	3	2	1	0	0
- moderate	2	0	1	1	0	0	1	0
- any severity (combined)	13	7	13	5	2	4	2	0
Mineralisation--secondary to renal disease, alveoli/septa:	0	2	1	42*	1	0	0	37*
Aggregates of alveolar macrophages, multifocal: - very slight	3	1	1	0	2	0	3	0
- slight	1	0	1	15*	0	0	0	6*[T]
- moderate	1	0	0	34*	0	0	0	42*
- any severity	5	1	2	49*	2	0	3	48*
Aspiration pneumonia, bronchioloalveolar, multifocal: - slight	1	0	0	2	0	0	0	0
- moderate	1	0	1	0	0	0	0	0
- any severity	2	0	1	2	0	0	0	0
Adenocarcinoma, uterus, malignant, secondary:	0	0	0	0	1	0	0	0
Adenoma, bronchila/bronchiolar epithelium, benign, primary:	2	0	2	0	0	0	1	0
Carcinoma, head, malignant, secondary:	0	0	1	0	0	0	0	0
Carcinoma, thyroid, malignant, secondary:	0	0	0	0	1	0	0	0

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
Pheochromocytoma, medulla, malignant, secondary:	1	0	0	0	0	0	0	0
Squamous cell carcinoma, gingiva, malignant, secondary:	0	0	1	0	0	0	0	0
Osteogenic sarcoma, hind foot, malignant, secondary:	0	1	0	0	0	0	0	0
Oral Tissues (# of tissues examined)	50	50	50	50	50	50	50	50
Within normal limits:	13	24	9	0	35	37	30	0
Abscess, tooth - unilateral, focal:	1	0	1	3	0	0	1	0
- bilateral, focal:	0	1	0	0	0	0	0	0
- any symmetry, focal: (combined)	1	1	1	3	0	0	1	0
Cyst, hard palate, unilateral, focal:	1	0	0	0	2	0	0	0
Dysplasia, upper incisors, bilateral, focal:	1	0	0	1	0	0	0	0
Epidermal inclusion cyst, lip, focal:	1	0	0	0	0	0	0	0
Fracture - old, tooth - unilateral, focal:	6	2	4	3	0	0	0	1
- bilateral, focal:	0	1	0	0	0	0	0	0
- any symmetry, focal: (combined)	6	3	4	3	0	0	0	1
Hyperplasia, hard palate, focal:	1	1	0	0	0	0	0	0
Inflammation - acute, gingiva - unilateral, focal:	1	3	3	7	1	1	2	1
- bilateral, focal:	0	0	0	0	0	1	0	1
- any symmetry, focal: (combined)	1	3	3	7	1	2	2	2
Inflammation - acute, tooth - unilateral, focal:	10	3	6	9	4	6	8	5
- bilateral, focal:	8	4	11	2	1	1	3	1
- any symmetry, focal: (combined)	18	7	17	11	5	7	11	6
Inflammation - acute, lip, unilateral, focal:	0	0	0	0	0	1	0	0
Inflammation - chronic active, gingiva - unilateral, focal:	6	5	7	1	3	1	3	0
- bilateral, focal:	5	1	2	0	0	0	0	0
- any symmetry, focal: (combined)	11	6	9	1*^{T}	3	1	3	0
Inflammation - chronic active, tooth - unilateral, focal:	2	5	1	2	5	1	2	1
- bilateral, focal:	3	4	4	0	0	0	0	0
- any symmetry, focal: (combined)	5	9	5	2	5	1	2	1
Ulcer, lip, focal:	0	0	1	0	0	0	0	0
Ulcer, muzzle, focal:	0	0	0	2	0	0	0	0
Fluorosis -dental, upper incisors, bilateral: - very slight	0	0	10*	12*$_{T}$	0	0	2	4^{T}
- slight	0	0	0	38*	0	0	0	46*
- any severity (combined)	0	0	10*	50*	0	0	2	50*
Squamous cell carcinoma, gingiva, malignant, primary - no metastasis:	2	0	2	0	0	0	0	0
- metastasis:	1	0	1	0	0	0	0	0

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**
- metastasis or no metastasis: (combined)	3	0	3	0	0	0	0	0
Squamous cell carcinoma, muzzle, malignant, primary, metastasis:	0	0	1	0	0	0	0	0
Squamous papilloma, hard palate, benign, primary:	0	0	2	0	0	0	1	0
Squamous papilloma, lip, benign, primary:	1	1	0	0	0	1	0	0
Squamous cell carcinoma and/or squamous papilloma:	*4*	*1*	*6*	*0*	*0*	*1*	*1*	*0*

*Statistically identified difference from control mean by Yate's Chi-Aquare pairwise test, alpha = 0.05.
T Linear trend by Cochran-Armitage Linear Trend Test, alpha = 0.02, two-sided.

Tumor Incidence:

For ease of evaluating the tumour incidences in the various tissues of males and females, they are presented separately from other histopathologic observations. In addition to statistical analyses of the individual tumours, those of similar cell types were combined when appropriate, according to procedures recommended *by McConnell et al., JNCI, 76(2) (1986)*, and reanalysed for statistical significance. There were no individual or combined tumour types which were statistically-identified as increased in any exposure group, as shown in Table 5.5a/01-15 and 5.5a/01-16.

Because of increased mortality in males and females in the 80 ppm group, mortality adjusted statistical procedures described by *Peto, Br. J. Cancer, 29: 101-105, (1974)* were applied. The statistical procedures were applied to any tumour types where an incidence greater than two was observed in any group. Based upon this criterion, the following tumours were analysed:

Tissue	Tumour Types
Adrenal Gland	Pheochromocytomas
Liver	Adenoma, hepatocellular
Mammary Gland	Adenocarcinoma, adenoma and fibroadenoma
Oral Tissues	Squamous cell carcinoma and squamous papilloma
Pituitary Gland	Adenoma and carcinoma, anterior *pars distalis*
Skin	Fibroma, fibrosarcoma and undifferentiated sarcoma
Thyroid Gland	Adenoma and carcinoma, parafollicular cells
Uterus	Endometrial stromal polyp
Multiple organs	Fischer rat leukaemia, any site

Fischer rat leukaemia is a frequently occurring neoplastic disease involving many tissues in this strain of rat. The spleen and liver are tissues usually affected earliest in the disease process. In the later stages nearly every tissue can be involved leading to anaemia, icterus, morbidity and death. The general observations at necropsy indicated that fewer of the 80 ppm exposed rats were icteric. The incidence of Fischer rat leukaemia was presented under multiple organs and, when combined with those with splenic involvement as the only tissue affected, the values were found to be statistically significantly decreased in the 80 ppm exposed males and 20 ppm exposed females. Although the incidence was also decreased in the 80 ppm exposure group of females it was not statistically identified.

The results of the mortality-adjusted trend test (negative Z value) statistics (alpha = 0.05, one-sided) found the tumours of the following tissues to be decreased:

Tissue	Males	Females
Adrenal Gland	X	X

Liver	X	N.S.
Mammary Gland	N.S.	X
Oral Tissues	X	X
Pituitary Gland	X	X
Skin	X	N.S.
Thyroid Gland	X	X
Uterus	N.A.	X
Multiple Organs	X	X

(N.S: not significant ; N.A.: not applicable)

The level of statistical significance for tumours of the various tissues following mortality adjustment are presented in the report.

There were no tumours in male and female rats with a statistically-identified increased incidence.

