CASWELL

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM **6(a)(2) Data**

Subject: **SULFURYL FLUORIDE. ID # 078003**. Evaluation of Rat Chronic Toxicity/Oncogenicity, Dog Chronic Toxicity and Mouse Oncogenicity Inhalation Studies.

Tox. Chem. No.: 816A
PC Code No.: 078003
DP Barcode Nos.: D210874, D226103, D227298
Submission Nos.: S479737, S504536, S506750

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I. CONCLUSIONS

The 3 submitted long-term inhalation studies on sulfuryl fluoride are considered Acceptable for regulatory purposes. Executive summaries are provided below, under the "Discussion" section and DERs are attached to this memorandum. However, these data were not required for reregistration of sulfuryl fluoride, but were submitted as additional information on the toxicology of this chemical. A reregistration eligibility decision (RED) document on sulfuryl fluoride was completed in September, 1993.

No imminent hazard was identified in these studies. The 6(a)(2) status was addressed previously in a memorandum dated May 1, 1995 from L. Hansen to K. O'Malley

(see brief comments below under "Discussion". There was no observed increase in incidence of neoplastic lesions due to long-term exposure to sulfuryl fluoride.

II. ACTION REQUESTED

DowElanco submitted for evaluation the following inhalation toxicity studies on sulfuryl fluoride as 6(a)(2) data: a two-year chronic toxicity/ oncogenicity study in the rat (Guideline 83-5; MRID 43354902), an 18-month carcinogenicity study in the mouse (Guideline 83-2b; MRID 4354903) and a one-year chronic toxicity study in the dog (Guideline 83-1b, MRID 43354901). The 6(a)(2) status was flagged based on neuropathology in all 3 species at the highest doses tested.

III. DISCUSSION

Sulfuryl fluoride caused brain lesions in all 3 species at the highest dose level tested. However, these effects were observed at doses that were higher (80 ppm, mice and rats; 200 ppm, dogs) than the current endpoint used for human risk assessment for repeated exposure (30 ppm, based on electrophysiological disturbances at 100 ppm in a 13-week inhalation neurotoxicity study in the rat, MRID 40839902; reviewed in HED Doc. No. 009479). Significant mortality and other toxic effects were also observed in the 3 chronic studies at the highest doses tested. Although the NOEL for rats was 5 ppm, the effect observed, very slight to slight fluorosis of the teeth, is not considered relevant for adult human exposure (teeth are no longer growing) or for children (repeated exposure not anticipated under current use patterns).

Brief summaries of the 3 studies reviewed are provided below:

1. Guideline 83-1(b), Dog Chronic Inhalation Toxicity Study. MRID 43354901

EXECUTIVE SUMMARY: In a chronic inhalation toxicity study (MRID 43354901), four Beagle dogs/sex/exposure group were exposed to sulfuryl fluoride gas (technical grade, 95.1 - 98.8% a.i.; Lot Nos. WP 910826-929, WP 920131-940, and WP 920619-953) at target exposure concentrations of 0, 20, 80, or 200 ppm for 6 hours/day, 5 days/week. The first three exposure groups (0, 20, and 80 ppm) were exposed for one year, while the fourth exposure group (200 ppm) was exposed to 200 ppm for 6 hours/day, 5 days/week for only up to 9 months due to the development of moribund conditions and death of those animals.

The male dogs in the 80 ppm exposure group had decreased cumulative body weight gains during the study, ranging from -2.4 to -26.7%. The female dogs in the 80 ppm exposure group had a steady decrease in cumulative body weight gains, ranging from -12.2 to -25.7%, beginning on Day 278 through the end of the study. Three male dogs and one female dog from the 80 ppm exposure group had a slight increase in the aggregates of alveolar macrophages and concentric circles in the teeth. Organ weights and evaluations of

Exposure to 200 ppm of sulfuryl fluoride resulted in deaths and moribund conditions in both male and female dogs after approximately 9 months. Prior to death or sacrifice, one male and three female dogs in the 200 ppm exposure group exhibited labored breathing, shallow rapid respiration, and pale or blue mucous membranes. One male dog died on Day 267, and another became moribund and was necropsied on Day 271. Three female dogs were sacrificed due to a moribund condition on Day 278 or 281. All remaining male and female dogs in the 200 ppm were sacrificed and necropsied on Day 282. Animals from the 200 ppm exposure group had darkened areas on the lungs which appeared consolidated and were firm upon palpation. An inflammation of the pulmonary system was evident from inflammatory cell infiltrates, hypertrophied type II pneumoncytes, hypertrophied and hyperplastic epithelial cells lining the respiratory and alveolar ducts, focal thickening of the pleura, and thickening of the interalveolar septae. The nasal turbinates, larynx, trachea, and major portions of the bronchial tree were not affected.

The brain was also affected in the 200 ppm exposure group. Two of the male dogs and three of the female dogs had a focus of malacia in the head of the caudate nucleus. The thyroid gland in all of the male dogs and three female dogs had a slight hypertrophy of the follicular epithelium with no degenerative or inflammatory changes.

No exposure-related changes were found in dogs from the 20 ppm exposure group.

The LOEL is 80 ppm, based on the decreases in the body weight gains, the slight increase in aggregates of alveolar macrophages, and the dental fluorosis. The NOEL is 20 ppm.

This chronic inhalation toxicity study is **acceptable** (83-1*(b)*) and does satisfy the guideline requirement for a chronic inhalation study (83-1*(b)*) in dogs.

2. Guideline 83-2(b), Mouse Inhalation Oncogenicity Study. MRID 43354903

EXECUTIVE SUMMARY: In a carcinogenicity toxicity study (MRID 43354903), sulfuryl fluoride (99.8% a.i., Lot #WP 880329-752, WP 901011-907, WP 910321-918, WP 910826-929) was administered by inhalation to 50 CD-1 mice/sex/dose at dose levels of 0, 5, 20, and 80 ppm in air 6 hours/day, 5 days/week for 18 months (estimated doses of 0, 4.96, 19.84, and 79.35 mg/kg/day)[1]. An additional 10 animals/sex/dose were treated for 12 months in a satellite study.

[1]Calculated by the reviewer by converting ppm to mg/m^3 by: mg/m^3 = ppm X MW/24.5 @ 25°C and 760 mm Hg; and calculating mg/kg/day using a breathing rate of 0.01 m^3/6 hours for a 0.03 kg mouse and adjusting for 5/7 day treatment periods. Example: mg/m^3 = 20 ppm x 102.07/24.5 = 83.32 mg/m^3; 83.32 mg/m^3 x 0.01 m^3 x 5/7 = 0.595 mg; 0.595 mg/0.03 kg = 19.84 mg/kg/day.

Mortality was significantly increased ($p \leq 0.05$)in females after treatment for 18 months at 80 ppm sulfuryl fluoride. Mortality was also increased in males, but the increase was not statistically significant (mortality: 80 ppm females, 72%; controls, 36%; 80 ppm males, 64%; controls, 46%). The mean body weight gain was decreased in both sexes at 80 ppm (males decreased 37%; females 35% compared to controls). The absolute weights of brain, kidney, and liver were significantly ($p \leq 0.05$) decreased in both sexes at 80 ppm compared to controls. Increased incidence of vacuolation in the cerebrum of the brain were seen in both sexes at 80 ppm (13/50 and 12/50 for males and females, respectively; 0/50 for both controls; $p \leq 0.001$). An increased incidence of thyroid epithelial hypertrophy was seen in both sexes compared to the control group, but was observed more frequently in males ($p \leq 0.01$) than females ($p \leq 0.05$)(males: 80 ppm, 20/50; females: 80 ppm, 6/50; controls, 1/50 for both sexes). Thymus atrophy was significantly increased ($p \leq 0.05$) in males at 80 ppm (80 ppm, 6/50; control 1/50). Significantly ($p \leq 0.01$) increased incidences of heart thrombus and chronic lung congestion were seen in females at 80 ppm (heart thrombus: 80 ppm, 14/50; controls, 4/50; lung congestion: 19/50; controls, 6/50). Amyloidosis, commonly seen in aging CD-1 mice was a major cause of death in the study. The incidence of severe to very severe kidney glomerular amyloidosis was significantly ($p \leq 0.05$) increased in females at 80 ppm (80 ppm, 32/50; control, 20/50).

The LOEL is 80 ppm (79.35 mg/kg/day)for both sexes, based on decreased survival, especially in females, decreased body weight gain and cerebral vacuolation in the brain in both sexes, thyroid epithelial hypertrophy, especially in males, and increased incidences of heart thrombus and lung congestion in females. The NOEL is 20 ppm (19.84 mg/kg/day) for both sexes.

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls. Major organs examined included liver, thyroid, kidney, testes, ovary, bladder, lung, and brain. Dosing was considered adequate based on decreased weight gain and increased microscopic brain lesions in both sexes, increased thyroid hypertrophy in males, and increased heart thrombus and lung congestion in females at the high dose.

This carcinogenicity study in the mouse is acceptable, and does satisfy the guideline requirement for a carcinogenicity study (83-2b)in mice.

3. Guideline 83-5, Rat Chronic Toxicity/Oncogenicity Inhalation Study. MRID 43354902

EXECUTIVE SUMMARY: In a combined chronic/oncogenicity study (MRID 43354902), groups of 50 male and 50 female Fisher 344 rats were subjected to whole-body inhalation exposure to sulfuryl fluoride (93.6 - 99.7% a.i.; Lot No. WP 880329-752, WP 901011-907, WP 910321-918, WP 910826-929, WP 920131-940) at concentrations of 0, 5, 20, or 80 ppm for 6 hours/day, 5 days/week for 2 years (main study). Fifteen animals of each sex per group were similarly exposed to sulfuryl fluoride for 12 months (satellite study)

for interim evaluation of toxicity. The satellite study identified the kidneys, lungs, and teeth as targets of sulfuryl fluoride.

All main study male and female rats exposed to 80 ppm of sulfuryl fluoride died before termination at 2 years; the death in more than 90% of each sex was due to very severe chronic glomerulonephropathy (advanced chronic renal disease), which resulted in renal failure. Other effects related to renal failure or directly to exposure to 80-ppm of the test material included decreased body weight gain, decreased specific gravity of urine, serum chemistry changes indicative of renal failure (elevated urea nitrogen, creatinine, triglycerides, and cholesterol, and phosphorus and depressed levels of total protein, albumin, and chloride). Pathologic lesions in 80-ppm group rats related to renal failure included parathyroid hyperplasia, osteodystrophy, splenic and lymph node atrophy, gastric erosion, cardiac thrombosis, lung congestion, and mineralization in a variety of tissues. Pathologic lesions considered to be related directly to exposure to 80 ppm of sulfuryl fluoride in male and female rats included adrenal cortical hemorrhage, hepatocellular atrophy, reactive hyperplasia and inflammation of the respiratory epithelium of the nasal turbinates, vacuolation of the cerebrum and thalamus/ hypothalamus, adrenal cortical hemorrhage, aggregates of alveolar macrophages, and dental fluorosis. The only treatment-related effects occurring at 20 ppm was a significantly increased incidence of dental fluorosis in males (10/50 vs 0/50 in controls, $p<0.01$). There was no increase in histopathologic lesions in females exposed to 20 ppm of sulfuryl fluoride. No exposure-related toxic effects occurred in male or female rats exposed to 5 ppm of sulfuryl fluoride.

The LOELs are 20 ppm for male rats based on dental fluorosis and 80 ppm for female rats based on primary and secondary renal toxicity; effects in the adrenal cortex, brain, eyes, liver, nasal tissue, respiratory tract; and dental fluorosis. The corresponding NOELs are 5 ppm for male rats and 20 ppm for female rats.

At the concentrations tested, there were no increases in exposure-related tumor incidences when compared with control incidences. Dosing was considered adequate based on the survival of adequate numbers of animals in the 20-ppm groups (76% in males and 66% in females) at study termination despite the fact all 80-ppm group rats died before termination of the study.

This combined chronic/oncogenicity study in the rat is classified as **Acceptable** (§83-5) and satisfies the Subdivision F guideline requirement for a combined chronic/oncogenicity study in rats (§83-5).

2/2/97

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DATA EVALUATION REPORT

SULFURYL FLUORIDE

STUDY TYPE: CHRONIC INHALATION TOXICITY - DOG

Prepared for

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Prepared by

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Quality Assurance: Susan Chang, M.S.	Signature:	Susan Chang
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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: W. Greear, M.P.H., D.A.B.T. W. Greear, Date 5/13/97
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EPA Secondary Reviewer: Marion Copley, Date 7/3/97
M. Copley, D.V.M., D.A.B.T., Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Inhalation Toxicity - Dog
OPPTS 870.4100 [§83-1b]

DP BARCODE:D210874 SUBMISSION CODE: S479737
P.C. CODE:078003 TOX. CHEM. NO.: 816A

TEST MATERIAL: Sulfuryl fluoride

SYNONYMS: VIKANE™ gas fumigant, SO_2F_2

CITATION: Quast, J., Beekman, M., and Nitschke, K. (1993) Sulfuryl Fluoride: One-year inhalation toxicity study in Beagle dogs. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan 48674. Laboratory project study identification K-016399-044, October 21, 1993. MRID 43354901. Unpublished.

SPONSOR: DowElanco, 9330 Zionsville, Indianapolis, IN 46268

EXECUTIVE SUMMARY: In a chronic inhalation toxicity study (MRID 43354901), four Beagle dogs/sex/exposure group were exposed to sulfuryl fluoride gas (technical grade, 95.1 - 98.8% a.i.; Lot Nos. WP 910826-929, WP 920131-940, and WP 920619-953) at target exposure concentrations of 0, 20, 80, or 200 ppm for 6 hours/day, 5 days/week. The first three exposure groups (0, 20, and 80 ppm) were exposed for one year, while the fourth exposure group (200 ppm) was exposed to 200 ppm for 6 hours/day, 5 days/week for only up to 9 months due to the development of moribund conditions and death of those animals.

The male dogs in the 80 ppm exposure group had decreased cumulative body weight gains during the study, ranging from -2.4 to -26.7%. The female dogs in the 80 ppm exposure group had a steady decrease in cumulative body weight gains, ranging from -12.2 to -25.7%, beginning on Day 278 through the end of the study. Three male dogs and one female dog from the 80 ppm exposure group had a slight increase in the aggregates of alveolar macrophages and concentric circles in the teeth. Organ weights and evaluations of hematological, clinical chemistry, and urinalysis data demonstrated no changes from exposure to sulfuryl fluoride.