Table 5.5a/01-15: Tumour Incidence--12-Month Study

Sex	**Males**				**Females**			
Exposure Conc. (ppm)	**0**	**5**	**20**	**80**	**0**	**5**	**20**	**80**
Number of Rats Examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Brain - olfactory lobe (# of tissues examined)	10	10	10	10	10	10	10	10
Meningioma, malignant, primary, metastasis:	0	0	0	0	0	0	1	0
Brain - thalamus/hypothalamus (# of tissues examined)	10	10	10	10	10	10	10	10
Meningioma, olfactory lobe, malignant, secondary:	0	0	0	0	0	0	1	0
Pituitary (# of tissues examined)	10	0	0	10	10	0	0	10
Adenoma, anterior (*pars distalis*), benign, primary:	1	--	--	0	0	--	--	0
Skin and Subcutis (# of tissues examined)	10	0	0	10	10	0	0	10
Basal cell tumour, benign, primary:	1	--	--	0	0	--	--	0
Testes (# of tissues examined)	10	3	0	10	--	--	--	--
Leydig cell tumour, benign, primary:	0	1	--	1	--	--	--	--
Uterus (# of tissues examined)	--	--	--	--	1	1	2	10
Endometrial stromal polyp, benign, primary:	--	--	--	--	1	0	1	0

Table 5.5a/01-16: Tumour Incidence--24-Month Study (Adrenals, Liver, Mammary Gland, Oral Tissues, Pituitary Gland, Skin, Thyroid Gland, Uterus)

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**
Adrenals (# of tissues examined)	50	50	50	50	50	50	50	50
Adenoma, cortex, benign, primary:	0	0	1	1	0	0	0	0
Pheochromocytoma, medulla, benign, primary: (one)	4	3	6	4	0	2	0	1
Pheochromocytoma, medulla, benign, primary: (two)	1	2	1	2	0	0	0	0
Pheochromocytoma, medulla, malignant, primary, metastasis:	1	0	0	0	0	0	0	0
Pheochromocytoma, medulla, benign or malignant, primary, metastasis: (combined)	*6*	*5*	*7*	*6*	*0*	*2*	*0*	*1*

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**
Ganglioneuroma, medulla, benign, primary:	0	1	0	0	0	0	0	0
Complex pheochromo, medulla, benign, primary:	0	0	0	0	1	0	0	0
Liver (# of tissues examined)	50	50	50	50	49	50	50	50
Adenoma, hepatocellular, benign, primary: (one)	2	0	2	0	1	0	0	1
Adenoma, hepatocellular, benign, primary: (two)	0	0	1	0	0	0	0	0
" " " (one or two) (combined)	2	0	3	0	1	0	0	0
Osteogenic sarcoma, hind foot, malignant, secondary:	0	1	0	0	0	0	0	0
Mammary Gland (# of tissues examined)	49	50	49	48	50	50	50	49
Adenocarcinoma, malignant, primary, no metastasis:	0	0	0	0	1	0	0	0
Adenoma, benign, primary:	1	0	1	0	1	0	0	0
Fibroadenoma, benign, primary: - (one)	2	1	1	2	7	8	2	1*[T]
Fibroadenoma, benign, primary: - (two)	0	0	0	0	0	0	1	0
- (one or two) (combined)	2	1	1	2	7	8	3	1*[T]
Adenocarcinoma, adenoma and/or fibroadenoma: (combined)	*2*	*1*	*2*	*2*	*9*	*8*	*3*	*1*[T]*
Oral Tissues (# of tissues examined)	50	50	50	50	50	50	50	50
Squamous cell carcinoma, gingiva, malignant, primary - no metastasis:	2	0	2	0	0	0	0	0
- metastasis:	1	0	1	0	0	0	0	0
- metastasis or no metastasis: (combined)	3	0	3	0	0	0	0	0
Squamous cell carcinoma, muzzle, malignant, primary, metastasis:	0	0	1	0	0	0	0	0
Squamous papilloma, hard palate, benign, primary:	0	0	2	0	0	0	1	0
Squamous papilloma, lip, benign, primary:	1	1	0	0	0	1	0	0
Squamous cell carcinoma and/or squamous papilloma: (combined)	*4*	*1*	*6*	*0*	*0*	*1*	*1*	*0*
Pituitary (# of tissues examined)	49	49	50	50	48	50	50	50
Adenoma, anterior (*pars distalis*), benign, primary: - (one)	15	19	17	4*[T]	22	19	20	7*[T]
- (two)	1	0	0	0	1	1	2	0
- (one or two) (combined)	16	19	17	4*[T]	23	20	22	7*[T]
Adenoma, *pars intermedia*, benign, primary:	0	0	0	0	1	1	0	0
Carcinoma, anterior (*pars distalis*), malignant, primary, metastasis:	1	0	0	0	0	0	0	0
Adenoma and/or carcinoma, anterior (pars distalis): (combined)	*17*	*19*	*17*	*4*[T]*	*23*	*20*	*22*	*7*[T]*
Ganglioneuroma, *pars nervosa*, benign, primary:	0	0	0	0	0	0	1	0
Haemingiosarcoma, brain, malignant, secondary:	0	1	0	0	0	0	0	0
Craniopharyngioma, *par nervosa*, benign, primary:	0	0	0	1	0	0	0	0
Skin and Subcutis (# of tissues examined)	50	50	49	48	50	50	50	49
Basal cell adenoma, hind foot, benign, primary:	0	0	0	0	0	1	0	0

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
Carcinoma, head, malignant, primary, metastasis:	0	0	1	0	0	0	0	0
Carcinoma, sebaceous glands, malignant, primary, no metastasis:	0	0	1	0	0	0	0	0
Carcinoma, Zymbal's gland, malignant, primary, no metastasis:	0	0	0	0	1	0	0	0
Keratoacanthoma, head, benign, primary:	1	0	0	0	0	0	0	0
Squamous cell carcinoma, gingiva, malignant, secondary:	1	0	0	0	0	0	0	0
Squamous cell carcinoma, umbilicus, malignant, primary, no metastasis:	1	0	0	0	0	0	0	0
Fibroma, benign, primary:	5	3	4	0	1	0	1	2
Fibrosarcoma, pinna, malignant, primary, no metastasis:	0	0	0	0	0	0	1	0
Fibrosarcoma, scrotum, malignant, primary, no metastasis:	0	0	1	0	0	0	0	0
Fibrosarcoma, abdomen, malignant, primary, no metastasis:	0	0	0	0	1	0	0	0
Fibrosarcoma, back, malignant, primary, no metastasis:	0	1	0	0	0	0	0	0
Undifferentiated sarcoma, head, malignant, primary, no metastasis:	0	0	1	0	0	0	0	0
Fibroma, fibrosarcoma and/or undifferentiated sarcoma: (combined)	5	4	6	0	2	0	2	2
Amelanotic melanoma, eyelid, benign, primary:	0	0	0	0	0	1	0	0
Basosquamous cell CA, abdomen, malignant, primary, no metastasis:	0	0	1	0	0	0	0	0
Thyroid Gland (# of tissues examined)	50	50	50	50	50	50	50	50
Adenoma, follicle(s), benign, primary:	1	0	0	2	1	0	0	1
Adenoma, parafollicular cells, benign, primary: - (one)	8	7	11	1	6	3	8	3
- (two)	0	1	0	0	0	0	0	0
- (one or two) (combined)	8	8	11	1	6	3	8	3
Carcinoma, parafollicular cells, malignant, primary - no metastasis:	0	0	0	0	0	2	0	0
- metastasis:	0	0	0	0	1	0	0	0
- metastasis or no metastasis: (combined)	0	0	0	0	1	2	0	0
Adenoma and/or carcinoma, parafollicular cells: (combined)	*8*	*8*	*11*	*1*	*7*	*5*	*8*	*3*
Uterus (# of tissues examined)	--	--	--	--	50	50	50	50
Adenocarcinoma, malignant, primary, metastasis:	--	--	--	--	1	0	1	0
Endometrial stromal polyp, benign, primary: - (one)	--	--	--	--	17	20	12	10
- (two)	--	--	--	--	5	2	2	2
- (three)	--	--	--	--	1	0	1	0

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
- (one, two, or three) (combined)	--	--	--	--	23	22	15	12*T
Haemangiopericytoma, serosa, benign, primary:	--	--	--	--	0	0	1	0

*Statistically identified difference from control mean by Yate's Chi-Square pairwise test, alpha = 0.05.
T Linear trend by Cochran-Armitage Linear Trend Test, alpha = 0.02, two-sided.

Conclusions: **In this 2-year inhalation combined chronic toxicity/oncogenicity study of sulfuryl fluoride in Fischer 344 rats no evidence for oncogenicity was observed in either sex at any exposure concentration.**

The NOEL was 5 ppm based on very slight fluorosis of incisor teeth in males. The NOAEL for toxicity was 20 ppm based on effects on the kidney and irritation of the respiratory tract at 80 ppm.

The kidney effects comprised chronic progressive glomerular nephropathy (CPGN) which increased in severity during the second year with all 80 ppm exposed rats moribund or dead prior to study termination, primarily due to renal failure. Following prolonged exposure there were minor lung changes characterised by increases in aggregates of alveolar macrophages suggestive of a response to irritation.

The chronic neurotoxicity study results following 12 months of exposure were negative and were the subject of a separate report (IIA 5.8.2c, G06).

Section A6.5/01 **Annex Point AII, VI. 6.5**	**Evaluation by Competent Authorities**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2004
Materials and Methods	The applicant's version is adopted with the amendment that the rats were of the strain Fischer 344. No samples for urinalysis and haematology at 3 months were included.
Results and discussion	The applicant's version is adopted with some revisions. In Table 5.5a/01-13 the number of females with very slight fluorosis in the 80 ppm group should be 9. In Table 5.5a/01-15 the number of tissues examined for uterus in the 0 ppm group should be 10. The table named Table 5.5.2/01-7 should be renamed to 5.5a/01-6.
Conclusion	The applicant's version is adopted. The dental fluorosis can be considered an adverse effect. However, it is not considered relevant in this case since the product is for professional use only and children should not be exposed
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 results for chronic neurotoxicity study following 12-month exposure were the subject of a separate report (III-A6.9/03, G06). The purity of the test substance was occasionally lower than the minimum purity stated in section A2.7 (99.4 %). However, the lowest purity was still rather high (93.6 %) and the impurities were identified.

Section A6.5/02 **Chronic Toxicity (Combined with Carcinogenicity A6.7)**
Annex Point AII, VI. 6.5

Carcinogenicity study in the mouse (Mouse, 5.5c/01, I04)

Report: [redacted] (1993)
Sulfuryl Fluoride: 18-Month Inhalation Oncogenicity Study in CD-1 Mice
[redacted]
Report DECO-HET-K-016399-039, dated 19/8/93; study began 24/7/90.

Guidelines: US EPA 83-2
OECD 451
87/302/EEC
MAFF Guideline: Oncogenicity Test
Deviations from EC test guideline B.32. Carcinogenicity Test: None, except no food consumption measured--but this was not a dietary study. This study meets and exceeds the guideline requirements. This study actually meets the guidelines for a combined Carcinogenicity and Chronic Toxicity study, B. 33. In that case there was no urinalysis conducted, but that is more difficult, and not frequent, in mice.

GLP: Yes

Methodology: Test material: Several smaller quantities (lot #'s WP 880329-752, WP 901011- 907, WP 910321-918 and WP 910826-929) of sulfuryl fluoride were obtained from DowElanco, Pittsburg, CA, during the course of the 18-month 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 that 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 mice/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 18 months. A satellite group of an additional 10 mice/sex/exposure level was randomly predesignated at the beginning of the study for necropsy after approximately 12 months of exposure.

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. 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, and platelets) and clinical chemistry (AP, AST, ALT, UN, creatinie, total protein, albumin, globulin, glucose, cholesterol, triglycerides, total bilirubin, Na, K, P, Cl and Ca) determinations from 10 animals/sex/exposure level when necropsied at 12 months, and from 20 animals/sex/exposure level at the 18-month

terminal necropsy. Palpation for masses was conducted during the weekly clinical examinations.

At each scheduled necropsy a wide range of tissues was collected and major organs (brain, heart, liver, kidneys, lungs (18 months only) and testes) weighed. In general, all tissues including gross lesions from control and 80 ppm exposed mice were examined microscopically. Target tissues and grossly visible lesions were also examined from mice in the lower exposure groups.

The chambers were 14.5 m^3 stainless steel and glass and were operated under dynamic airflow conditions. The test atmospheres were generated using the J-tube method and were analysed using a Miran 1A IR.

Findings: The chambers ran according to plan. Concentrations were found to be 5.1, 20.1 and 79.7 ppm average.

Mortality: Satellite Group: A few male mice and a single female mouse scheduled for the 12-month necropsy were removed from the study due to morbidity or death. A 5 ppm exposed male mouse (90A4765) was found dead on day 70 due to trauma resulting from deposition or migration of the animal identification microchip into the thorax. There were abdominal organs herniated into the thoracic cavity with chronic adhesion formation resulting in inanition. There were two male mice in the 20 ppm group removed moribund. One mouse (90A4820) was removed on day 329 in a moribund condition due to a transmural abscess in the wall of the ileum resulting in inanition. The second mouse (90A4822) in this group was removed from test moribund on day 358 because of a preputial abscess and septicaemia. A single female mouse (90A5120) in the 80 ppm group was found dead on test day 217 due to starvation as a result of broken and inflamed incisor teeth which occluded the nasal passages with exudate.

Oncogenicity Group: Male and female mice from all groups were removed from study when they became moribund or were found dead. During the first year of exposure a slightly earlier onset of mortality was observed in the 80 ppm males (Table 5.5c/01-1); however, mortality at the end of 18 months of exposure was not statistically identified as increased in any of the sulfuryl fluoride exposed groups.

The female mortality rate during the first year of exposure was comparable in all groups (Table 5.5c/01-1). During days 455 to 545 the 80 ppm exposed females became moribund or died at an increasing rate when compared to other exposure groups and controls. The increased mortality rate in the 80 ppm exposure group of females was statistically identified at the end of 18 months.

There were no statistically identified differences in mortality of lower exposed groups of female mice.

There were only a few mice predesignated for the 12-month necropsy that did not survive until the scheduled necropsy, and their removal from study was not due to sulfuryl fluoride exposure.

The mice assigned to the 18-month study were removed for a variety of normally occurring age-related disease conditions that caused morbidity and/or death. Systemic amyloidosis, urinary tract infections, inflammatory reactions, tissue abscesses and trauma were the most common observations in the male mice that resulted in morbidity or death. In the 80 ppm exposed males, 7/32 mice had inflammatory lesions involving tissues of the oral cavity and head region, compared to fewer affected mice in other groups. In addition, 5/32 mice in this group had an oesophagus impacted with feed. The impacted oesophagus frequently was associated with exudative rhinitis and aspiration pneumonia leading to starvation. An explanation for the greater frequency of oral inflammatory lesions and the impacted oesophagus was not apparent.

The spectrum of changes described in the male mice were also seen in females. Neoplasms were more frequently a cause of morbidity or mortality in females

compared to males. Occasionally, there was haemorrhage associated with the tumour that resulted in blood loss either externally or internally. In the 80 ppm group, 8/36 female mice apparently starved due to an impacted oesophagus with exudative rhinitis and aspiration pneumonia, as was observed in males.

Another significant contribution to the number of mice removed from study in this group was the apparent increase in the incidence of severe systemic amyloidosis. The laboratory historical experience with this mouse strain consists of one other currently conducted study. The cause of death/moribund condition for female CD-1 mice from a currently conducted study was compared to the sulfuryl fluoride exposed mice (see Table 5.5c/01-2 below).

These data reflect the variable incidence of the disease in all groups from the two studies. However, based upon limited experience with CD-1 mice at the testing laboratory, the incidence of amyloidosis and death appeared to be exacerbated by exposure to sulfuryl fluoride in the 80 ppm female mice. The lower exposed groups were interpreted to be unaffected when compared to their controls and the controls from the other current study.

There is an apparent genetic predisposition in CD-1 mice for the development of systemic amyloidosis which contributes significantly to their early demise. This is in stark contrast to the very rare occurrence of amyloid deposits in any tissues in the B6C3F1 mouse, which is the mouse strain historically used in our laboratory since 1979, and has never been the cause of death in controls or treated mice. Furthermore, systemic amyloidosis is not a disease condition observed in chronic studies using Sprague-Dawley and Fischer 344 rats.

In addition, information obtained from discussions with pathologists from other laboratories routinely using CD-1 mice for oncogenicity testing indicated that systemic amyloidosis was a very common cause of morbidity and death of this mouse strain (Quast, personal communication).