Exposure to 200 ppm of sulfuryl fluoride resulted in deaths and moribund conditions in both male and female dogs after approximately 9 months. Prior to death or sacrifice, one male and three female dogs in the 200 ppm exposure group exhibited labored breathing, shallow rapid respiration, and pale or blue mucous membranes. One male dog died on Day 267, and another became moribund and was necropsied on Day 271. Three female dogs were sacrificed due to a moribund condition on Day 278 or 281. All remaining male and female dogs in the 200 ppm were sacrificed and necropsied on Day 282. Animals from the 200 ppm exposure group had darkened areas on the lungs which appeared consolidated and were firm upon palpation. An inflammation of the pulmonary system was evident from inflammatory cell infiltrates,

hypertrophied type II pneumoncytes, hypertrophied and hyperplastic epithelial cells lining the respiratory and alveolar ducts, focal thickening of the pleura, and thickening of the interalveolar septae. The nasal turbinates, larynx, trachea, and major portions of the bronchial tree were not affected.

The brain was also affected in the 200 ppm exposure group. Two of the male dogs and three of the female dogs had a focus of malacia in the head of the caudate nucleus. The thyroid gland in all of the male dogs and three female dogs had a slight hypertrophy of the follicular epithelium with no degenerative or inflammatory changes.

No exposure-related changes were found in dogs from the 20 ppm exposure group.

The LOEL is 80 ppm, based on the decreases in the body weight gains, the slight increase in aggregates of alveolar macrophages, and the dental fluorosis. The NOEL is 20 ppm.

This chronic inhalation toxicity study is **acceptable** (83-1*(b)*) and does satisfy the guideline requirement for a chronic inhalation study (83-1*(b)*) in dogs.

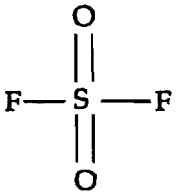
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Sulfuryl fluoride

Description: colorless gas
CAS No.: 2699-79-8
Lot No.: WP 910826-929, WP 920131-940, WP 920619-953
Purity: 95.1 to 98.8% a.i.
Contaminants: [redacted]
Stability of compound: Stable for the duration of thestudy (Table 3, p. 50, MRID 43354901)
Structure:



2. Vehicle and/or positive control

Not applicable

3. Test animals

Species: dog
Strain: Beagle
Age and weight at study initiation: four months old; males: 8274-13,534 g, females: 7360-9249 g
Source: Marshall Farms, North Rose, NY
Housing: The dogs were group housed during prestudy and nonexposure study periods. During the study, the dogs were housed two per pen in runs, except during exposure, when the dogs were singly housed in the exposure chambers.
Diet: Purina Certified Canine Chow #5007, ad libitum except during exposure
Water: Municipal water, ad libitum except during exposure
Environmental conditions (exposure chambers):
Temperature: approximately 22°C
Humidity: approximately 50%
Air changes: not given, but airflow rate was 2900 L/min
Photoperiod: 12 hr light/12 hr dark
Acclimation period: at least 30 days

B. STUDY DESIGN

1. In life dates

Start: January 13, 1992; end: October 21, 1993

2. Animal assignment

The number of animals assigned to the exposure groups is listed in Table 1. The animals were assigned by their pre-study body weight using a computer randomization program. Litter mates were not assigned to the same exposure group.

TABLE 1: Animal assignment

Exposure Group	Target Exposure Concentration (ppm)	Mean Exposure Concentrations (ppm)	Animals	
			Male	Female
Control	0	0	4	4
Low	20	21	4	4
Mid	80	79	4	4
High	200	198	4	4

Data taken from Table 1, p.48 and p. 27, MRID 43354901.

3. Dose selection rationale

In a two-week inhalation study, one Beagle dog/sex/exposure group was exposed to sulfuryl fluoride gas at target exposure concentrations of 0, 30, 100, or 300 ppm for 6 hours/day, 5 days/week. Beginning with the 5th exposure, infrequent intermittent episodes of tremors and tetany were present in both dogs from the 300 ppm exposure group. These effects were considered reversible, since they were not observed during the time between exposures, and no histopathologic changes of the nervous system were found. A slight body weight decrease was observed in a female dog from the 300 ppm exposure group. Histopathologically, minimal microscopic inflammatory changes were noted in the nasal turbinates and the trachea of both dogs in the 300 ppm exposure group. The NOEL was suggested to be 100 ppm for this two-week exposure period.

In a thirteen-week inhalation study, four Beagle dogs/sex/exposure group were exposed to sulfuryl fluoride gas at target exposure concentrations of 0, 30, 100, or 200 ppm for 6 hours/day, 5 days/week. Effects were only noted in the 200 ppm exposure group. Shortly after the beginning of exposures through the 19th exposure, one male dog exhibited transient tremors and tetany. In the male dogs, a slight body weight decrease was observed; and, to a lesser degree a body weight loss occurred in the female dogs. Small bilaterally symmetrical microscopic focal inflammatory and degenerative changes were noted in the caudate nucleus of the basal ganglia in one male and one female dog. This male dog had displayed the tremors and tetany once on Day 19 of the study. No other exposure-related histopathologic effects were observed. The NOEL was suggested to be 100 ppm for this 13-week exposure period.

4. Diet

A basal diet of Purina Certified Canine Chow #5007 (Purina Mills, Inc., St. Louis, MO) in pelleted form was available *ad libitum*, except during exposure. Analysis of the Purina Certified Canine Chow #5007 was supplied by Purina Mills, Inc., confirming that the diet provided adequate nutrition and that the levels of selected contaminants associated with the formulation process were within acceptable limits. Drinking water from the municipal water supply of the City of Midland, MI was available *ad libitum*, except during exposure. Analyses of the water were performed by the City of Midland, MI and by an independent laboratory. These results were within acceptable limits.

5. Exposure chambers

The volume of the exposure chambers were 14.5 m^3 (2.4 m wide x 2.4 m high x 2.4 m deep with a pyramidal top). The airflow rate through the chambers was approximately 2900 L/min, which the authors stated was sufficient to provide the normal concentration of oxygen to the animals. No indication was given that the oxygen concentrations were measured.

6. Generation system

Sulfuryl fluoride was mixed with air (from a compressed air source) prior to entering the exposure chamber. The mixing occurred in a J-tube packed with glass beads. These beads facilitated the mixing of the sulfuryl fluoride and the air prior to entering the chamber (see Figure 1, page 47, MRID 43354901).

7. Chamber monitoring

The distribution of sulfuryl fluoride was determined at 8 points within the animals' breathing zone of each chamber, plus the reference point within each chamber. The concentrations were approximately ± 10% of the reference concentration which was within acceptable limits.

A single cylinder of sulfuryl fluoride was used to supply as many as six chambers. The cylinder of sulfuryl fluoride was weighed prior to and after each exposure to determine the amount of sulfuryl fluoride used. A combined nominal concentration for all exposure concentrations was calculated from the amount of sulfuryl fluoride used and the total chamber airflow volume through the chambers. The concentration of sulfuryl fluoride was measured spectrophotometrically every 30 minutes in each chamber.

Airflow rates through each chamber were determined at 60 minute intervals with a Universal Venturi tube (Series 180, BIF, 345 Harris Ave., Providence, RI) coupled with a Setra Differential Pressure Transmitter (Setra Systems, Inc., Acton, MA). Chamber temperatures were measured with a thermometer or a resistance temperature device, and relative

humidities were measured with relative humidity gauges or humidity sensors (HMP 112A, Vaisala, Helsinki, Finland) at 60 minute intervals. The temperature and relative humidity in the chambers were maintained at approximately 22°C and 50%, respectively.

8. Statistics

Only descriptive statistics (means and standard deviations) of chamber concentrations, temperatures, relative humidities, and airflow rates, as well as, leukocyte (white blood cell) differential counts were conducted.

All parameters examined statistically were tested first for equality of variance using Bartlett's test. If the results from Bartlett's test were significant, then the data for the parameter were subjected to a transformation to obtain equality of the variances. The transformations examined were the common log, the inverse, and the square root, in that order with a Bartlett's test following each transformation. When Bartlett's test was satisfied no further transformations were applied, or, if none of the transformations resulted in homogeneous variances the transformed data or raw data with the lowest Bartlett's statistic were used. The selected form of the data was subjected to the appropriate parametric analysis as described below.

In-life body weight, hematological parameters (excluding differential WBC and nucleated RBC's), and clinical chemistry parameters were evaluated using a three-way repeated measures (RM) analysis of variance (ANOVA) for time (the repeated factor), sex, and dose (Winer, 1971). In the three-way RM-ANOVA, differences between the groups were detected primarily by the time-dose interaction. Because the 200 ppm exposed dogs became moribund and were removed from study prior to the one-year schedule termination, their data were not included in the three-way repeated measures analysis.

Parameters analyzed by a three-way RM-ANOVA involved several preliminary examinations. The first was an examination of the time-sex-dose interaction; if significant, the analysis was repeated separately for each sex without examining the results of other factors. If the time-sex-dose interaction was not statistically identified then the sex-dose interaction was reviewed for significance. A significant finding was then reexamined separately for each sex. After accounting for the influence of sex on the response to treatment, the time-dose interaction was examined. If the time-dose interaction was statistically identified, the analysis was repeated for each dose against controls. In a few instances, a sex-dose interaction may have been identified for a particular dose versus control comparison which led to separation by sex and reanalysis. A Bonferroni correction was used to compensate for the multiple comparison with the control group. This was applied only when comparisons were made to the control group and was applied for the time-dose interaction.

Terminal body weights, organ weights (absolute and relative, excluding ovaries and testes), and urine specific gravities were evaluated using a two-way ANOVA with the factors of sex and dose; differences between the groups were primarily detected by the dose factor. For these parameters, the first determination was whether the sex-dose interaction was significant; if it was, a one-way ANOVA was done separately for each sex. Comparisons of individual dose groups to the control group were made with a Dunnett's test only when a statistically significant dose effect existed; this was subsequent to the evaluation of the sex-dose interaction. The form of the ANOVA, one-way or two-way, was determined by whether or not the analysis had been separated by sex or not.

Results for ovaries and testes weight (absolute and relative) were analyzed using a one-way ANOVA. If significant dose effects were determined in the one-way ANOVA then separate doses were compared to controls using a Dunnett's test.

The reviewer has no objections to the statistical analyses performed.

C. METHODS

1. Observations

Animals were observed twice daily for mortality, morbidity, availability of feed and water, and signs of clinical toxicity. During the exposure period, the animals were observed more frequently. Clinical examinations were performed weekly and at the time of each blood collection.

2. Body weight

Animals were weighed prior to the initial exposure, at weekly intervals for the first thirteen weeks, and monthly up to nine months. Body weights were then collected biweekly from nine months to the end of the study.

3. Food consumption

Food consumptions were not determined.

4. Ophthalmoscopic examination

Eyes were examined prior to the initial exposure and shortly before the scheduled one-year necropsy. The examination was performed by a clinical veterinarian using a Fison Binocular indirect ophthalmoscope.

5. Blood was collected twice prior to the initial exposure, at three, six, and nine months, and once again during the last two weeks of the study for hematology and clinical analysis from all surviving animals. The blood was collected from the jugular vein of the dogs after an overnight fast. Additional blood samples were collected from the animals in the 200 ppm exposure group as they became moribund during

the latter part of the study. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected from the animals at 6 months via catheterization and at necropsy via transmural aspiration from the bladder. Urine specimens were only obtained from the dogs in the 200 ppm exposure group at six months. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Occult Blood*
X	Sediment		Nitrate
X	(microscopic)*	X	Urobilinogen
	Protein*		

* Required for chronic studies

7. Sacrifice and pathology

All animals from the 0, 20, and 80 ppm exposure groups were sacrificed and necropsied following 52 weeks of exposure to sulfuryl fluoride and were subjected to gross pathological examination. Fasted terminal body weights were recorded for the 0, 20, and 80 ppm exposure groups. Each animal was euthanatized with sodium pentobarbital administered intravenously. A complete necropsy examination was conducted on each dog by the study pathologist (Diplomate, American College of Veterinary Pathologists). The CHECKED (X) tissues listed below were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	X	Brain*
X	Salivary glands*	XX	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*		Eyes*
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*
X	Colon*	XX	Kidneys*+	X	Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland*
XX	Liver*+	XX	Testes*+	XX	Parathyroids*++
X	Gall bladder*	X	Epididymides	XX	Thyroids*++
X	Pancreas*	X	Prostate		
X	Oral tissue		Seminal vesicle		OTHER
		XX	Ovaries*+	X	Bone*
	RESPIRATORY	X	Uterus*	X	Skeletal muscle*
X	Trachea*	X	Cervix	X	Skin*
XX	Lung*	X	Oviducts	X	All gross lesions and
	Nose	X	Vagina	X	masses*
	Pharynx			X	Mediastinal tissue
X	Larynx			X	Mesenteric tissue
X	Nasal tissue			X	Tonsils
				X	Lower jaw (one-half) with teeth**
				X	Femur**

Data taken from Table 2, p.49, MRID 43354901.
* Required for chronic studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.
** Saved for fluorosis analysis which was not performed(p. 21, MRID 43354901)

The animals in the 200 ppm exposure group became moribund or died after approximately 40 weeks of exposure. Consequently, they were removed from the study and necropsied. Since no control data existed for comparison, the organ weights of these dogs were not recorded. However, two male dogs and one female dog were sacrificed without being declared "moribund" or "dead." No record was made of their organ weights, and their organ weights could have been collected for some degree of comparison. Consequently, the authors statement should indicate that the organs from all of the dogs in the 200 ppm exposure group were not weighed.

The presence of an apparent exposure-related microscopic effect in the brain of an occasional dog in the subchronic study resulted in a more extensive evaluation of the brains in this study. Following formalin fixation, the brain of each dog was grossly examined and approximately 15 transverse sections were cut using a brain mold for consistency of the region sectioned. In addition, the sections were grossly evaluated using a dissecting microscope to ascertain whether a lesion could be detected. The study pathologist cut the 15 sections and examined them prior to selecting the 9 sections which were prepared for microscopic examination from the anterior cerebrum through the medulla oblongata. The

sections of brain evaluated histologically were selected and examined from sequential sections which were cut 5 μm thick and stained with hematoxylin and eosin (H&E), Luxol Fast Blue Cresyl Violet (myelin), and Sevier-Munger (axons).

Upon initial light microscopic examination of these brain sections, an exposure-related effect was observed in one or more of the first three sections of several dogs exposed to 200 ppm sulfuryl fluoride. Therefore, in an effort to increase the probability of detecting any microscopic effects in the unaffected dogs, an additional recut of the first three sections of brain from each dog was prepared and stained with H&E for microscopic evaluation. A minimum of 30 sections of brain was examined from each dog.

Grading of histopathologic findings was done to reflect the severity of a specific lesion on the health status of the animal, or to evaluate exacerbations of commonly occurring lesions as a result of exposure. The grading system used for lungs, brain, thyroid gland, and the canine tooth, was described separately since they were the only tissues considered to be primarily affected by exposure to sulfuryl fluoride.

The microscopic pathologic changes in the lung characterized by multifocal chronic active inflammation of the alveoli were graded very slight, moderate, or severe and primarily involved the periphery of the respiratory tree. The grade of very slight indicated only minimal microscopic involvement without clinical symptoms and only an occasional corresponding gross correlate. In the moderate degree, the lungs contained diffuse focal changes suggestive of consolidation which were associated with clinical symptoms. The severe grade of chronic inflammation was associated with gross evidence of a significant portion of all lobes being consolidated and with clinical symptoms. The well being of the dogs with a moderate or severe degree of the chronic active inflammatory process was compromised and resulted in labored breathing, anorexia, and marked loss in body weight.

The lesion in the brain was graded as very slight, slight, or moderate and was located in the section of cerebrum containing the caudate nucleus. The lesion was bilaterally symmetrical and consisted of a malacic focus within the head (body and tail unaffected) of the caudate nucleus. The grade of very slight was used to indicate the lesion involved only a minimal localized amount of the caudate nucleus evaluated in the multiple sections. The grade of slight was used to indicate a larger size of the focal malacia with involvement of approximately 5 to 10% of the caudate nucleus. A moderate grade was used when greater than 10% of the caudate nucleus was affected.

Dental fluorosis of the canine tooth was defined as concentric rings which stained slightly darker and corresponded with each exposure day. The very slight grade was used to indicate the rings were barely visible, whereas, in the slight grade they were clearly visible.

II. RESULTS

A. CHAMBER CONDITIONS

The sulfuryl fluoride concentrations, airflow rates, temperatures, and relative humidities were within the target range for each parameter. The average analytical concentrations of sulfuryl fluoride for the target concentrations of 0, 20, 80, or 200 ppm were 0, 21, 79, or 198 ppm, respectively. The mean chamber airflow rates ranged from 2822 to 2942 L/min. The mean temperatures and relative humidities ranged from 21.3 to 22.1°C and 45.9 to 52.1%, respectively.

B. OBSERVATIONS

1. Toxicity

No clinical observations for dogs in the 20 and 80 ppm exposure groups were noted during the study. After approximately 9 months, one male and three female dogs in the 200 ppm exposure group exhibited labored breathing, shallow rapid respiration, and pale or blue mucous membranes. The body temperature of some dogs from the 200 ppm exposure group was occasionally elevated, but this was not consistently observed in the same dog on repeated examinations, nor within the group. The heart rate and sounds and the intensity of the femoral pulse appeared to be normal. These latter findings did not suggest that cardiac arrhythmia was the cause of the altered respiratory function. (A few dogs were administered an antibiotic and a diuretic which did not significantly alter the clinical outcome of the disease process.)

2. Mortality

One male dog from the 200 ppm exposure group died on Day 267; and another became moribund and was necropsied on Day 271. Three female dogs from the 200 ppm exposure group were sacrificed due to a moribund condition on Day 278 or 281. All remaining male and female dogs in the 200 ppm were sacrificed and necropsied on Day 282. No spontaneous deaths occurred in the other exposure groups.

C. BODY WEIGHT

No statistically significant body weight differences were observed in the male or female dogs from the 20 and 80 ppm exposure groups.

Mean body weights for the 0 and 200 ppm exposure groups are shown in Table 2. The male dogs in the 200 ppm exposure group did not have any body weight decreases until Day 89 (Week 12), whereas the female dogs were almost immediately affected on Day 5.

TABLE 2: Mean body weight (g) for animals exposed to sulfuryl fluoride

Exposure Day	Exposure Group (ppm)			
	Males		Females	
	0	200	0	200
1	10082 ± 1390	11174 ± 1583 (10.8%)[a]	8217 ± 705	8256 ± 491 (0.5%)
12	10575 ± 1594	11375 ± 1388 (7.6%)	8584 ± 838	8540 ± 727 (-0.5%)
26	11305 ± 1754	11622 ± 1336 (2.8%)	9169 ± 810	8588 ± 801 (-6.3%)
40	11635 ± 1640	11801 ± 1564 (1.4%)	9492 ± 890	8766 ± 876 (-7.6%)
54	12054 ± 1722	12267 ± 1383 (1.8%)	9746 ± 1077	8934 ± 830 (-8.3%)
68	12160 ± 1651	12370 ± 1322 (1.7%)	9926 ± 1119	9026 ± 1099 (-9.1%)
89	12488 ± 1923	12373 ± 1246 (-0.9%)	10294 ± 1198	9429 ± 1096 (-8.4%)
124	12792 ± 2080	12670 ± 1484 (-1.0%)	10835 ± 1361	9470 ± 1135 (-12.6%)
180	13159 ± 2097	12665 ± 1462 (-3.8%)	10920 ± 1655	9622 ± 1232 (-11.9%)
236	13430 ± 2501	12608 ± 1654 (-6.1%)	11239 ± 1929	9242 ± 792 (-17.8%)
278[b]	13266 ± 2570	12636 ± 3384 (-4.7%)[c]	11392 ± 2238	7730 ± 1032 (-32.1%)

Data taken from Tables 7 and 8, pp. 54-59, MRID 43354901.
[a]Parentheses: percent increase or decrease of the treated group body weight as compared to the control body weight (calculated by the reviewer).
[b]Dogs in the 200 ppm exposure group were sacrificed before the next day of measuring of body weights.
[c]Based on two animals.

Cumulative body weight gains for the 0, 20, 80, and 200 ppm exposure groups are presented in Table 3. The male dogs in the 80 ppm exposure group had decreases in the cumulative body weight gains during the study, ranging from -2.4 to -17.6%. The female dogs in the 80 ppm exposure group had a steady decrease in cumulative body weight gains, ranging from -12.2 to -25.7%, beginning on Day 278 and continuing through the end of the study. The male and female dogs from the 200 ppm exposure group were affected on Day 5 with -165 and -110 g body weight gains, respectively. Some initial recovery was evident; however, the animals never fully recovered. A total decrease in the body weight gain of -54.1 and -116.6% was present for the male and female dogs from the 200 ppm exposure group.

TABLE 3: Mean cumulative body weight gain (g) for animals exposed to Sulfuryl Fluoride

Exposure Day	Exposure Group (ppm)							
	Males				Females			
	0	20[a]	80[a]	200[c]	0	20[a]	80[a]	200[c]
5[a]	237	167 (-29.5%)[b]	281 (18.6%)	-165 (-169.6%)	173	565 (226.6%)	176 (1.7%)	-110 (-163.6%)
19[a]	811	673 (-17.0%)	899 (10.9%)	534 (-34.2%)	714	1007 (41.0%)	852 (19.3%)	378 (-47.1%)
33	1375	1387 (0.9%)	1154 (-16.1%)	786 (-42.8%)	1109	1510 (36.2%)	1092 (-1.5%)	386 (-65.2%)
47[a]	1683	1726 (2.6%)	1608 (-4.5%)	990 (-41.2%)	1439	1726 (19.9%)	1501 (4.3%)	559 (-61.2%)
61	1954	1664 (-14.8%)	1637 (-16.2%)	1071 (-45.2%)	1666	1745 (4.7%)	1883 (13.0%)	722 (-56.7%)
75[a]	2143	2218 (3.5%)	1858 (-13.3%)	1144 (-46.6%)	1911	2123 (11.1%)	1981 (3.7%)	889 (-53.5%)
89	2406	2642 (9.8%)	1982 (-17.6%)	1199 (-50.2%)	2077	1847 (-11.1%)	2161 (4.0%)	1173 (-43.5%)
152[a]	2891	3526 (22.0%)	2465 (-14.7%)	1593 (-44.9%)	2576	2884 (12.0%)	2500 (-3.0%)	1235 (-52.1%)
208	2921	4058 (38.9%)	2718 (-6.9%)	1686 (-42.3%)	2764	3632 (31.4%)	2544 (-8.0%)	1415 (-48.8%)
278	3184	4554 (43.0%)	3225 (1.3%)	1462 (-54.1%)	3175	3360 (5.8%)	2788 (-12.2%)	-526 (-116.6%)
292[a]	3522	4922 (39.75%)	3559 (1.1%)		3663	3539 (-3.4%)	3118 (-14.9%)	
306[a]	3811	4711 (23.6%)	3347 (-12.2%)		3544	3684 (4.0%)	2971 (-16.2%)	
320[a]	3710	4995 (34.6%)	3550 (-4.3%)		3518	3750 (6.6%)	2809 (-20.2%)	

TABLE 3: Continued

Exposure Day	Exposure Group (ppm)							
	Males				Females			
	0	20[a]	80[a]	200[c]	0	20[a]	80[a]	200[c]
334[a]	3772	5201 (37.9%)	3446 (-8.6%)		4079	4101 (0.5%)	2814 (-31.0%)	
362[a]	3984	5456 (36.9%)	3759 (-5.6%)		3906	4392 (12.4%)	3243 (-17.0%)	
368[a]	3439	4882 (42.0%)	3355 (-2.4%)		3896	4451 (14.2%)	2894 (-25.7%)	

Some of the data taken from p. 28, MRID 43354901.
[a]Body weight gains were calculated by the reviewer from data in Tables 7 &8, pp. 54-59.
[b]Parentheses: percent change of the treated group body weight as compared to the control body weight (calculated by the reviewer).
[C]Dogs in the 200 ppm exposure group were sacrificed prior to Day 292.

D. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

This measurement was not collected.

2. Food efficiency

This measurement could not be calculated.

E. OPHTHALMOSCOPIC EXAMINATION

No exposure-related changes were observed in any animal.

F. BLOOD

1. Hematology

No exposure-related effects were observed in the male and female dogs from the 20 and 80 ppm exposure groups throughout the study, in the male dogs from the 200 ppm exposure group through 6 months of exposure, and in the female dogs from the 200 ppm exposure group through 9 months of exposure. Prior to termination of the male and female dogs in the 200 ppm exposure group, blood samples were collected for analysis. These results are given in Table 4 (males) and Table 5 (females).

TABLE 4: Hematological values for male dogs in the 200 ppm exposure group prior to removal from the study

Exposure Day	Exposure Group (ppm)	Animal Number	RBC ($x10^6/m^3$)	Platelet ($x10^3/m^3$)	WBC ($x10^3/m^3$)
270[a]	0	Mean value	7.34	273	7.5
270[b]	200	91A6410	6.32 (-13.9%)[c]	220 (19.4%)	27.9 (272.0%)

TABLE 4: Hematological values for male dogs in the 200 ppm exposure group prior to removal from the study					
267	200	91A6413	9.99 (36.1%)	408 (49.5%)	46.1 (514.7%)
271	200	91A6410	6.40 (-12.8%)	233 (-14.7%)	36.2 (382.7%)
278	200	91A6411	6.57 (-10.5%)	557 (104.0%)	12.9 (72.0%)
282	200	91A6411	7.09 (-3.4%)	569 (108.4%)	13.1 (74.7%)
282	200	91A6412	6.76 (-7.9%)	215 (-21.2%)	8.9 (18.7%)

Data taken from Table 21, p. 125, MRID 43354901.
[a]Control mean value at the 9-month bleeding (Table 19, p. 121) is presented for comparison.
[B]This dog was bled at 9 months, and the data were presented for comparison with values from the following day.
[C]Parentheses: percent change of the exposure group's hematological values as compared to the control hematological values (calculated by the reviewer)

Generally, the RBC values were slightly decreased for the males, except for an increase in the RBC value for animal #91A6413; and the platelet and WBC values were increased. For animal #91A6410, the segmented WBC count was increased. The authors suggested that the increased RBC value in animal #91A6413 was due to clinical dehydration; however, no clinical signs or water consumption values were presented to provide evidence of dehydration. The decreased RBC and increased WBC values were an indication of the general debility and pneumonia of the animals.

TABLE 5: Hematological values for female dogs in the 200 ppm exposure group prior to removal from the study					
Exposure Day	Exposure Group (ppm)	Animal Number	RBC ($x10^6/m^3$)	Platelet ($x10^3/m^3$)	WBC ($x10^3/m^3$)
270[a]	0	Mean value	7.32	288	8.6
278	200	91A6429	6.53 (-10.8%)[b]	425 (47.6%)	11.7 (36.0%)
281	200	91A6426	8.39 (14.6%)	541 (87.8%)	23.7 (175.6%)
281	200	91A6427	7.87 (7.5%)	459 (59.4%)	25.3 (194.2%)
282	200	91A6428	6.75 (-7.8%)	621 (115.6%)	20.5 (138.4%)

Data taken from Table 28, p. 139, MRID 43354901.
[a]Control mean value at the 9-month bleeding (Table 26, p. 135) are presented for comparison.
[B]Parentheses: percent change of the exposure group's hematological values as compared to the control hematological values (calculated by the reviewer)

For the female dogs, the RBC values were either below or above the control mean value. The platelet and WBC values were elevated. These results indicated a moribund condition and were secondary to the histopathologic findings in the lungs of the dogs.

2. Clinical chemistry

No exposure-related changes in the clinical chemistry values were observed.

G. URINALYSIS

No exposure-related changes were observed.

H. SACRIFICE AND PATHOLOGY

1. Organ weight

The mean relative heart weight in the female dogs from the 20 ppm exposure group was significantly ($p < 0.05$) decreased (-10.8%). This was probably due to a heavier mean body weight for this exposure group. In addition, since higher exposure groups did not have a similar finding and no histopathological changes were noted, this weight change probably had no toxicological significance. Not cited by the authors was a statistically significant ($p < 0.05$; Dunnett's test) decrease (-23.8%) in the relative testicular weight in the male dogs from the 20 ppm exposure group. Male dogs in the 80 ppm exposure also had a 15% decrease (non-statistically significant) in relative testicular weight. However, no toxicological significance was placed on these weight changes because of no histopathological evidence.