Table 5.5c/01-1: Percentage Cumulative Mortality (18-Month Study)

Conc. (ppm)	Males				Females			
	0	5	20	80	0	5	20	80
Days on Test								
1-7	0	0	0	0	0	0	0	0
8-14	0	0	0	2	2	0	0	0
15-21	0	0	0	2	2	0	0	0
22-28	2	0	2	2	2	0	0	0
29-35	2	0	2	2	2	0	0	0
36-42	2	0	2	2	2	0	0	0
43-49	2	0	2	2	2	0	0	0
50-56	2	0	2	2	2	0	0	0
57-63	2	0	4	2	2	0	0	0
64-70	2	0	4	2	2	0	0	0
71-77	2	0	4	2	2	0	0	0
78-84	2	0	4	2	2	0	0	0
85-91	2	0	4	2	2	0	0	0
92-98	2	0	4	2	2	0	0	0
99-105	2	2	4	2	2	0	0	0
106-112	2	2	4	2	4	0	0	0

Conc. (ppm)	Males				Females			
	0	5	20	80	0	5	20	80
113-119	2	2	4	2	4	0	0	0
120-126	2	2	4	2	4	0	0	0
127-133	2	2	4	2	4	0	0	0
134-140	2	2	4	2	4	0	0	0
141-147	2	2	4	4	4	0	0	0
148-154	2	4	4	4	4	0	0	0
155-161	4	4	4	4	6	0	0	0
162-168	4	4	4	4	6	0	2	0
169-175	4	4	4	6	6	4	2	0
176-182	4	4	4	6	6	4	2	0
183-189	4	4	6	8	6	4	2	0
190-196	4	4	6	8	6	4	2	0
197-203	4	6	6	10	8	6	2	0
204-210	4	6	6	10	10	6	2	0
211-217	4	6	6	10	10	6	2	0
218-224	4	6	6	10	10	6	2	2
225-231	4	6	6	10	10	6	2	2
232-238	4	8	6	10	10	6	2	2
239-245	4	8	6	10	10	6	2	2
246-252	4	8	6	10	10	8	4	2
253-259	4	8	6	10	10	8	4	2
260-266	4	8	6	12	10	8	4	2
267-273	4	8	6	12	10	8	6	2
274-280	4	8	6	12	10	8	6	4
281-287	4	8	6	12	10	8	6	6
288-294	4	8	6	12	10	8	6	6
295-301	4	8	6	14	10	8	6	6
302-308	4	8	6	14	10	8	6	6
309-315	6	8	6	14	10	8	8	6
316-322	6	8	6	14	10	8	8	6
323-329	8	8	8	16	10	8	8	6
330-336	8	8	8	16	10	10	8	6
337-343	8	8	8	16	10	10	8	8
344-350	8	8	8	16	10	10	8	8
351-357	8	8	8	16	10	10	8	8
358-364	8	8	8	16	12	10	8	10
365-371	8	8	8	16	14	10	8	10
372-378	8	8	8	16	14	10	8	10
379-385	8	8	12	16	14	10	8	16
386-392	8	8	12	18	14	10	8	16
393-399	8	8	14	18	16	10	8	16

Conc. (ppm)	Males				Females			
	0	5	20	80	0	5	20	80
400-406	10	8	14	18	16	10	8	16
407-413	10	10	14	22	18	10	8	16
414-420	12	10	16	24	18	10	10	18
421-427	12	12	16	24	18	10	10	24
428-434	14	12	16	28	18	10	10	24
435-441	14	12	16	28	18	10	10	26
442-448	14	20	24	30	20	12	12	32
449-455	20	20	26	32	20	12	14	42
456-462	22	22	28	32	20	12	14	44
463-469	22	22	30	36	22	12	16	46
470-476	22	24	32	38	26	12	18	50
477-483	26	26	34	38	26	12	18	54
484-490	30	26	36	40	26	14	20	56
491-497	34	30	38	42	28	16	20	58
498-504	36	32	38	46	28	16	26	62
505-511	36	34	38	50	32	16	26	64
512-518	42	34	40	50	34	18	26	64
519-525	42	34	40	52	34	20	28	66
526-532	44	34	42	56	34	20	32	68
533-539	44	38	44	60	34	22	36	68
540-546	44	38	46	64	36	22	38	68
547-553	46	40	50	64	36	24	40	72
554-555(m)/7(f)	46	40	50	64	36	24	40	72*

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

Table 5.5c/01-2: Incidence of Amyloidosis in This Study and Concurrent Study for Females *(number of mice removed from study moribund or dead due to systemic amyloidosis/number removed from study for any cause/number of mice in the group)*

Group	Other Current Study	Sulfuryl Fluoride
Control	15/23/60	6/18/50
Low Dose	11/18/60	5/12/50
Middle Dose	8/14/60	13/20/50
High Dose	13/26/60	26/36/50

Clinical signs: A relatively frequent observation in mice was swelling, reddening and abscess formation involving the preputial or clitoral gland. Skin lesions characterised by alopoecia of different body regions, abrasions and occasional tumours involving the skin or subcutaneous tissue were also present. Changes observed in the eyes included opaque, lacrimation, phthisis bulbi and exophthalmus. An occasional mouse from the various groups, including controls, would exhibit tremors, circle or convulse while being handled. These nervous system signs were infrequently observed and were not exposure related.

None of the in-life observations made in the various exposure groups suggested an effect due to sulfuryl fluoride.

Palpable Masses: Part of the evaluation of in-life examinations consists of detecting palpable masses which could suggest an early onset of an oncogenic effect. The location and date of initial appearance of clinically observed swellings or masses and their final outcome based upon gross and histopathology were recorded. A number of the swellings involved the preputial or clitoral gland and were frequently no longer observed at necropsy. This was most likely due to the fact that most of the inflammatory conditions were abscesses of the gland which drained to the exterior and rapidly healed. In some mice, they were still present at the time of necropsy and were found microscopically to be abscesses. On occasion, a mouse with a swollen abdomen had an internal neoplasm. There were no indications that sulfuryl fluoride exposure resulted in an increased incidence or an earlier onset of tumours.

Since the increased mortality rate in the 80 ppm exposed female mice was statistically identified, mortality-adjusted statistical procedures were applied to their tumour incidences. Interpretation of these findings will be covered in the histopathology section.

Body weight: **Males**: Although the body weights of all exposed groups of males were comparable to the controls on day -5, they were lower in the 80 ppm group on day -1. The slight but statistically-identified, decreased male body weight in the 80 ppm group on day -1 increased with exposure. The difference in body weight of 80 ppm male mice exceeded 10% when compared to controls prior to necropsy at 12 and 18 months (Table 5.5c/01-3).

Females: Although the body weights of all groups were comparable on day -5, the 80 ppm group body weight was lower than the others on day -1. The difference in body weight of the 80 ppm group compared to the controls continued to diverge with exposure and was statistically identified throughout most of the study. The difference between 0 ppm and 80 ppm female body weights at approximately 12 months was less than the difference exhibited by the male mice in these two groups. However, during the last several months of exposure the decreased female body weights were slightly greater than in the males. The body weight difference between the female high-exposed group and controls approached 15% by study termination (Table 5.5c/01-3). An occasional sporadic body weight difference was statistically identified in the middle- and low-exposed groups of females and was not interpreted to be due to sulfuryl fluoride exposure.

The body weight suppression in the 80 ppm exposed male and female mice was considered due to sulfuryl fluoride exposure and was consistent with expectations based upon the subchronic study findings. There were no changes in body weight in the other exposure groups interpreted to be due to sulfuryl fluoride exposure.

Table 5.5c/01-3: Mean Body Weights

	Male				Female			
Conc. (ppm)	**0**	**5**	**20**	**80**	**0**	**5**	**20**	**80**
Days on Test								
-5	26.5	26.5	26.5	26.5	20.2	20.3	20.3	20.2
-1	27.8	27.3	27.6	27.0*	21.3	21.2	21.5	20.9
6	30.3	29.8	30.0	29.5*	23.1	23.4	23.4	22.8
13	32.2	31.7	32.3	31.0*	24.3	24.6	24.7	24.3
20	33.6	33.0	33.4	31.8*	25.9	26.0	26.0	25.1
27	34.6	34.0	34.2	32.5*	27.1	26.5	26.3	25.4*