2. Gross pathology

No tissues from the 20 and 80 ppm exposure groups were found to have any gross findings related to the sulfuryl fluoride exposures.

One male dog in the 200 ppm exposure group was found dead on Day 267 and one was sacrificed moribund on Day 271. The other two male dogs in the 200 ppm exposure group were sacrificed on Day 282. Three female dogs in the 200 ppm exposure group were sacrificed moribund on Day 278 or 281. The fourth female dog in the group was sacrificed on Day 282.

The gross necropsy of the dogs found dead or sacrificed moribund revealed that all had decreased ingesta which the authors suggested was consistent with the in-life observation of inappetence. The "inappetence" was somewhat lacking in documentation, since only the two male dogs that died or sacrificed moribund were noted as having "...ate some moist dog food" (pp. 110 & 111) one or two days prior to their necropsy.

The authors reported that the lungs of most of the dogs from the 200 ppm group had scattered darkened areas with pale areas. These dark areas appeared to be consolidated

and were firm upon palpation. However, these findings were not well-documented in the gross pathology section (pp.545-548; 561-564, MRID 43354901).

Diffuse consolidation of the lungs was found in the male dog found dead on Day 267. Consolidation of the right cardiac lobe, hydrothorax, fibrinous pleuritis, and fibrinous pericarditis were present in one of the male dogs which had been sacrificed moribund on Day 271. One of the two sacrificed male dogs had diffuse pulmonary consolidation, while the other male dog had normal looking lungs. Three female dogs were sacrificed moribund on Day 278 or 281 and had evidence of pulmonary consolidation. The fourth female dog sacrificed on Day 282 also had consolidation of the lungs.

No other tissues from the 200 ppm exposure group appeared to be grossly affected.

3. Microscopic pathology

a) Non-neoplastic-

Inflammation of the pulmonary system was evident primarily in the peripheral portions of dogs in the 200 ppm exposure group. Two male dogs and one female dog were noted to have very slight inflammation. One male dog had moderate inflammation. Of the remaining dogs, one male and three females had severe inflammation. This inflammation was characterized by a variable number of inflammatory cell infiltrates. Chronically active inflammatory foci were increased in size and hypertrophied type II pneumocytes were present. Also, the epithelial cells lining the respiratory and alveolar ducts of these foci were hypertrophied and hyperplastic. In areas in which these changes existed as a continuum, pulmonary consolidation resulted. A focal thickening of the pleura and thickening of the interalveolar septae were present with the chronic inflammatory response. These two findings were due to collagen deposition. Other portions of the pulmonary system (nasal turbinate, larynx, trachea and major portions of the bronchial tree) were not affected.

One of the male dogs from the 200 ppm exposure group had developed an acute suppurative pulmonary inflammatory process in one of the pulmonary lobes. This inflammatory process involved the pleura and the thoracic viscera. A Hemophilus species was isolated from a microbiological culture of this lesion. This was not found in the lungs of the other two dogs that were cultured. The authors considered this bacterial infection to be opportunistic and not the primary etiology of the lung pathology.

According to the pathology report (pp. 561-564, MRID 43354901), one female dog from the 200 ppm exposure group had a slight degree of chronic active inflammatory process. No clinical symptom or gross consolidation was observed in this dog that could be associated with this microscopic finding.

Three of the four male dogs and one of the four female dogs from the 80 ppm exposure group had very slight increases in the aggregates of alveolar macrophages that were multifocal in distribution. Occasional granulomatous foci were observed in the lungs of two female dogs in the 80 ppm exposure group which were attributed to aspiration of feed particles and hairshafts. No exposure-related pulmonary lesions were found in the 20 ppm exposure group.

Two of the four male dogs and three of the four female dogs from the 200 ppm exposure group had a focus of malacia in the caudate nucleus. The severity of these lesions ranged from very slight to slight for the male dogs and slight to moderate for the female dogs. Vessels and neuropil were present in the lesion; however, the inflammatory cells were not considered significant. The authors stated that the lesions did not appear to be a recent occurrence. Also, the characteristics of the lesion suggested that the etiology was due to ischemia and not cytotoxicity. However, no documentation was present in the pathology section to substantiate these conclusions. No clinical manifestations of neurotoxicity were noted during the study. The other exposure groups did not have any evidence of histopathological changes in the brain.

All male dogs and three of the four female dogs in the 200 ppm exposure group presented a very slight hypertrophy of the follicular epithelium in the thyroid gland. Also, the follicles were decreased in size, and their colloid was not as intensely stained. However, the intensity of staining was not documented in the pathology section (pp. 545-548 and 561-564, MRID 43354901). These changes were not found in the dogs from the 20 and 80 ppm exposure groups.

All dogs in the 200 ppm exposure group and three male dogs and one female dog from the 80 ppm exposure group demonstrated changes suggestive of dental fluorosis. These changes, ranging from very slight to slight, consisted of slightly dark concentric rings corresponding to days of exposure. The outer portion of the tooth which had formed prior to exposure lacked these concentric rings since this consisted of dentin which had formed prior to study initiation. The presence of the rings due to each day of exposure was not associated with other changes in the teeth. No other changes with the teeth were noted, and no dogs in the 20 ppm exposure group displayed these changes.

The authors noted other changes (liver and lymphoid tissue atrophy; bone marrow myeloid hyperplasia) in the 200 ppm exposure group as secondary to the effects in the lungs.

b) Neoplastic - No neoplastic evidence was reported in any of the animals.

III. DISCUSSION

A. DISCUSSION

These data demonstrated that 200 ppm was not well-tolerated by beagle dogs in a chronic study. By approximately the 9th month of exposure, one male and three female dogs in the 200 ppm exposure group exhibited labored breathing, shallow rapid respiration, and pale or blue mucous membranes. After which, two male and three female dogs were found in a moribund condition or dead. Animals from the 200 ppm exposure group had darkened areas on the lungs which appeared consolidated and were firm upon palpation. An inflammation of pulmonary system was evident from inflammatory cell infiltrates, hypertrophied type II pneumoncytes, hypertrophied and hyperplastic epithelial cells lining the respiratory and alveolar ducts, focal thickening of the pleura, and thickening of the interalveolar septae. One of the male dogs developed a suppuratory inflammation response, but this was suggested to be due to an opportunistic bacteria and not the primary etiology of the lung pathology. The nasal turbinates, larynx, trachea, and major portions of the bronchial tree were not affected.

The brain was also affected in the 200 ppm exposure group. Two of the male dogs and three of the female dogs had focus of malacia in the head of the caudate nucleus. This lesion was described as not being a recent event, and possibly from an ischemic etiology and not due to cytotoxicity of sulfuryl fluoride. No clinical manifestations were observed that correlated with this lesion.

Another organ affected in all of the male dogs and three female dogs from the 200 ppm exposure group was the thyroid gland. This was manifested as a slight hypertrophy of the follicular epithelium with no degenerative or inflammatory changes.

Concentric circles in the canine teeth of all dogs in the 200 ppm exposure group were noted. These circles were suggestive of dental fluorosis.

In the 80 ppm exposure group, two effects were reported in three male dogs and one female dog; a slight increase in the aggregates of alveolar macrophages and concentric circles in the teeth. Additional effects, not noted by the authors, for the 80 ppm exposure group were decreases in the cumulative body weight gains of the male dogs (ranging from -2.4 to 17.6%) during the study and steady decreases in the cumulative body weight gains of the female dogs (ranging from -12.2 to 25.7%) from Days 278 to 334.

Dogs from the 20 ppm exposure group had no exposure-related findings.

Based on the decreases in the body weight gains, the slight increases in aggregates of alveolar macrophages, and the dental fluorosis in the 80 ppm exposure group, the LOEL value is 80 ppm, and the NOEL value is 20 ppm.

B. STUDY DEFICIENCIES

Minor: The protocol and the protocol deviations were not included in the report. Some of the protocol deviations that

should be recorded are: (1) cessation of exposures for the 200 ppm exposure group, (2) failure to record organ weights, failure to collect urinalysis data, and failure to collect ophthalmic data from the dogs in the 200 ppm exposure group, (3) change from the collection of monthly to biweekly body weights after 9 months, (4) administration of antibotic and diuretic.

The following observations are additional study deficiencies. Perhaps an explanation should be provided as to why urine volume was not measured, since it is required for chronic studies. The pages were not numbered according to FIFRA format. Additional documentation for details described in the report are needed, such as inappetence, dehydration, and some pathology descriptions. Oxygen concentrations were not measured in the exposure chambers. In-life dates were not provided. The study initiation date (1/13/92) was prior to the final protocol date (2/7/92), which is not according to GLP guidelines.

Classification: Acceptable.

2/2/94

DATA EVALUATION REPORT

012468

SULFURYL FLUORIDE

STUDY TYPE: ONCOGENICITY INHALATION - MOUSE (83-2b)

Prepared for

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U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Disclaimer

This Review may have been altered subsequent to signing by the contractor's signature above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

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EPA Secondary Reviewer:
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Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Inhalation - Mouse
OPPTS 870.4200 [§83-2b]

DP BARCODE: D210874
P.C. CODE: 078003

SUBMISSION CODE: S479737
TOX. CHEM. NO.: 816A

TEST MATERIAL (PURITY): Sulfuryl fluoride (99.8%)

SYNONYMS: Vikane®

CITATION: Quast, J., G. Bradley, and K. Nitschke (1993) Sulfuryl fluoride: 18-month inhalation oncogenicity study in CD-1 mice. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan. Laboratory project study ID number K-016399-039, August 19, 1993. MRID 43354903. Unpublished.

SPONSOR: DowElanco 9002 Purdue Road, Quad IV, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a carcinogenicity toxicity study (MRID 43354903), sulfuryl fluoride (99.8% a.i., Lot #WP 880329-752, WP 901011-907, WP 910321-918, WP 910826-929) was administered by inhalation to 50 CD-1 mice/sex/dose at dose levels of 0, 5, 20, and 80 ppm in air 6 hours/day, 5 days/week for 18 months (estimated doses of 0, 4.96, 19.84, and 79.35 mg/kg/day)[1]. An additional 10 animals/sex/dose were treated for 12 months in a satellite study.

Mortality was significantly increased ($p \leq 0.05$) in females after treatment for 18 months at 80 ppm sulfuryl fluoride. Mortality was also increased in males, but the increase was not statistically significant (mortality: 80 ppm females, 72%; controls, 36%; 80 ppm males, 64%; controls, 46%). The mean body weight gain was decreased in both sexes at 80 ppm (males decreased 37%; females 35% compared to controls). The absolute weights of brain, kidney, and liver were significantly ($p \leq 0.05$) decreased in both sexes at 80 ppm compared to controls. Increased incidences of vacuolation in the cerebrum of the brain were seen in both sexes at 80 ppm (13/50 and 12/50 for males and females, respectively; 0/50 for both controls; $p \leq 0.001$). An increased incidence of thyroid epithelial hypertrophy was seen in both sexes compared to the control group, but was observed more frequently in males ($p \leq 0.01$) than females ($p \leq 0.05$) (males: 80 ppm, 20/50; females: 80 ppm, 6/50; controls, 1/50 for both sexes). Thymus atrophy was significantly increased ($p \leq 0.05$) in males at 80 ppm (80 ppm, 6/50; control 1/50). Significantly ($p \leq 0.01$) increased incidences of heart thrombus and chronic lung congestion

[1]Calculated by the reviewer by converting ppm to mg/m^3 by: mg/m^3 = ppm X MW/24.5 @ 25°C and 760 mm Hg; and calculating mg/kg/day using a breathing rate of 0.01 m^3/6 hours for a 0.03 kg mouse and adjusting for 5/7 day treatment periods. Example: mg/m^3 = 20 ppm x 102.07/24.5 = 83.32 mg/m^3; 83.32 mg/m^3 x 0.01 m^3 x 5/7 = 0.595 mg; 0.595 mg/0.03 kg = 19.84 mg/kg/day.

were seen in females at 80 ppm (heart thrombus: 80 ppm, 14/50; controls, 4/50; lung congestion: 19/50; controls, 6/50). Amyloidosis, commonly seen in aging CD-1 mice was a major cause of death in the study. The incidence of severe to very severe kidney glomerular amyloidosis was significantly ($p \leq 0.05$) increased in females at 80 ppm (80 ppm, 32/50; control, 20/50).

The LOEL is 80 ppm (79.35 mg/kg/day) for both sexes, based on decreased survival, especially in females, decreased body weight gain and cerebral vacuolation in the brain in both sexes, thyroid epithelial hypertrophy, especially in males, and increased incidences of heart thrombus and lung congestion in females. The NOEL is 20 ppm (19.84 mg/kg/day) for both sexes.

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls. Major organs examined included liver, thyroid, kidney, testes, ovary, bladder, lung, and brain. Dosing was considered adequate based on decreased weight gain and increased microscopic brain lesions in both sexes, increased thyroid hypertrophy in males, and increased heart thrombus and lung congestion in females at the high dose.

This carcinogenicity study in the mouse is acceptable, and does satisfy the guideline requirement for a carcinogenicity study (83-2b) in mice.

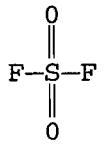
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Sulfuryl fluoride

   Description: colorless gas
   Lot/Batch #: WP 880329-752, WP 901011-907, WP 910321-918, and WP 910826-929
   Purity: 99.8% a.i. (stated by manufacturer on all lots)
   Stability of compound: stable at room temperature
   CAS #: 2699-79-8
   Structure:



2. Vehicle and/or positive control

   Test substance was mixed with air.

3. Test animals

   Species: mouse
   Strain: CD-1
   Age and weight at study initiation: males and females, 6-7 weeks; mean weight males, 27.4 g (~25.5-28.9 g); mean weight females, 21.2 g (~9.1-23.0 g).
   Source: Charles River Breeding Laboratories, Inc., Kingston, NY.