	Male				Female			
Conc. (ppm)	**0**	**5**	**20**	**80**	**0**	**5**	**20**	**80**
Days on Test								
34	34.6	34.2	34.6	32.8*	26.9	26.6	26.4	25.8*
41	35.5	35.1	35.3	33.7*	27.6	27.1	27.2	26.5*
48	36.6	36.0	36.7	34.3*	28.3	27.7	28.2	27.4
55	36.8	36.2	36.3	34.0*	28.7	27.4*	27.8*	27.2*
62	37.6	36.9	37.5	35.2*	29.1	28.3	28.9	28.2
69	38.0	37.0	37.6	35.0*	29.1	28.2	28.6	27.9*
76	38.4	37.6	38.3	35.9*	29.4	28.8	29.0	28.6
83	37.8	37.3	37.7	34.8*	29.6	28.9	29.2	28.4*
90	37.4	37.2	37.7	34.8*	29.4	28.6	29.2	28.3*
117	38.4	37.9	38.2	34.8*	29.7	28.9	29.7	28.5*
145	38.4	39.0	38.8	35.3*	30.5	29.4	30.2	28.8*
173	38.9	38.8	39.1	35.0*	30.9	29.9	30.7	28.9*
201	40.2	40.3	39.9	35.9*	31.9	30.7*	31.3	29.3*
229	39.8	40.4	39.9	35.6*	31.5	31.2	31.6	30.0*
257	39.9	40.1	39.9	36.0*	32.0	31.0	31.4	30.1*
285	40.3	40.4	39.7	35.6*	32.2	30.9*	32.4	30.0*
313	41.3	41.6	41.1	36.6*	33.2	32.4	33.1	30.7*
341	41.7	42.0	41.8	36.9*	33.7	33.3	33.8	31.4*
369	41.9	42.1	41.6	36.9$	34.5	33.2	34.1	31.8*
397	42.4	42.5	42.3	36.6*	35.0	33.9	34.8	31.8*
425	41.2	41.9	41.9	35.5*	34.8	33.5	34.6	31.2*
453	41.0	41.8	41.0	35.1$	35.4	33.9	34.3	30.3*
481	40.8	40.4	41.4	34.6*	34.3	33.8	34.3	30.9*
509	42.0	41.5	42.0	35.6*	34.7	35.1	35.2	20.4$
537	41.8	42.6	41.3	35.9$	34.7	34.8	35.3	30.9*
551	41.0	41.6	40.8	35.3$	34.9	34.1	35.0	29.8*

*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: Not conducted

Haematology: **Males**: A statistically-identified, increased platelet count was noted in the male 80 ppm mice at 12 months, but not at 18 months (Table 5.5c/01-4). The erythrocyte count in the 80 ppm exposed group at 18 months was slightly increased and was statistically identified. Neither of these observations were considered toxicologically significant nor exposure-related effects.

Females: A statistically-identified, increased platelet count was observed at all exposure concentrations at 12 months; however, they were normal at 18 months. The platelet counts were not considered abnormal and likely reflected normal variation. There were no other haematological values which were statistically identified as different at either time interval.

Evaluation of individual mouse differential counts and morphology of blood smears from each time period failed to detect any exposure-related effects. The absence of an exposure-related effect on the peripheral blood of male and female mice at 12 and 18 months was consistent with the normal appearing bone marrow sections evaluated histopathologically.

Table 5.5c/01-4: Platelets ($x10^3$/cu mm)

Conc. (ppm)	0	5	20	80
Males: 12-Month Study	1383	1393	1541	1660*
Males: 18-Month Study	1120	1099	1043	1180
Females: 12-Month Study	968	1280*	1356*	1446*
Females: 18-Month Study	971	951	894	964

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

Clinical chemistry:

Data are presented inTable 5.5c/01-5.

Males: At 12 months, a marginally lower serum potassium level was statistically identified but was not seen at 18 months. The serum glucose levels in the 80 ppm group and the serum albumin in the 5 ppm group were also statistically identified as decreased in mice at 18 months. These identified differences in clinical chemistry parameters lacked a consistent dose-response pattern and were not interpreted to be due to exposure.

Females: At 12 months the serum urea nitrogen value in the 80 ppm group was statistically identified as increased. Although statistically identified, this value was considered well within the normal physiological range and was not associated with an increase in creatinine that would suggest a renal effect. Furthermore, the values were not affected as the study progressed. There were no other statistically identified changes in any parameter at either time interval.

Results of the clinical chemistry determinations at 12 and 18 months failed to identify any abnormalities indicative of target organ toxicity in any exposure group.

Table 5.5c/01-5: Clinical Chemistry (Males: K and Glucose; Females: UN and Creatinine)

Conc. (ppm)	Male				Females			
	Glucose 12 Month mg/dl	**Glucose 18 Month mg/dl**	**K 12 Month mmol/L**	**K 18 Month mmol/L**	**UN 12 Month mg/dl**	**UN 18 Month mg/dl**	**Creatinine 12 Month mg/dl**	**Creatinine 18 Month mg/dl**
0	199	149	5.8	6.0	21	27	0.4	0.5
5	188	129	5.2	6.0	22	32	0.4	0.4
20	164	159	5.5	5.9	20	34	0.4	0.5
80	169	112*	4.9$	6.0	27*	37	0.4	0.5

*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: Not conducted.

Terminal Body and Organ Weights 12-Month Study:

Males: The 80 ppm male body weights were statistically identified as decreased. None of the organ weights in this group were statistically identified as different; however, all of the absolute organs weighed in this group, except the testes, were decreased, while the relative organ weights for brain, heart, liver, and testes were

increased, as a reflection of the decreased body weight (Table 5.5c/01-6). In the 5 ppm group, the absolute brain weight and the relative liver weight were statistically identified as increased and were considered to be a reflection of normal variability in aged mice. There were no other organ weight changes identified as statistically different from the controls. None of the identified organ weight changes were interpreted to be indicative of a target organ effect due to exposure.

Females: The body and relative brain weights of 20 ppm (Table 5.5c/01-7) exposed females were increased and statistically identified; however, their body weights were comparable to the entire group weights on days 341 and 369. Therefore, the observed body and relative brain weight differences were due to the non-representative values from the smaller subpopulation necropsied at this time, and were not exposure- or dose-related.

The body weights of all other groups of mice necropsied at 12 months were low when compared to the entire group weights on days 341 and 369. Body weights of the entire 80 ppm group of mice were statistically identified as decreased throughout the study; however, those necropsied at 12 months were decreased but not sufficiently to be statistically identified. The absolute organ weights in the 80 ppm female group were also decreased, as were the males at this exposure.

The decreased body weights of 80 ppm exposed male and female mice were consistent with the results of the subchronic study. The organ weight changes were reflective of the decreased body weight and did not identify any target organ toxicity.

Table 5.5c/01-6: Terminal Body Weights and Organ Weights (Males)--12-Month Study

Conc. (ppm)	Final Body Wt	Brain		Heart		Kidneys	
	(g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	40.5	0.496	1.241	0.195	0.483	0.750	1.843
5	40.0	0.526*	1.326	0.200	0.501	0.745	1.864
20	39.4	0.504	1.279	0.199	0.505	0.780	1.976
80	35.4*	0.476	1.350	0.180	0.510	0.640	1.815

Table 5.5c/01-6: Terminal Body Weights and Organ Weights (Males)--12-Month Study--Cont.

Conc. (ppm)	Liver		Testes	
	(g)	(g/100g)	(g)	(g/100g)
0	2.247	5.547	0.236	0.588
5	2.451	6.134*	0.218	0.550
20	2.363	6.004	0.214	0.546
80	2.041	5.749	0.244	0.695

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

Table 5.5c/01-7: Terminal Body Weights and Organ Weights (Females)--12-Month Study

Conc. (ppm)	Final Body Wt	Brain		Heart		Kidneys		Liver	
	(g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	29.9	0.515	1.729	0.153	0.511	0.451	1.509	1.598	5.341
5	31.3	0.510	1.638	0.147	0.471	0.471	1.508	1.639	5.250

20	33.2*	0.508	1.538*	0.164	0.494	0.467	1.410	1.778	5.339
80	28.2	0.479	1.717	0.143	0.511	0.403	1.434	1.559	5.508

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

Gross pathology 12-Month Study:

There were no gross observations indicative of a target organ effect at any exposure concentration. In a number of mice, there were adhesions present in the mesentery, kidneys, liver, lung and spleen 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 adhesions of visceral organs. The other gross observations were considered spontaneous in occurrence and not exposure related.

Histopathology 12-Month Study:

In 80 ppm exposed male and female mice the brain and the thyroid gland were the tissues affected by exposure (Table 5.5c/01-8). These were the only tissues microscopically affected by sulfuryl fluoride exposure at 12 months, and they had previously been identified as target organs in the subchronic study.