Housing: Mice were housed in pairs of the same sex during acclimation, individually during the study, in stainless steel wire cages. Cages were kept in 14.5 m^3 inhalation exposure chambers continuously throughout the study.
Diet: Purina Certified Rodent Chow #5002 from Purina Mills Inc. was provided ad libitum except during the 6-hour treatment periods.
Water: tap water ad libitum
Environmental conditions:
Temperature: 22.3-23.5°C (Inside exposure chambers.)
Humidity: 47.2-51.8% (Inside exposure chambers.)
Air changes: not supplied (Chamber air flow was given as 2900 L/minute, which would equate to an air change about every 5 minutes.)
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 7 days

B. STUDY DESIGN

1. In life dates

Start: 1990; end: 1992.

2. Animal assignment

Animals were assigned randomly within weight limitations to the test groups in Table 1.

TABLE 1: Study design

Test group	Conc.in air (ppm)	Dose to animal[a] main study mg/kg/day	Main study 18 months No. animals		Interim sac. 12 months No. animals	
			male	female	male	female
Control	0	0	50	50	10	10
Low (LDT)	5	4.96	50	50	10	10
Mid (MDT)	20	19.84	50	50	10	10
High (HDT)	80	79.35	50	50	10	10

Taken from Table 1, p. 59, MRID 43354903.
[a]Approximate mean daily intake for the entire administration period; calculated by the reviewer[1].

3. Dose selection

The dose selections were based on the results from a previous 13-week subchronic inhalation toxicity study with CD-1 mice. In this study, mice were shown to be more sensitive to sulfuryl fluoride than rats. The data indicated that decreased body weight gain and histopathologic changes in the brain and thyroid gland should result from chronic exposure to 80 ppm sulfuryl fluoride, and no effect would be expected at 5 ppm.

4. Dose delivery and analysis

Sulfuryl fluoride gas was metered from a cylinder into a J-tube connected to the air supply for each 14.5 m^3 exposure chamber. Compressed air was passed through the J-tube diluting the test gas. This mixture was then further diluted and mixed to achieve the desired test concentrations when it was passed into the main chamber airstreams. The airflow was measured for each exposure chamber every 60 minutes with a Universal Venturi Tube, which was calibrated at the factory. The resulting concentrations of sulfuryl fluoride in the chambers were measured every 30 minutes at a reference point using a MIRAN 1A infrared spectrophotometer. The mixing and distribution of sulfuryl fluoride was tested by sampling from 8 different sample points plus the standard reference point. The gas cylinders were weighed before and after each exposure session to document the amount of gas released.

Results - The airflow into each chamber was maintained at approximately 2900 L/minute. The time weighted average concentration of sulfuryl fluoride measured for each exposure chamber was 5.1 ± 0.6 ppm (range, 3.1-10.0 ppm), 20.1 ± 1.5 ppm (range, 13.6-26.3 ppm), and 79.7 ± 3.3 ppm (range, 64.5-87.6 ppm). The deviations of the concentrations measured at the eight sampling sites in each exposure chamber from the concentrations measured at the reference sites were: 96-102%, 100-102%, and 99-101% for the 5, 20, and 80 ppm chambers, respectively, indicating that distribution within each chamber was adequate. No sulfuryl fluoride was detected in the chamber containing the control mice.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Body and organ weights, hematology and clinical chemistry parameters were evaluated by Bartlett's test for equality of variances followed by an analysis of variance (ANOVA) and Dunnett's test or Wilcoxon Rank-Sum test. Histopathologic data was analyzed with the Cochran-Armitage test for linear trend followed by a pairwise chi-square test comparing the treated group with the control with the Yate's continuity correction.. Differences in mortality were tested by the Gehan-Wilcoxon procedure. Results were tabulated as significant at $p \leq 0.05$.

C. METHODS

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. Comprehensive clinical examinations and palpations of the animals were done at least once a week.

2. Body weight

Animals were weighed 5 days and 1 day prior to the initiation of treatment, then weekly for the first 13 weeks of treatment and at monthly intervals thereafter until study termination.

3. Food consumption and compound intake

Food consumption was not determined. Compound intake (mg/kg/ day) values were estimated from standard mouse breathing rates and the dose frequency.

4. Ophthalmoscopic examination

Examination of eyes with an ophthalmoscope is not required for carcinogenicity studies based on Subdivision F Guidelines.

5. Blood was collected for hematology and clinical analysis from the orbital sinus under methoxyflurane anesthesia. Animals were not fasted prior to blood sampling. Samples were taken from all surviving animals in the satellite study after 12 months of treatment and from 20 randomly selected animals in each group in the main study after 18 months of treatment. Blood samples were not taken from animals that died or were killed moribund during the treatment period. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count*
X	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

*Minimum required for carcinogenicity studies (only on Control and high dose groups unless effects are observed) based on Subdivision F Guidelines.

b. Clinical chemistry*

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)		
X	Serum aspartate amino-transferase (also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

*Not required for carcinogenicity studies based on Subdivision F Guidelines.

6. Urinalysis

Urinalysis is not required for carcinogenicity studies based on Subdivision F Guidelines and was not performed.

7. Sacrifice and pathology

All animals in the 12 and 18-month studies were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. Animals were killed under methoxyflurane anesthesia after blood samples were taken. The lungs, liver, kidneys, all gross lesions, and all tissues where effects were seen were examined from all animal groups; the other organs and tissues were examined from all animals in the control and high dose groups. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*
X	Colon*	XX	Kidneys*+	X	Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland*
XX	Liver*+	XX	Testes*+	X	Parathyroids*
X	Gall bladder*	X	Epididymides	X	Thyroids*
X	Pancreas*	X	Prostate	X	Auditory sebaceous glands
	RESPIRATORY	X	Seminal vesicle		
X	Trachea*	X	Ovaries*+		OTHER
XX	Lung*	X	Oviducts	X	Bone*
X	Nose	X	Uterus*	X	Skeletal muscle*
	Pharynx	X	Vagina	X	Skin*
X	Larynx			X	All gross lesions and masses*

* Required for carcinogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

No treatment-related clinical observations were recorded. The most common observation in males was swelling, reddening and abscess formation of the preputial gland. The most commonly seen clinical observation in females was opaque eyes. This observation was made in aging mice (> 1 year) of both sexes. The overall incidences of opaque eyes in females were: 21, 0, 59, and 46 for the control, 5, 20, and 80 ppm groups , respectively. The incidences of opaque eyes in males were: 9, 19, 31, and 2 for the control, 5, 20, and 80 ppm groups, respectively.

2. Mortality

The mortality seen during various time periods during the 18-month study is given in Table 2. Sulfuryl fluoride treatment affected mortality only at 80 ppm. Males showed increased mortality within the first year of treatment at 80 ppm, but were not affected as much as females later in the study. The increased mortality of females became statistically significant ($p \leq 0.05$) after 400 days of treatment at 80 ppm, but was unaffected during the first year of treatment.

TABLE 2: Cumulative mortality at various intervals in the 18-month study.

Time Period (Days)	Cumulative Mortality (No. mice dead/% of mice remaining in the group[a])							
	Control		5 ppm		20 ppm		80 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female
0-140	1/2%	2/4%	1/2%	0/0%	2/4%	0/0%	1/2%	0/0%
0-280	2/4%	5/10%	4/8%	4/8%	3/6%	3/6%	6/12%	2/4%
0-364	4/8%	6/12%	4/8%	5/10%	4/8%	4/8%	8/16%	5/10%
365-555	19/41%	12/27%	16/35%	7/16%	21/46%	16/35%	24/57%	31/69%
0-555	23/46%	18/36%	20/40%	12/24%	25/50%	20/40%	32/64%	36/72%*

Data extracted from Table 6, pp. 66-69 and Table 7, pp. 70-73, MRID 43354903.
[a]The percentages of dead mice were calculated by the reviewer as follows: dead mice/no. mice alive at the beginning of the time period X 100. The no. of live mice at day-0 was 50 in all groups.
*p ≤ 0.05 Significantly different from controls.

B. BODY WEIGHT

Group mean body weight gains at various intervals in the study are given in Table 3. The mean terminal body weights measured at necropsy are given in Table 5. Males exposed to 80 ppm sulfuryl fluoride had about 30% less weight gain than the control group by the end 12 months of treatment and 37% less by 18 months. Females at 80 ppm gained about 17% less than controls after 12 months of treatment and about 35% less at 18 months.

Although the mean body weight of male mice in the 80 ppm group was comparable with the control group when the study was designed (day -5), it became significantly ($p \leq 0.05$) less than the controls by day -1 (~3%). The difference in mean body weights remained significant ($p \leq 0.05$) throughout the study and increased to about 14%. No treatment-related decreases in body weights were seen in the remaining treated groups of male mice compared to the control group. The mean body weights of females in the control and 80 ppm groups were the same at the beginning of the study. The body weights of the 80 ppm group became significantly ($p \leq 0.05$) less than the control weights at most weighing points from day 27 until the end of the study where the mean body weights of the 80 ppm group were about 15% less than the control weights. The mean body weights of the low- and mid-dose female groups were significantly different from the control sporadically during the study, but the differences were not dose-related and the body weights in these groups were comparable to the control group by the end of the study. The mean terminal body weight at necropsy (Table 5) of males was significantly ($p \leq 0.05$) decreased by about 19%, and the mean terminal weight of females was decreased by about 14% at 80 ppm.

TABLE 3: Mean body weight gains at various intervals in the 18-month study.

Time Period (Days)	Group mean body weight gain (Grams)							
	Control		5 ppm		20 ppm		80 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female
-1-34	6.8	5.6	6.9	5.4	7.0	4.9	5.8	4.9
34-62	3.0	2.2	2.7	1.7	2.9	2.5	2.4	2.4
62-90	-0.2	0.3	0.3	0.3	0.2	0.3	-0.4	0.1
90-369	4.5	5.1	4.9	4.6	3.9	4.9	2.1	3.5
369-551	-0.9	0.4	-0.5	0.9	-0.8	0.9	-1.6	-2.0
-1-369	14.1	13.2	14.8	12.0	14.0	12.6	9.9	10.9
-1-551	13.2	13.6	14.3	12.9	13.2	13.5	8.3	8.9

Data calculated from Table 16, pp. 150-152 and Table 17, pp. 153-155, MRID 43354903.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

The time-weighted average food consumption levels were not measured.

2. Compound consumption

The time-weighted average compound consumption for each study group estimated from standard breathing rates for 0.03 kg mice and adjusted for the dose frequency is given in Table 1.

3. Food efficiency

The food efficiency was not calculated.

D. OPHTHALMOSCOPIC EXAMINATION

An ophthalmoscopic examination was not performed or required by Subdivision F guidelines.

E. BLOOD WORK

1. Hematology

Changes observed in hematology parameters are shown in Table 4. In the 12-month study, blood samples were evaluated from 10 mice/sex/group except for 9 males in the 5 ppm group, 7 males in the 20 ppm group, and 9 females in the control and 80 ppm groups. Differential counts were done on 9 males in the 5 ppm group, 8 males in the 20 ppm group, 9 females in the 5 ppm, and 8 females in the 20 ppm group. Differential counts were done on 10 animals in all other groups in the 12-month study. Blood samples from 20 preselected mice/sex were evaluated in the control, 5, and 20

ppm groups in the main 18-month study; blood samples from 18 males and 14 females at 80 ppm were evaluated. There were no significant differences or trends in the differential blood counts or morphological variations observed between the high dose and control groups either in the 12-month satellite animals or at the termination of the 18-month study. Although not statistically significant due to high standard deviations, segmented neutrophils were elevated in high dose males after 18 months of treatment (80 ppm, 51% ± 15; control, 38% ± 11). Platelet counts were significantly ($p \leq 0.05$) elevated (~20% greater than controls) at 80 ppm in males and at all treatment levels (32-49%) in females at 12 months, but were comparable to control counts after 18 months of treatment. Erythrocyte counts were significantly ($p \leq 0.05$) increased (~14%) in males at 80 ppm after 18 months of treatment, and slightly (~5%), but not significantly, at 20 ppm compared to the control group.

TABLE 4: Hematology parameters that changed as a possible result of treatment with sulfuryl fluoride

Parameter	Numerical values for hematology parameters							
	Control		5 ppm		20 ppm		80 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female
Platelets 12 mo. ($10^3/mm^3$)	1383	968	1393	1280*	1541	1356*	1660*	1446*
Platelets 18 mo. ($10^3/mm^3$)	1120	971	1099	951	1043	894	1180	964
RBCs 12 mo. ($10^6/mm^3$)	8.86	9.37	9.09	9.70	8.87	9.38	9.59	10.26
RBCs 18 mo. ($10^6/mm^3$)	7.70	8.94	7.63	8.21	8.07	8.83	8.74*	9.04
Hematocrit 18 mo. (%)	40.8	48.4	40.5	43.9	42.1	47.1	44.0	47.1
Segmented Neutrophil 12 mo. (%)	32	29	35	26	26	28	38	32
Segmented Neutrophil 18 mo. (%)	38	34	40	40	43	31	51	38

Data extracted from Table 18, pp. 156-157; Table 19, pp. 158-159; Table 20, pp. 160-161; and Table 21, pp. 162-163, MRID 43354903.
*$p \leq 0.05$ Significantly different from controls.

2. Clinical chemistry

Clinical chemistry parameters were measured in 9 and 8 samples from male mice in the 5 and 20 ppm groups, respectively, and from 10 males in the control and high dose groups in the 12-month study. Samples from 9 females in the control and high dose groups and from 10 females in the 5 and 20 ppm groups were also analyzed. No trends or significant differences compared to control values were seen for either sex in the 12-month study.

In the 18-month study, clinical chemistry parameters were analyzed in samples from either 19 or 20 mice/sex in the control, 5, and 20 ppm dose groups and from either 17 or 18 males and 13 or 14 females in the 80 ppm group. Blood glucose was significantly ($p \leq 0.05$) decreased (~25%) in males at 80 ppm, and albumin was significantly ($p \leq 0.05$) decreased (~15%) in males at 5 ppm. Blood glucose was also slightly, but not significantly, decreased (~11%) in females at 80 ppm compared to control values. These parameters were not affected at other doses.

F. URINALYSIS

Urinalysis is not required and was not performed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

The mean relative and absolute organ weights and the terminal body weights after 18 months of treatment with sulfuryl fluoride are given in Table 5. The absolute weights of brain, heart, kidneys, and liver were significantly ($p \leq 0.05$) decreased at 80 ppm sulfuryl fluoride in males and the brain, kidney, and liver weights were significantly decreased in females. At 80 ppm, the relative brain and lung weights were significantly ($p \leq 0.05$) increased in both sexes, and testes relative weight was significantly increased in males.

TABLE 5. Mean absolute and relative organ weights at termination of the 18-month study.

Organ	Mean absolute/relative organ weights in grams (No. mice)							
	Control		4 ppm		20 ppm		80 ppm	
	Male (27)	Female (32)	Male (30)	Female (38)	Male (25)	Female (30)	Male (18)	Female (14)
Brain	0.521/ 1.297	0.523/ 1.549	0.515/ 1.301	0.519/ 1.578	0.520/ 1.319	0.516/ 1.552	0.494*/ 1.515*	0.494*/ 1.698*
Heart	0.208/ 0.517	0.157/ 0.461	0.213/ 0.532	0.163/ 0.493	0.206/ 0.520	0.173/ 0.515*	0.181*/ 0.551	0.151/ 0.517
Kidneys	0.797/ 1.978	0.499/ 1.470	0.768/ 1.919	0.500/ 1.510	0.783/ 1.976	0.498/ 1.485	0.645*/ 1.973	0.406*/ 1.393
Liver	2.452/ 6.084	1.943/ 5.677	2.365/ 5.953	1.964/ 5.898	2.177/ 5.457	1.912/ 5.658	1.718*/ 5.185	1.612*/ 5.523
Lungs	0.245/ 0.609	0.230/ 0.675	0.253/ 0.638	0.244/ 0.740	0.243/ 0.613	0.233/ 0.700	0.245/ 0.749*	0.260/ 0.915*
Testes	0.214/ 0.532	---	0.204/ 0.511	---	0.206/ 0.524	---	0.210/ 0.643*	---
Terminal Whole Body	40.3	34.1	40.2	33.1	39.7	33.8	32.8*	29.2*

Data extracted from Table 31, p. 196 and Table 32, p. 197, MRID 43354903.
*p ≤ 0.05 Significantly different from controls.

2. Gross pathology

Gross lesions seen in the 18-month study that were different in treated groups compared to the control group are shown in Table 6. Significantly increased incidences of hemolyzed blood in the digestive system were noted in both sexes exposed to 80 ppm sulfuryl fluoride. An increased incidence of perineal soiling was seen in females, but not in males, at 80 ppm. More animals also had decreased fat at 80 ppm than the control and other treated groups, which could be expected from the decreased body weights at that dose. A few negative associations were seen such as an apparent dose-related decrease in the incidence of bilateral dilated kidney pelvis in males, decreased incidence of bilateral ovarian cysts and uterine endometrial cysts in females at 80 ppm, and decreased incidence of nodules or masses in the livers of males at 80 ppm.

TABLE 6: Number of animals with gross lesions in the 18-month study with sulfuryl fluoride

Finding or Lesion	Lesion incidence/50 animals							
	Control		5 ppm		20 ppm		80 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female
Hemolyzed blood in digestive system	1	0	2	3	4	1	15***	10***
Decreased fat	6	7	5	4	3	6	19**	10
Perineal soiling	5	0	7	2	6	1	4	11***
Liver mass/nodule	8	0	3	2	5	2	2*	0
Dilated kidney pelvis bilateral	10	1	8	0	4	0	0***	0
Ovarian cyst bilateral	---	10	---	5	---	8	---	2*
Uterus endometrial cyst	---	31	---	34	---	31	---	9***

Data extracted from Table 33, pp. 198-217, MRID 43354903.
*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 Significantly different from controls. Fisher exact test performed by reviewer.

3. Microscopic pathology

a) Non-neoplastic - Selected microscopic findings in the 18-month study that may be treatment-related are shown in Table 7. Significant ($p \leq 0.001$) increases in the incidences of vacuolation in the brains of both sexes were seen at 80 ppm. Vacuolation in the cerebrum was also seen in both sexes after 12 months of treatment at 80 ppm sulfuryl fluoride (incidence: controls 0/10, both sexes; 80 ppm males, 10/10, females, 9/10). The severity of this lesion was determined to be very slight in all cases and it did not progress with the longer (18-month) exposures. No increases in the incidences of cerebrum vacuolation were seen at the other doses. Significant decreases ($p \leq 0.001$) in mineralization in the thalamus and hypothalamus were seen in females at 20 and 80 ppm and in males at 80 ppm. Amyloidosis was seen in numerous tissues in the aging animals, and was increased ($p \leq 0.05$) in the kidney glomerulus of both sexes at 80 ppm; however, the severity was greater in females. The incidence of thyroid epithelial hypertrophy was significantly ($p \leq 0.001$) increased in high dose males and slightly increased ($p \leq 0.05$) in high dose females. Increased incidences of thyroid epithelial hypertrophy were also seen at 12 months in both sexes (incidences: controls 0, both sexes; 80 ppm males, 7/10, females, 4/10). No increases in thyroid

epithelial hypertrophy were seen at the other doses at either time interval and the lesions did not appear to progress from the 12-month to the 18-month exposure periods. The incidences of heart thrombus and chronic lung congestion were significantly ($p \leq 0.01$) increased in high dose females in the 18-month study compared to the controls, but not in males and were not increased in either sex in the 12-month study. The incidence of suppurative inflammation of the nasal lumen tissues was slightly increased in both sexes, significantly in males ($p \leq 0.05$), but was not statistically significant in females. The nasal inflammation was also more severe in males. Slight increases ($p \leq 0.05$) in the incidences of thymus atrophy in males and spleen atrophy in females were seen at 80 ppm. The incidence of uterine cystic endometrial hyperplasia was significantly ($p \leq 0.05$) decreased in high dose females compared to controls at 18 months and slightly decreased at 12 months. The decreased incidence in the 18-month study may be due to the increased early deaths of the females at 80 ppm.

TABLE 7: Number of animals with microscopic lesions in the 18-month study with sulfuryl fluoride

Organ/Lesion	Lesion incidence/50 animals							
	Control		5 ppm		20 ppm		80 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female
Brain, cerebrum/ Vacuolation	0	0	0	0	0	0	13***	12***
Brain, thalamus & hypothalamus/ Mineralization	39	30	34	23	33	10***	11**	2***
Kidney, glomerulus/ amyloidosis, slight	4	8	3	7	5	6	12*	5
Kidney, glomerulus/ amyloidosis, ≥ severe	22	20	25	23	24	28	14	32*
Thymus/Atrophy	1	7	0	4	1	8	6*	12
Thyroid/ Epithelial hypertrophy	1	1	0	1	1	1	20***	6^{+}
Heart/Thrombus	2	4	3	1	5	5	2	14**
Nasalcavity/ Suppurative inflammation	0	1	0	0	1	0	5*	4
Lungs/Chronic congestion	1	6	2	2	4	4	1	19**
Spleen/Atrophy	3	4	1	4	2	6	8	12*
Uterus/ Endometrial cystic hyperplasia	---	37	---	36	---	37	---	19***

Data extracted from Table 34, pp. 218-264, Table 36, pp. 272-279, and Table 37, pp. 280-287, MRID 43354903.
*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 Significantly different from controls; Yate's chi-square pairwise test performed by the study authors.
$^{+}$p ≤ 0.05 Significantly different from controls. Yate's chi-square pairwise test performed by the reviewer.

b) Neoplastic - There were no treatment-related increases in tumors in the study. The numbers of animals with neoplastic lesions in the liver, lung, or vascular tissue were negatively associated with treatment in some cases. Significant ($p \leq 0.05$) negative linear trends for lung adenoma and vascular hemangioma/hemangiosarcoma were seen for males (lung adenoma incidence: controls, 12/50; 80 ppm, 2/50; vascular tumor incidence: controls, 7/50; 80 ppm, 1/50; from Table 35, pp. 265-272, MRID No. 43354903).

III. DISCUSSION

A. DISCUSSION

Sulfuryl fluoride was given to groups of 50 male and 50 female CD-1 mice by inhalation at concentration levels of 0, 5, 20, or 80 ppm 6 hours/day, 5 days/week for 18 months. Satellite groups of 10 mice per sex per group were treated with the same

concentrations for 12 months. These concentrations amounted to estimated dose levels of 0, 4.96, 19.84, and 79.35 mg/kg/day for 0.03 kg mice[1].

There were no treatment-related effects found during the clinical examinations of the animal. Treatment at 80 ppm resulted in increased mortality in both sexes. Males were affected more than females in the first year of the study, but the male deaths did not reach statistical significance. Most of the females died during the last 6 months of the treatment period. A total of 72% of the females ($p \leq 0.05$) and 64% of males died at 80 ppm in the study compared to 36% of females and 46% of males in the control groups.

The mean group body weight gain in the main 18-month study was decreased in both sexes at 80 ppm compared to the control groups (males, 37%; females, 35%), but not at the other dose levels. Body weight gain was also decreased in both sexes at 80 ppm in the 12-month satellite study. The mean body weight of the 80 ppm male group was significantly ($p \leq 0.05$) less than that of the control group throughout the experiment and the difference increased from 3% on the day before treatment began to about 14% less than the control at 18 months. There was no explanation for the lack of weight gain in the high dose male group prior to the start of treatment.

No significant changes were seen in differential blood counts either at 12 or 18 months between treated and control animals. Although not statistically significant due to high standard deviations, segmented neutrophils were elevated in males at 80 ppm compared to control values (80 ppm, 51% ± 15; control, 38% ± 11). Erythrocyte counts from high dose males were significantly ($p \leq 0.05$) higher than the control counts in the 18 month study. The platelet counts after 12 months of treatment with sulfuryl fluoride were significantly higher in both sexes than the control counts at 80 ppm and at 5 and 20 ppm in females. However, the platelet count in the female control group was slightly below the normal range for CD-1 mice, and by 18 months, the platelet counts in the treated groups were comparable to the control values. No changes were seen in the bone marrow and the increases seen in hematology parameters were within or near the normal range of values reported for CD-1 mice[2]. No treatment-related changes were seen in clinical chemistry parameters in the 12-month study. Blood glucose was slightly, but not significantly, decreased (~11%) in females and significantly ($p \leq 0.05$) decreased (~25%) in males at 80 ppm compared to controls after 18 months of treatment.

The mean absolute weights of brain, kidney, and liver were significantly ($p \leq 0.05$) decreased in both sexes at 80 ppm; and the mean heart weight was also decreased in males at 80 ppm. The mean relative weights of brain and lungs were increased in both sexes and the relative testes weight was increased in males at 80 ppm. The increased relative weights were largely a result of the significantly ($p \leq 0.05$) decreased terminal

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[2]Charles River Laboratories. 1986. Baseline hematology and clinical chemistry values as a function of sex and age for Crl:CD-1 outbred mice. CRL Technical Bulletin, Summer 1986.

body weights of both sexes (19 and 14% for males and females, respectively).

Increased incidences of hemolyzed blood in the digestive system and decreased amount of abdominal fat were seen in both sexes at 80 ppm on gross necropsy. The incidences of liver masses or nodules and dilated kidney pelvis in males, and bilateral ovarian cysts and uterine endometrial cysts and cystic hyperplasia seen in females were negatively associated with treatment at the high dose. The earlier deaths of animals at the high dose partially account for these observations. Significant ($p \leq 0.001$) increases in the instances of cerebral vacuolation in the brain were seen in both sexes at 80 ppm (13/50 and 12/50 for males and females, respectively; 0/50 for both control groups). Significantly increased incidences of thyroid epithelial hypertrophy were seen in both sexes, but females were affected greater than males (females: 80 ppm, 20/50; control, 1/50; $p \leq 0.001$; Males: 80 ppm, 6/50; control, 1/50; $p \leq 0.05$). A comparison of the brain and thyroid lesions after 12 and 18 months of treatment indicated that the lesions did not progress with further exposure to sulfuryl fluoride. Increased incidence of atrophy of the thymus at 80 ppm was also seen in males (80 ppm, 6/50; control, 1/50; $p \leq 0.05$). The slight, but significant ($p \leq 0.05$) increase in nasal inflammation seen in males was found to be related to the presence of aspirated food particles and not a direct effect of exposure to sulfuryl fluoride. Females had significantly increased incidences of heart thrombus (80 ppm, 14/50; control, 4/50; $p \leq 0.01$), chronic lung congestion (80 ppm, 19/50; control, 6/50; $p \leq 0.01$), and atrophy of the spleen at 80 ppm (80 ppm, 12/50; control, 4/50, $p \leq 0.05$). The lung congestion seen in females was coincident with impaired cardiac circulation, which was related to renal failure and systemic amyloidosis. Amyloidosis, which is often seen in aging CD-1 mice, occurred in many tissues in all animals and was a major cause of death in the study. Although the overall incidence of amyloidosis did not significantly change with treatment, kidney glomerular amyloidosis in females was more severe in high dose animals. The combined incidence of severe and very severe kidney glomerular amyloidosis was significantly ($p \leq 0.05$) increased in females at 80 ppm (80 ppm, 32/50; control, 20/50). Since the severity of amyloidosis increases with age, the earlier deaths of more males than females could partially account for the difference in severity between the sexes at 80 ppm.

Specific target tissues identified for sulfuryl fluoride included the brain (cerebral vacuolation) and the thyroid (epithelial hypertrophy). The decreased weight gain accompanied by decreased fat content and lower blood sugar seen in high dose animals is consistent with a decrease in food intake; however, information on food consumption was not available. Systemic amyloidosis affecting multiple organ systems was the most common cause of death in all dose groups including the increased mortality seen in the high dose groups.

There were no treatment-related increases in neoplastic lesions in the study. However, there were lower incidences of liver adenoma, lung adenoma and adenocarcinoma, and vascular neoplasms in males at 80 ppm. The decreases in lung adenoma and vascular tumors were statistically significant ($p \leq 0.05$) after

adjusting for early deaths in the high dose group. Females had fewer lung carcinomas and vascular tumors as well at 80 ppm, but the decreased incidences were not statistically significant.

A No-Observed-Effect-Level (NOEL) of 20 ppm (19.84 mg/kg/day) in both sexes was identified. The Lowest-Observed-Effect-Level (LOEL) was 80 ppm (79.35 mg/kg/day) for both sexes based on decreased body weight gain, decreased survival (especially in females), cerebral vacuolation in the brain, and thyroid epithelial hypertrophy (especially in males). Females also had increased heart thrombus and chronic lung congestion, which could have been a secondary result of systemic amyloidosis.