Brain: Essentially all 80 ppm exposed mice had very slight or slight microscopic vacuolation of the cerebrum in the region of the external capsule, as was previously observed in the 100 ppm exposed group in the subchronic study. The caudate putamen was only affected in one male mouse in the 80 ppm group. The amygdaloid adjacent to the external capsule was not affected in the sections examined from 80 ppm exposed mice as it was in an occasional 100 ppm exposed mouse in the subchronic study. The amount of vacuolation present in any region of the brain examined after 12 months of exposure to 80 ppm was considerably less than observed at 100 ppm in the subchronic study. In fact, without the knowledge of the lesion location and character from the subchronic study it could have easily been considered normal histologic variation at 12 months. The vacuolation was suggestive of oedematous change and was not associated with an inflammatory cell reaction. The microscopic changes in the brain did not produce any recognisable abnormal clinical behaviour in these mice, nor in the subchronic study in which the microscopic changes were slightly more evident. The reasons for the decreased severity and incidence of microvacuolation are not known. In any event, the extent of microvacuolation in the brain of mice did not progress in severity with prolonged exposure.

Thyroid: The thyroid gland was also affected at 12 months as it was in the subchronic study. Microscopic changes were diagnosed as very slight hypertrophy of the follicular epithelial cells. A continuum of changes associated with the epithelial cell hypertrophy consisted of a decrease in follicular colloid which also stained less intensely eosinophilic. More male mice were affected with thyroid changes than females. The follicular epithelial cell and colloid effects were not associated with any degenerative changes or inflammatory cell infiltrates. The microscopic response was the same at 12 months as it was during the subchronic study except fewer mice were affected at 12 months. Whether the thyroid changes had an effect on normal metabolism and were responsible for the decreased body weights and other exposure-related effects is unknown.

The most common histopathologic change involving numerous tissues of both sexes of control and exposed mice was amyloid deposition. The amyloid deposits had a wide tissue distribution and were variable in degree in mice of the same sex and group and did not appear to be exposure related.

A variety of other microscopic changes characterised by degenerative and/or inflammatory reactions were present in many tissues and were considered to be normal for this mouse strain. No other organs besides the brain and thyroid gland were considered to be target tissues affected by sulfuryl fluoride exposure.

An occasional mouse was found to have a microscopic tumour present and the

results of these findings are presented below in Table 5.5c/01-9. There were no tumours present which were considered exposure related.

Table 5.5c/01-8: Histopathology--12-Month Study (Brain and Thyroid)

Sex	Males				Females			
Exposure Conc. (ppm)	0	5	20	80	0	5	20	80
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	0	10	9	10	1
Aggregate(s) of mononuclear (predominately lymphoid) cells, ependymal canal:	0	0	0	0	0	1	0	0
Vacuolation, caudate putamen, bilateral, focal: - very slight	0	0	0	1	0	0	0	0
Vacuolation, external capsule, bilateral, focal: - very slight	0	0	0	10	0	0	0	9
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:	3	4	6	9	8	4	8	10
Aggregate(s) of mononuclear (predominately lymphoid) cells, ependymal canal:	0	0	0	0	0	1	0	0
Mineralisation, unilateral, focal:	3	3	3	1	2	3	2	0
Mineralisation, bilateral, focal:	3	3	1	0	9	2	0	0
Mineralisation, any symmetry, focal: (combined)	6	6	4	1	2	5	2	0
Mineralisation, ependymal canal, focal:	1	0	0	0	0	0	0	0
Thyroid Gland (# of tissues examined)	10	10	10	10	10	10	10	10
Within normal limits:	9	5	4	0	6	6	3	2
Amyloid, interstitium: - very slight	1	2	2	2	4	3	3	3
- slight	0	2	4	2	-	1	4	1
- moderate	0	1	0	0	0	0	0	0
- any severity (combined)	1	5	6	4	4	4	7	4
Hypertrophy, epithelial cells: - very slight	0	0	0	7	0	0	0	4

Table 5.5c/01-9: Tumour Incidence--12-Month Study

Sex	Males				Females			
Exposure Conc. (ppm)	0	5	20	80	0	5	20	80
Number of Mice Examined	10	10	10	10	10	10	10	10
Lacrimal/Harderian Gland(s) (# of tissues examined)	10	1	2	10	10	0	0	10
Adenoma, benign, primary:	0	0	0	0	0	--	--	1

Liver (# of tissues examined)	10	10	10	10	10	10	10	10
Adenoma, hepatocellular, benign, primary:	0	0	1	0	0	0	0	0
Lungs (# of tissues examined)	10	10	10	10	10	10	10	10
Adenoma, bronchioloalveolar, benign, primary:	0	1	0	1	1	0	0	0
Multiple organs (# of tissues examined)	0	0	0	0	1	0	0	0
Lymphosarcoma, malignant, primary:	--	--	--	--	1	--	--	--

Terminal Body and Organ Weights 18-Month Study:

Males: The terminal body weights of the 80 ppm exposed males were decreased and statistically identified. The difference from the control group was greater than they were at 12 months (Table 5.5c/01-10). Although there were no organ weights in the 80 ppm group, statistically identified as different from the controls at 12 months, all of them were statistically identified, on either an absolute and/or relative weight basis, at the 18-month necropsy. None of these organ weight differences were interpreted to be an indication of target organ toxicity; however, they were reflective of the expected changes associated with the marked body weight suppression. The body weights and the organ weights of the lower-exposure groups of male mice were unaffected.

Females: The female mice body weights at termination of the study were decreased and statistically identified in the 80 ppm exposed group. This was in contrast to the slightly decreased, but not statistically identified, weight difference in the mice necropsied at 12 months. Consistent with the female in-life body weights, the difference from controls became greater as the study progressed, especially during the last several months of exposure. The majority of the organ weights for these mice were statistically identified as different on an absolute and/or relative basis (Table 5.5c/01-11). None of these were considered to be indicative of a target organ effect, but rather were reflective of their decreased body weights. A statistically-identified increased relative heart weight associated with a slightly decreased body weight was observed in the 20 ppm group and was not considered exposure related. There were no other statistically-identified differences in organ or body weights in the lower exposed groups of female mice.

Table 5.5c/01-10: Terminal Body Weights and Organ Weights (Males)--18-Month Study

Conc. (ppm)	Final Body Wt	Brain		Heart		Kidneys	
	(g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	40.3	0.521	1.297	0.208	0.517	0.797	1.978
5	40.2	0.515	1.301	0.213	0.532	0.768	1.919
20	39.7	0.520	1.319	0.206	0.520	0.783	1.976
80	32.8*	0.494*	1.515$	0.181*	0.551	0.645$	1.973

Table 5.5c/01-10: Terminal Body Weights and Organ Weights (Males--18-Month Study)--Cont.

Conc. (ppm)	Liver		Lungs		Testes	
	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
0	2.452	6.084	0.245	0.609	0.214	0.532
5	2.365	5.953	0.253	0.638	0.204	0.511
20	2.177	5.457	0.243	0.613	0.206	0.524

80	1.718$	5.185	0.245	0.749$	0.210	0.643*

*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 5.5c/01-11: Terminal Body Weights and Organ Weights (Females)--18-Month Study

Conc. (ppm)	Final Body Wt	Brain		Heart	
	(g)	(g)	(g/100g)	(g)	(g/100g)
0	34.1	0.523	1.549	0.157	0.461
5	33.1	0.519	1.578	0.163	0.493
20	33.8	0.516	1.552	0.173	0.515*
80	29.2*	0.494*	1.698*	0.151	0.517

Table 5.5c/01-11: Terminal Body Weights and Organ Weights (Females)--18-Month Study--Cont.

Conc. (ppm)	Kidneys		Liver		Lungs	
	(g)	(g/100)	(g)	(g/100)	(g)	(g/100)
0	0.499	1.470	1.943	5.677	0.230	0.675
5	0.500	1.510	1.964	5.898	0.244	0.740
20	0.498	1.485	1.912	5.658	0.233	0.700
80	0.406*	1.393	1.612*	5.523	0.260	0.915$

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

Gross pathology 18-Month Study:

There were adhesions involving visceral organs due to the microchip as was seen at the 12-month necropsy. In all exposed groups and controls, a number of expected changes were observed in the tissues of moribund and dead mice. Since these conditions were usually associated with inanition, the digestive tract of 80 ppm exposed mice contained less ingesta, with their contents being more fluid in nature and containing haemolysed blood. Similarly, the stomachs also contained haemolysed blood in these mice and distended gallbladders were occasionally observed in females. Because of the inanition of these mice, more of them contained less abdominal fat. A number of male mice from all groups had a distended urinary bladder due to paraphimosis and urethral obstruction.

The 80 ppm exposed female mice had an increased number with perineal staining because of their moribund condition and lack of preening. A marked decrease in the number of mice in this group with cystic ovaries and cystic endometrial hyperplasia of the uterus was also noted. This may have been due to their morbidity and early mortality.