B. STUDY DEFICIENCIES

The statistical significance of the decrease in mean body weights of high dose males compared to the control group is misleading since they were significantly less from the start of treatment. The weight gain, which would have minimized the initial differences, was not calculated by the study authors. The cage or individual food consumption and water intake was not measured. Some entries in the summary tables for gross and microscopic necropsy findings were confusing and required checking in the individual animal data. An example of this is the listing for "perineal soiling," which was also listed under "soiling, perineal" with different numbers for the same group and sex.

These deficiencies were minor and do not detract significantly from the value of the oncogenicity study. The weight gain was calculated by the reviewer. The food consumption was not necessary for calculating dose in an inhalation study, although it might have been helpful in assessing the non-neoplastic toxicological effects of treatment.

2/2/98

012468

DATA EVALUATION REPORT

SULFURYL FLUORIDE

STUDY TYPE: COMBINED CHRONIC/ONCOGENICITY INHALATION - RAT (83-5)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Life Sciences Division
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Task Order No. 96-12C

Primary Reviewer:
K.A. Davidson, Ph.D, D.A.B.T. Signature: K.A. Davidson
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Susan Chang, M.S. Signature: Susan Chang
Date: 2-28-97

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under Contract No.DE-AC05-96OR11464

SULFURYL FLUORIDE Chronic/Oncogenicity Inhalation Study (83-5)

EPA Reviewer: W. Greear, M.P.H., D.A.B.T. W. Greear, Date 6/5/97
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, Marion Copley, Date 7/2/97
D.V.M., D.A.B.T.
Toxicology Branch I

DATA EVALUATION RECORD

STUDY TYPE: Combined Chronic/Oncogenicity Inhalation - Rat OPPTS 870.4300 [§83-5])

DP BAR CODE: D210874 SUBMISSION CODE: S479737
P.C. CODE: 078003 TOX. CHEM. NO.: 816A

TEST MATERIAL (PURITY): Sulfuryl fluoride (93.6 - 99.7% a.i.)

SYNONYMS: VIKANE™

CITATION: Quast, J., G. Bradley, K. Nitschke (1993) Sulfuryl fluoride: 2-year inhalation chronic toxicity/oncogenicity study in Fischer 344 rats. The Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Company, Midland, MI. Laboratory project study ID K-016399-040, August 18, 1993. MRID 43354902. Unpublished.

SPONSOR: DowElanco, 9002 Purdue Road, Quad IV, Indianapolis, IN 46268-1189

EXECUTIVE SUMMARY: In a combined chronic/oncogenicity study (MRID 43354902), groups of 50 male and 50 female Fisher 344 rats were subjected to whole-body inhalation exposure to sulfuryl fluoride (93.6 - 99.7% a.i.; Lot No. WP 880329-752, WP 901011-907, WP 910321-918, WP 910826-929, WP 920131-940) at concentrations of 0, 5, 20, or 80 ppm for 6 hours/day, 5 days/week for 2 years (main study). Fifteen animals of each sex per group were similarly exposed to sulfuryl fluoride for 12 months (satellite study) for interim evaluation of toxicity. The satellite study identified the kidneys, lungs, and teeth as targets of sulfuryl fluoride.

All main study male and female rats exposed to 80 ppm of sulfuryl fluoride died before termination at 2 years; the death in more than 90% of each sex was due to very severe chronic glomerulonephropathy (advanced chronic renal disease), which resulted in renal failure. Other effects related to renal failure or directly to exposure to 80-ppm of the test material included decreased body weight gain, decreased specific gravity of urine, serum chemistry changes indicative of renal failure (elevated urea nitrogen, creatinine, triglycerides, and cholesterol, and phosphorus and depressed levels of total protein, albumin, and chloride). Pathologic lesions in 80-ppm

group rats related to renal failure included parathyroid hyperplasia, osteodystrophy, splenic and lymph node atrophy, gastric erosion, cardiac thrombosis, lung congestion, and mineralization in a variety of tissues. Pathologic lesions considered to be related directly to exposure to 80 ppm of sulfuryl fluoride in male and female rats included adrenal cortical hemorrhage, hepatocellular atrophy, reactive hyperplasia and inflammation of the respiratory epithelium of the nasal turbinates, vacuolation of the cerebrum and thalamus/ hypothalamus, adrenal cortical hemorrhage, aggregates of alveolar macrophages, and dental fluorosis. The only treatment-related effects occurring at 20 ppm was a significantly increased incidence of dental fluorosis in males (10/50 vs 0/50 in controls, $p<0.01$). There was no increase in histopathologic lesions in females exposed to 20 ppm of sulfuryl fluoride. No exposure-related toxic effects occurred in male or female rats exposed to 5 ppm of sulfuryl fluoride.

The LOELs are 20 ppm for male rats based on dental fluorosis and 80 ppm for female rats based on primary and secondary renal toxicity; effects in the adrenal cortex, brain, eyes, liver, nasal tissue, respiratory tract; and dental fluorosis. The corresponding NOELs are 5 ppm for male rats and 20 ppm for female rats.

At the concentrations tested, there were no increases in exposure-related tumor incidences when compared with control incidences. Dosing was considered adequate based on the survival of adequate numbers of animals in the 20-ppm groups (76% in males and 66% in females) at study termination despite the fact all 80-ppm group rats died before termination of the study.

This combined chronic/oncogenicity study in the rat is classified as **Acceptable** (§83-5) and satisfies the Subdivision F guideline requirement for a combined chronic/oncogenicity study in rats (§83-5).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Sulfuryl fluoride

   Description: colorless gas
   Lot/Batch #:WP 880329-752, WP 901011-907, WP 910321-918, WP 910826-929, WP 920131-940
   Purity: 93.6 - 99.7% a.i.
   Stability of compound: not reported

CAS #: 2699-79-8

2. Vehicle and/or positive control

No vehicle required; a positive control was not included.

3. Test animals

Species: rat
Strain: Fisher 344
Age and weight at study initiation: 6-7 weeks of age; 95.8±7.6 g (males), 83.9±3.4 g (females)
Source: Charles River Breeding Laboratories, Inc., Kingston, NY
Housing: 2 per cage in exposure chambers continuously throughout study
Diet:Purina Certified Rodent Chow #5002, ad libitum, except the 6-hour/day exposure period
Water: tap water, ad libitum
Environmental conditions:
Temperature: 22.7 - 23.4°C
Humidity: 47.9 - 51.6%
Air changes: not reported; air flow = 2858 - 2913 L/min
Photoperiod: 12 hours light/12 hours dark
Acclimation period: one week

B. STUDY DESIGN

1. In life dates

Start: July 17, 1990; end: July 22, 1992

2. Animal assignment

Animals were assigned randomly (based on body weights) to the test groups in Table 1; they were subjected to whole-body exposure for 6 hours/day, 5 days/week for 2 years or until sacrifice due to moribundity or until natural death.

TABLE 1. Study design

Test Group	Target concentration (ppm)	Analytical concentration[a] (ppm)	Main study 24 months	Satellite study 12 months
			rats/sex	rats/sex
Control	0	0.0	50	15
Low (LDT)	5	5.1±0.6	50	15
Mid (MDT)	20	20.2±1.5	50	15
High (HDT)	80	79.6±3.4	50	15

Data taken from Table 1, pp. 77, Table 4, pp. 81, MRID 43354902.
[a]Time-weighted average (TWA) concentration

3. Dose selection

Exposure concentrations were selected based on previous toxicity studies described in the study report (page 12, MRID 43354902). A 14-day acute toxicity study in Fischer 344 rats exposed to 0, 100, 300, or 600 ppm of sulfuryl fluoride 6 hour/day, 5 days/week for 2 weeks showed renal papillary necrosis and respiratory effects at 600 ppm and minimal renal effects at 300 ppm. A 13-week subchronic study in rats exposed to 0, 30, 100, or 300 ppm for 6 hours/day, 5 days/week showed effects on body weight, teeth, respiratory tract, and brain (vacuolation of the caudate putamen in the region of the cerebrum) at 300 ppm. Effects on the teeth were seen at 100 ppm and no effects were observed at 30 ppm. Therefore, concentrations for the 2-year study were selected such that dental, renal, and respiratory effects were expected at the highest concentration (80 ppm) and no effects were expected at the lowest concentration (5 ppm).

4. Generation of the test atmosphere and description of the chamber

Sulfuryl fluoride was supplied in cylinders during the course of the study as needed. The test atmospheres were generated using the glass J-tube method, in which sulfuryl fluoride was metered from the cylinder into a J-tube connected to each chamber. Compressed air was passed through the same J-tube to dilute the test

material to the desired concentration for each chamber. Six chambers were connected to a single cylinder.

Each group of rats was exposed to the test material under dynamic flow conditions in chambers measuring 2.4 m wide x 2.4 m high x 2.4 m deep with a pyramidal top (14.5 m^3). Air flow was monitored at 60-min intervals and was maintained at about 2900 L/min. Chamber temperature was measured at 60-min intervals using a thermometer or resistance temperature device; relative humidity was also monitored at 60-min intervals using relative humidity gauges or humidity sensors.

5. Test atmosphere concentration and analysis

The concentration of sulfuryl fluoride was measured at 30-min intervals by infrared spectrophotometry at a wavelength of 11.8 μm. The distribution of sulfuryl fluoride was measured in the breathing zones of the rats at eight sampling points plus a reference point. Nominal concentrations were determined daily based on the amount of test material used divided by the total chamber airflows.

Concentration analysis - The time-weighted average analytical concentrations of sulfuryl fluoride ranged from 0.0 to 0.0, 3.1 to 10.0, 13.6 to 26.3, and 64.5 to 87.6 ppm for the 0-, 5-, 20-, and 80-ppm exposure chambers.

Distribution analysis - The sample line concentrations of sulfuryl fluoride was 96 -102% (5 ppm), 100 - 102% (20 ppm), and 99 = 101% (80 ppm) relative to the reference line average.

Stability analysis - The purity of the sulfuryl fluoride in each cylinder was determined before and after using the cylinder. The purity did not vary by more than 6.4%, indicating stability during the time of delivery to the chambers.

6. Statistics

Body weights, organ weights, urinalysis, clinical chemistry, and hematology data were analyzed by Bartlett's test for equality of variances (α = 0.01), followed by a parametric analysis of variance (ANOVA) (α = 0.10) or nonparametric ANOVA (α = 0.10) depending on the outcome of Bartlett's test. Significant differences between test groups and the corresponding control were determined by Dunnett's (α = 0.05, two-

sided) or Wilcoxon Rank-Sum test (α = 0.05, two-sided) with a Bonferroni correction. Mortality data were analyzed by the Gehan-Wilcoxon procedure. The incidences of gross pathologic lesions were not analyzed statistically. The cumulative incidences of histopathologic lesions were first tested for deviation of linearity using ordinal spacing of doses followed by the Cochran-Armitage Trend test (α = 0.02, two-sided) if linearity was not rejected. If the trend was significant or if linearitiy was found, then exposure groups were compared with controls using chi-square test with Yates continuity correction (α = 0.05, one-sided). Tumor incidences were adjusted for mortality using the Peto method.

C. METHODS

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. A detailed clinical examination was conducted prior to study initiation and once weekly thereafter; particular attention was focused on signs of central nervous system toxicity. The rats were palpated once weekly for evidence of masses.

2. Body weight

Animals were weighed prior to study initiation, once weekly for the first 13 weeks, and at monthly intervals thereafter.

3. Food consumption was not measured in this inhalation study.

4. Ophthalmoscopic examination

The eyes of each rat were examined before initiation of exposure and at necropsy.

5. Blood was collected from the orbital sinus for hematology and clinical analysis. Blood samples were collected from all surviving satellite animals at 6 and 12 months, 10 rats of each sex per group at 19 and 21 months, and 20 randomly preselected rats per sex from the surviving groups at study termination. Blood smears were prepared for differential leukocyte counts and erythrocyte, leukocyte, and platelet morphology. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements	X	Erythrocyte morphology
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for combined chronic/oncogenicity studies based on Subdivision F Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin*
		X	Total serum protein (TP)*
X	ENZYMES	X	Triglycerides
	Alkaline phosphatase (ALK)		Serum protein electrophorese
X	Cholinesterase (ChE)		
	Creatine phosphokinase*		
X	Lactic acid dehydrogenase		
X	(LDH)		
	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for combined chronic/oncogenicity studies based on Subdivision F Guidelines.

6. Urinalysis

Urine was collected from ten nonfasted animals 1 to 2 weeks before blood sampling at 6, 12, 19 and 21 months and from the first 20 surviving rats in each group at study termination. A second urine sample was collected from the same animals 2 weeks after the first 6 months sample to verify results of first 6-month samples. Urine was collected by spontaneous micturition or manual compression of the urinary bladder. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

*Required for combined chronic/oncogenicity studies based on Subdivision F Guidelines.

7. Sacrifice and pathology

All animals that died and those sacrificed due to moribundity or on schedule were subjected to gross pathological examination, except for five satellite rats that were whole-body perfused for neuropathological screening. The CHECKED (X) tissues were collected for microscopic examination. All tissues collected from control and the 80-ppm satellite animals were examined microscopically; liver, kidneys, lungs, nasal tissues, oral tissues, trachea, brain, and all gross lesions were examined in the lower exposure groups. In addition, Periodic acid-Schiff (PAS) stain was used for visualization of granular cell tumors in the meninges of satellite animals. All tissues collected from all rats assigned to the main study were examined microscopically. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary	XX	Heart*	X	Periph. nerve*
X	glands*	X	Bone marrow*	X	Spinal cord (3
X	Esophagus*	X	Lymph nodes*	X	levels)*
X	Stomach*	X	Spleen*	X	Pituitary*
X	Duodenum*	X	Thymus*		Eyes (optic n.)*
X	Jejunum*				
X	Ileum*		UROGENITAL		GLANDULAR
X	Cecum*	XX	Kidneys*+	XX	Adrenal gland*+
X	Colon*	X	Urinary	X	Lacrimal gland
X	Rectum*	XX	bladder*	X	Mammary gland*
X	Liver*+	X	Testes*+	X	Parathyroids*
X	Pancreas*	X	Epididymides*	X	Thyroids*
X	Oral tissues	X	Prostate*	X	Harderian Gland
		X	Seminal		
	RESPIRATORY	XX	vesicle*		OTHER
X	Trachea*	X	Coagulating	X	Bone*
X	Lung*	X	glands	X	Skeletal muscle*
X	Nose	X	Ovaries*	X	Skin*
X	Pharynx	X	Uterus*	X	All gross lesions
X	Larynx		Oviducts		and masses*
			Cervix		
			Vagina		

*Required for combined chronic/oncogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in combined chronic/oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

No exposure-related clinical signs of toxicity were observed in either male or female rats from any group.