In all exposed groups of male mice, but especially in the 80 ppm group, the number with a dilated kidney pelvis was considerably decreased. There were no other exposure-related kidney findings in either exposed or control male mice. The livers of male mice also were unaffected by exposure, with the possible exception of a decrease in the number of 80 ppm exposed mice with a mass/nodule.

Based upon the gross necropsy findings, there were no target organs of toxicity identified in the mice after 18 months. The observed changes were those generally expected in mice which are moribund and/or die from a variety of age-related conditions.

Histopathology 18-Month Study:

Since there were numerous statistically-identified differences in many tissues, the findings will be discussed in order of significance of target organ effects (Table

5.5c/01-12). Brain and thyroid gland were affected by exposure and were previously identified as target organs in the subchronic and 12-month studies. Exposure-related effects in these tissues were confined to mice in the 80 ppm group.

Brain: Microscopic vacuolation in the external capsule of the brain of mice from the 18-month study was only observed in 80 ppm exposed mice. The extent of involvement of the brain was less at 18 months than at 12 months, and less than in mice from the subchronic study exposed to 100 ppm sulfuryl fluoride. In the brains of mice examined microscopically at 12 months, 10/10 males and 9/10 females were affected, in contrast to 13/50 males and 12/50 females at 18 months. In addition, a recognisable change was not observed in the caudate putamen or amygdaloid regions of 18-month exposed mice compared to earlier studies. There were no special stains used to further characterise the microvacuolation since they had previously been performed in the subchronic rat study without any additional meaningful findings. Rat brains from males and females with microvacuolation in the subchronic study were stained with luxol-fast blue Periodic acid-Schiff-haematoxylin and Sevier- Munger silver stains and examined light microscopically. The special stains used in the subchronic rat study did not contribute to the diagnosis or elucidation of the pathogenesis of the vacuoles. Microvacuolation was not observed in the brain sections of mice exposed to 5 or 20 ppm sulfuryl fluoride.

In the brain sections from the region of the thalamus and hypothalamus, there was a statistically-identified decreased incidence of focal mineralisation in the 80 ppm group of males and females and in the 20 ppm females. The presence or absence of these mineralised foci does not have any known toxicological significance. In our laboratory experience, these foci of mineralisation are generally small and of limited distribution in CD-1 and B6C3F1 mice, and rarely observed in Sprague-Dawley and Fischer 344 rats. In the 80 ppm group, the decreased incidence may be due to the slightly higher mortality during the first year of the study, which was not statistically identified at the termination, in contrast to the females, for which mortality was statistically identified as increased. This interpretation was based upon the fact that few mice necropsied at 12 months contain mineralised foci. The exposure-related microscopic changes in the brain were not associated with an increased incidence of nervous system tumours.

Thyroid: The thyroid gland was also affected in the 18-month mice exposed to 80 ppm sulfuryl fluoride. The microscopic changes were identical to those previously observed and were characterised primarily by hypertrophy of follicular epithelial cells. The colloid within the follicles was decreased in amount and stained less intensely eosinophilic. There were no degenerative or inflammatory changes present in the affected thyroids. The incidence was only statistically identified as increased in the male mice; however, the thyroids of female mice were also interpreted to be slightly affected by exposure. The incidence of mice affected at 18 months was considerably decreased when compared to 12 months. At both time intervals, the changes were present in a greater number of males compared to females. The decreased incidence of mice with affected thyroids in the 18- month study paralleled the decreased incidence of microvacuolation in the brain of mice at this time. The exact reasons for these observations are unknown, but clearly indicates that chronic exposure to sulfuryl fluoride does not result in an increased incidence or severity of the exposure-related effect in either primary target organ. The microscopic thyroid changes were not associated with development of follicular cell tumours in either sex.

Other/Amyloidosis: Although many other tissues in the 80 ppm exposed mice contained microscopic changes which were statistically identified as increased or decreased, they were not interpreted to be due to sulfuryl fluoride exposure. One of the most common microscopic findings in numerous tissues was amyloid deposition.

In tissues examined with the light microscope and stained with haematoxylin, the amyloid appeared as an amorphous, eosinophilic, hyaline, extracellular substance that, with progressive accumulations, encroaches on and produces pressure atrophy of adjacent cells. Amyloidosis occurs in a variety of species of animals, including humans. *Cohen and Connors, The Pathogenesis and Biochemistry of Amyloidosis. J. Pathol. 151: 1-10, (1987)* reviewed the pathogenesis and biochemistry of amyloidosis in humans. It was their conclusion that all of the mechanisms which lead to the disease have not been elucidated. They further report that in all types of amyloid fibrils, 3 common features are displayed by the major protein constituents. The common features are: that the fibril proteins have a serum precursor, a high degree of anti-parallel beta-sheet conformation and a distinctive ultrastructure on electron microscopy. The serum apoprotein amyloid originates from hepatocytes (*Takahashi et al., Ultrastructural Evidence for the Synthesis of Serum Amyloid A Protein by Murine Hepatocytes. Lab. Invest. 52: 220-223, 1985*) and is carried on high density lipoprotein 3 (HDL3) as an apoprotein *(Hebert and Gervais, Scand. J. Immunol. 31: 167-173, 1990*). Normally, in amyloid resistant animals, the apoprotein serum amyloid is catabolised, and not deposited as amyloid (*Cohen and Connors, 1987*). However, in amyloid susceptible strains, apoprotein serum amyloid undergoes limited cleavage and is deposited in tissues as polymerised fibrillar amyloid protein (*Hebert and Gervais, 1990*). In humans, as in animals, the clinical circumstances under which amyloidosis occurs, as well as the distribution of the disease, are extremely varied (*Cohen and Connors, 1987*).

The incidence, distribution and morphology of amyloidosis in Charles River CD-1 mice at various ages has been reported by *Frith and Chandra, Toxicol. Path., 19(2): 124-127, (1991)*. According to these authors, the primary cause of death of CD-1 mice was renal amyloidosis, but the effects on endocrine organs (adrenal glands, thyroid and parathyroid glands), and other tissues cannot be discounted. Genetics appears to play a role in the pathogenesis of amyloidosis in the CD-1 mouse, since amyloid occurs at such a low incidence in many other mouse strains. This commonly occurring background disease condition of CD-1 mice was more prevalent in our mice in comparison to the incidence reported by Frith and Chandra, (1991). This may partly be due to the source of animal supplier and different environmental housing conditions. In addition, the number of tissue sections examined, which differs from laboratory to laboratory, has a significant bearing on the reported or observed incidence of a disease process which normally shows considerable variability. The tissues shown below (Table 5.5c/01-13) were most commonly affected microscopically with amyloidosis in the control male and female mice from the sulfuryl fluoride study.

It is readily apparent that amyloidosis is a multisystemic disease process with a significant incidence in numerous tissues critical to the normal physiology and well-being of the mouse.

There were a number of tissues listed in Table c/01-13 below with a statistically-identified increased or decreased incidence of amyloid of a certain grade of severity in the 80 ppm exposed group of mice (Table 5.5c/01-12). For the most part, the total number of mice with amyloid deposits in the various tissues were not identified as different. In fact, only in the **ileum and jejunum** were there statistically identified differences in the 20 and 80 ppm groups of females. According to Frith and Chandra (1991), amyloid deposits were only observed in the ileum, jejunum, duodenum, mesenteric lymph nodes and ovaries of mice between 0-8 months of age. With increasing age, the number of other tissues affected also increased. They reported a higher incidence of amyloidosis in females in contrast to males. The observed statistically identified differences in our 20 and 80 ppm exposed females suggest a lower than expected occurrence in our control females.

Kidneys/Amyloidosis: The kidneys were frequently involved with amyloidosis in

all control and sulfuryl fluoride exposed mice. Our findings that renal amyloidosis was a primary cause of death of the CD-1 mice were consistent with the findings reported by Frith and Chandra (1991). Close examination of the incidence of renal amyloidosis in the 80 ppm exposed group failed to identify a difference in the total number of mice with the condition; however, when the two most severe grades of renal amyloidosis of the glomerulus were combined there was a slight but statistically-identified increase in female mice. This was in contrast to the slight decrease which was not statistically identified in the males of this group. The lower incidence of renal amyloidosis in the males was interpreted to be a result of more males dying in the first year of the study, in comparison to the females of this group, which died or were removed primarily during the last several months of test. Therefore, the females had a longer time to develop the more severe grades of amyloidosis.

The kidneys and many other tissues were involved with amyloid deposits; however, to attribute morbidity and death to renal disease alone is somewhat suspect, since the clinical chemistry and organ weight data did not suggest a renal effect. In fact, a number of mice from all exposure groups and controls at termination of the study contained as severe a renal amyloidosis as others which were removed moribund and/ or dead. It would appear that a constellation of effects in various tissues, including the kidneys, were required for the morbidity and/or mortality observed in controls and exposed mice. In any event, the increased degree of renal amyloidosis in the 80 ppm exposed females, and the greater degree of other tissue involvement, appeared to be a reasonable explanation for their increased morbidity and mortality.

Heart/Amyloidosis: In the 80 ppm exposed female mice, several other organ systems were secondarily involved in pathological processes that likely contributed to morbidity and/or mortality. The incidence of cardiac amyloidosis was not different from controls; however, more mice contained an atrial thrombus of relatively acute onset. An occasional thrombus was also observed in other locations of the heart and may have had a more chronic appearance. The formation of the atrial thrombus was considered secondary to the possible renal failure and systemic amyloidosis rather than a direct effect on the heart. Associated with impaired cardiac circulation were chronic passive congestive lung changes.

Lungs: There were no primary microscopic changes in the lung considered exposure related. A decrease in the incidence of focal chronic inflammation of the pleura was coincidental, since this inflammatory reaction was usually associated with the presence of the microchip used for animal identification. There was a slight increase in the number of female mice in the high-exposure group with microscopic hyperplasia of the larynx and trachea and focal ulceration of the larynx. These changes were not considered to be due to irritative effects of sulfuryl fluoride exposure since they were primarily found in mice which had aspirated food due to an impacted esophagus. Furthermore, the larynx and trachea were not previously affected in the 13-week or 12-month studies.

Liver/Spleen: More of the 80 ppm exposed mice were found to have hepatic and splenic atrophy due to stress-related, generalised systemic amyloidosis, inanition and the associated debility. A statistically-identified decrease in the number of mice with macrophages containing pigment in the liver was noted and not considered of any biological significance. These pigmentary accumulations are frequently more prominent in older mice and the observed decrease may be a reflection of the earlier mortality in the 80 ppm group.

Mesentery: The mesenteric vessels were one of the more common sites showing degenerative fibrinoid changes in females. A decreased incidence of this vascular change was noted in all exposed groups and was statistically identified in the 80 ppm group. Generally, if morbidity and/or death are as a result of kidney failure, there is an increased incidence of degenerative vascular disease involving many

tissues.

Reproductive: The only reproductive system effect observed was a statistically-identified decrease in the number of mice with cystic endometrial hyperplasia.

Tumours: There were no tissues in female mice with a statistically identified increased rate of tumours when compared to the control group.

Male mice had a number of statistically-identified increased or decreased pathological observations in non-target tissues in the 80 ppm exposed group.

Larynx/Trachea: A number of mice with feed impacted in their oesophagus were observed in all groups, but were statistically identified as increased only in the 80 ppm exposed group. As in the female mice, there were some inflammatory changes in the larynx and trachea of the male mice. Occasional mice also had some epithelial hyperplasia of the trachea. The changes in the larynx and trachea of male mice were usually the result of feed particles present in association with the impacted oesophagus. Likewise, the increased incidence of suppurative inflammation in the nasal turbinates was related to feed particles present within the lumen.

Mediastinal Lymph Node/Thymus: As expected, stress-related atrophy of the mediastinal lymph node and thymus were observed more frequently in the high-exposed group of mice suffering from other debilitating conditions.

Oesophagus: The cause for the impacted oesophagus was not determined on gross or histopathologic examination of tissues from these mice. Generally, this was the best explanation for the earlier deaths of a number of the mice in the 80 ppm group. What relation this has to sulfuryl fluoride exposure in this group, if any, was undetermined, since female control rats in the concurrently conducted 2-year study had the same condition, and it was also a significant cause of their death (IIA 5.5a/01, **I03**).

Kidney/Amyloidosis: Renal amyloidosis of male mice essentially was detected in the glomerulus of all male mice. More of the 80 ppm exposed males had very slight or slight amounts of amyloid deposited in their glomerulus, in contrast to the females with severe to very severe amounts present. The total number of male mice with the two most severe categories of glomerular amyloidosis combined, was decreased although not statistically identified, in contrast to the increase in females. Not unexpected, the incidence of interstitial amyloid deposits in the male high-exposed group was also decreased. A statistically-identified decrease in renal mineral deposition was also noted in the high-exposed male group. The observed decrease in renal amyloidosis and mineralisation in males may have been the result of earlier mortality of some mice in this group during the first year, prior to the onset of significant age-related changes. The renal pelvis of 20 ppm and 80 ppm male mice was less frequently dilated compared to the controls and was consistent with their gross observation. This was considered normal variation since it was not observed in female mice in these groups. Furthermore, male mice frequently have this condition and there was no reason to expect a beneficial effect of exposure to sulfuryl fluoride.

Adrenals/Testes: There were fewer 80 ppm exposed male mice with a focus of altered cells in the adrenal cortex, as well as, tubular atrophy, fibrinoid vascular degeneration and mineralisation of the testes. All of these changes were likely to be a reflection of the earlier deaths of some mice in this group during the first year of exposure.

Peripheral Nerve: Fewer mice in the 20 ppm and 80 ppm groups had a microscopic observation of degenerative changes in their peripheral nerve. There may be an age-related effect in the 80 ppm exposed mice, but more likely, it was a result of histological variation of the very slight changes normally present. This may also be due to the small amount of peripheral nerve available for examination

from mice due to their small size. In any event, the changes in the peripheral nerve were not increased in severity or incidence which may have suggested an exposure-related effect.

Table 5.5c/01-12: Histopathology--18-Month Study (Various Tissues--Mentioned Above)

Sex	Males				Females			
Exposure Conc. (ppm)	0	5	20	80	0	5	20	80
Number of Mice Examined	50	50	50	50	50	50	50	50
Adrenals (# of tissues examined)	50	50	50	50	48	50	50	50
Missing:	0	0	0	0	2	0	0	0
Within normal limits:	2	3	4	4	2	1	0	2
Amyloid, cortico-medullary junction: - very slight	4	2	3	2	4	3	4	3
- slight	10	14	12	10	18	16	15	26
- moderate	10	7	11	5	3	4	9	4
- severe	3	3	0	3	0	0	0	0
- any severity (combined)	27	26	26	20	25	23	28	33
Focus(i) of altered cells, cortex, focal:	8	12	12	2	1	3	1	1
Focus(i) of altered cells, cortex, multifocal:	6	7	8	3	0	0	0	0
- focal or multifocal: (combined)	14	19	20	5*	1	3	1	1
Hyperplasia - spindle cell, cortex: - very slight	14	19	21	13	34	39	32	36
- slight	3	2	3	2	8	8	11	2
- moderate	1	0	1	0	0	0	0	0
- any severity (combined)	18	21	25	15	42	47	43	38
Mineralisation, cortex, unilateral, focal:	1	0	0	0	0	0	0	0
Mineralisation, corticomedullary junction, unilateral - focal:	1	0	0	0	0	0	0	0
- multifocal:	1	0	0	1	1	0	0	0
any symmetry - focal or multifocal: (combined)	2	0	0	1	1	0	0	0
Pigment, corticumedullary junction, multifocal: - very slight	20	22	9*	24	15	15	15	12
- slight	12	7	15	7	14	8	13	16
- moderate	8	4	5	6	7	10	7	1
- severe	1	0	1	0	3	1	1	0
Pigment, corticomedullary junction, multifocal: - any severity (combined)	41	33	30	37	39	34	36	29
Adenoma, cortex, benign, primary:	1	0	1	0	0	0	0	0
Brain - Cerebellum (# of tissues examined)	50	50	50	50	50	50	50	50
Within normal limits:	49	50	50	50	50	50	49	50
Aggregate(s) of mononuclear (predominately lymphoid) cells, ependymal canal:	0	0	0	0	0	0	1	0
" " " meninges, focal:	1	0	0	0	0	0	0	0
Mineralisation, meninges, focal:	1	0	0	0	0	0	0	0
Brain – Cerebrum (# of tissues examined)	50	50	50	50	50	50	50	50
Within normal limits:	48	50	49	37	49	50	47	38