2. Mortality

One male rat from the control satellite group died on day 198 of the study due to causes unrelated to treatment. Mortality data for male and female rats in the main study are presented in Table 2 and Figures 1 and 2. Exposure-related mortality was observed in both male and female rats. The mortality rate markedly increased after week 75 in male rats exposed

to 80 ppm of sulfuryl fluoride; all males in this group were dead by the end of week 101. Females in the high-exposure group showed a similar pattern of death, with mortality increasing markedly after week 85; all females were dead by the beginning of week 101. Renal disease was the primary cause of death in both sexes, affecting 47 males and 46 females. Nine female controls and four 5-ppm group females had impacted esophagi that presumably caused the death of these animals. The mortality rates in male rats exposed to 5 and 20 ppm were similar to that of controls. Significantly fewer deaths occurred in females exposed to 5 and 20 ppm of sulfuryl fluoride than in the control group.

TABLE 2. Cumulative mortality in male and female rats exposed by inhalation to Sulfuryl Fluoride for 2 years

Week of Study	Concentration (ppm)							
	0	5	20	80	0	5	20	80
	Males				Females			
No. Animals[a] per group	50	50	50	50	50	50	50	50
52	1 (2%)[b]	1 (2%)	0 (0%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)
75	2 (4%)	1 (2%)	2 (4%)	5 (10%)	5 (10%)	4 (8%)	1 (2%)	1 (2%)
85	5 (10%)	8 (16%)	4 (8%)	26 (52%)	9 (18%)	4 (8%)	3 (6%)	14 (28%)
90	9 (18%)	9 (18%)	7 (14%)	43 (86%)	14 (28%)	6 (12%)	6 (12%)	41 (82%)
101	18 (36%)	17 (34%)	14 (28%)	50 (100%)	23 (46%)	10 (20%)	11 (22%)	50 (100%)
105	21 (42%)	18 (36%)	22 (44%)	50* (100%)	25 (50%)	13* (26%)	12* (24%)	50* (100%)

Data taken from Tables 5 and 6, pp. 82-89, MRID 43354902.
[a]Number of animals at study initiation.
[b]The numbers in parentheses are the percentages of mortality.
*$p<0.05$ compared with controls; Gehan-Wilcoxon procedure.

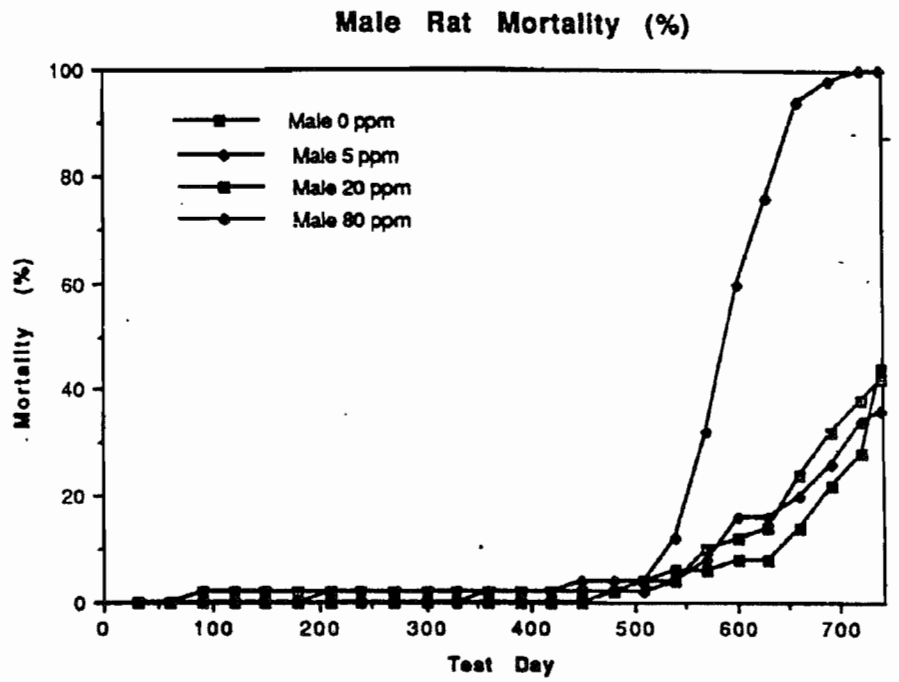

Figure 1. Taken from page 28, MRID 43354902.

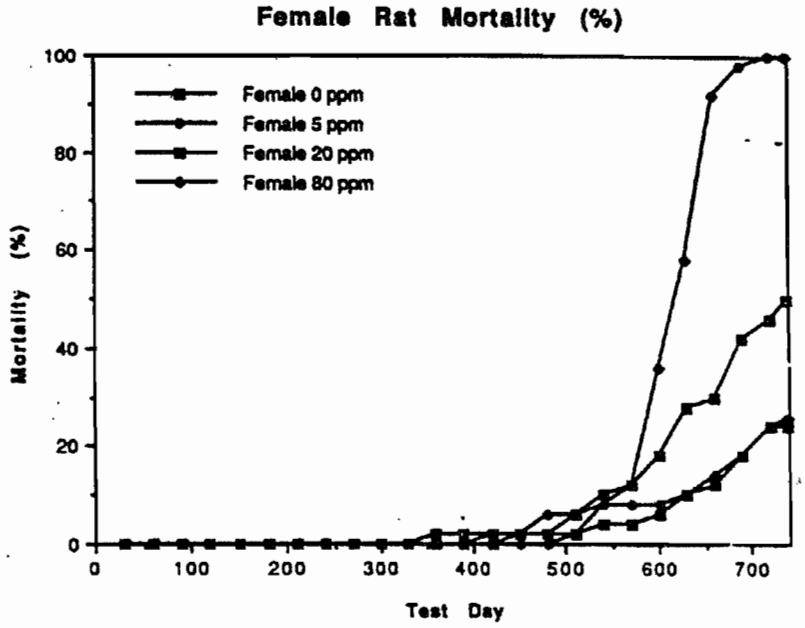

Figure 2. Taken from page 29, MRID 43354902.

B. BODY WEIGHT

Group mean body weights and body weight gain are summarized in Table 3. Body weight data are also presented graphically in Figures 3 and 4. Inadequate numbers of 80-ppm group animals were alive to calculate weight gain after week 89. No exposure-related effects on body weights were observed during the first year of the study as all three exposure groups showed only sporadic statistically significant deceases when compared with control body weights. Body weight gain was also similar in exposed and control groups during the first year of the study. During the second year, however, mean body weights of both sexes exposed to 80 ppm of sulfuryl fluoride showed consistent and severe decreases compared with controls, particularly after week 73 (day 509). By week 89 (day 621), mean body weights of males and females were about 80% of the corresponding control body weight. Weight loss occurred in all groups during the second year, but was more pronounced in the 80-ppm groups as reflected by a net weight loss, which was quite severe in males.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Food consumption was not measured in this study.

2. Compound consumption

This is an inhalation study; therefore, compound consumption is not relevant.

3. Food efficiency

Food efficiency could not be calculated as food consumption was not measured

D. OPHTHALMOSCOPIC EXAMINATION

No ophthalmic abnormalities were observed.

TABLE 3. Selected group mean body weights and body weight gain in male and female rats exposed to sulfuryl fluoride by inhalation for 2 years

	Concentration (ppm)							
Week	0	5	20	80	0	5	20	80
	Males				Females			
Mean body weight (g)								
-1 day (Pretest)	98.1	97.7 (100)	95.8 (98)	96.1 (98)	84.4	84.9 (101)	84.7 (100)	83.9 (99)
8	272.9	267.4 (98)	268.0 (98)	268.8 (98)	165.5	164.8 (100)	161.9* (98)	156.8* (95)
13	314.6	307.9 (98)	307.3* (98)	307.4* (98)	180.3	180.3 (100)	177.6 (99)	171.7* (95)
25	359.7	361.5 (101)	359.9 (100)	358.4 (100)	200.2	199.7 (100)	198.7 (99)	192.8* (96)
53	414.2	414.2 (100)	416.7 (101)	407.0 (98)	222.8	220.0 (99)	220.8 (99)	216.5* (97)
65	432.1	435.5 (101)	434.3 (101)	414.4* (96)	237.0	231.8 (98)	232.3 (98)	225.3* (95)
77	432.4	429.1 (99)	429.4 (99)	384.9* (89)	254.4	245.9 (97)	245.3* (96)	219.9* (86)
89	435.9	430.4 (99)	427.1 (98)	347.5* (80)	266.2	263.2 (99)	262.6 (99)	214.4* (81)
105	401.7	388.7 (97)	378.7 (94)	0	255.9	265.4 (104)	258.4 (101)	0
Body weight gain (g)[b]								
P-53	316.1	316.5 (100)	320.9 (102)	310.9 (98)	138.4	135.1 (98)	136.1 (98)	132.6 (96)
53-89	21.7	16.2 (75)	10.4 (48)	-59.5 (-274)	43.4	43.2 (100)	41.8 (96)	-2.1 (-4.8)
P-89	337.8	332.7 (98)	331.3 (98)	251.4 (74)	181.8	178.3 (98)	177.9 (98)	130.5 (72)

Data taken from Tables 13 and 14, pp. 225-230, MRID 43354902.
[a]Numbers in parentheses are percents of control calculated by the reviewer.
[b]Body weight gain calculated by the reviewer; P - 53 = pretest to week 53.
*$p<0.05$ compared with controls

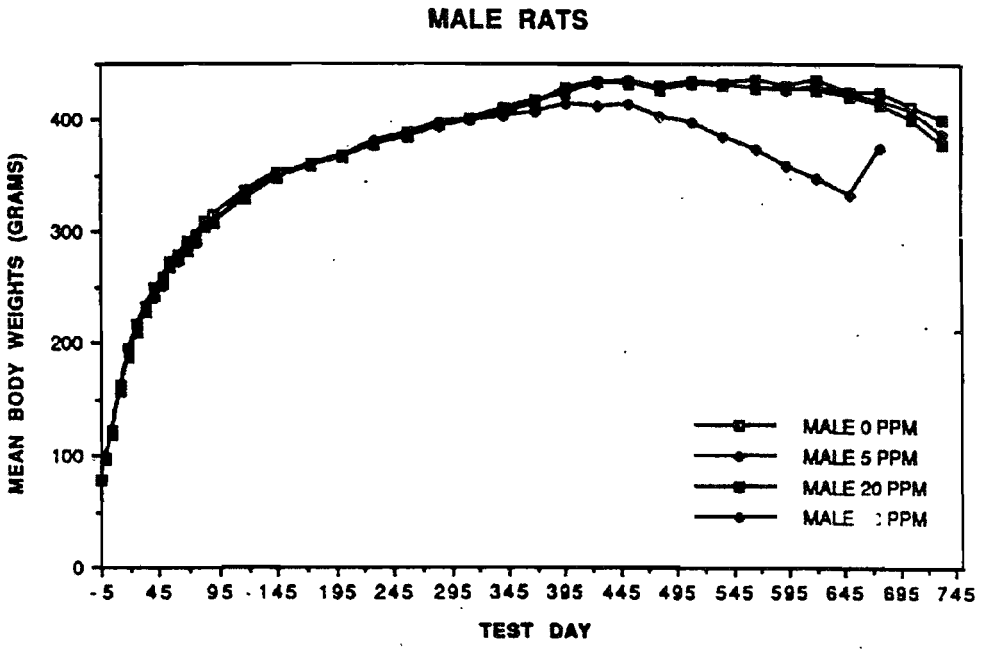

Figure 3. Taken from page 32, MRID 43354902.

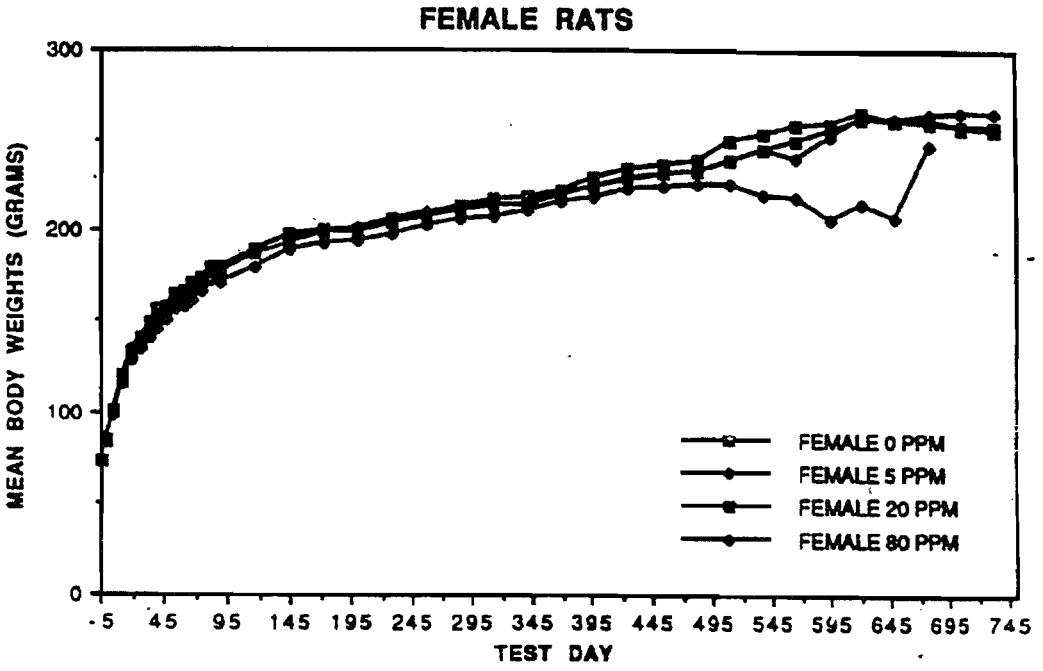

Figure 4. Taken from page 33, MRID 43354902.

E. BLOOD WORK

1. Hematology

No treatment-related effects on hematologic parameters were observed. Statistically significant changes were noted, but the values did not show a consistent increase or decrease with duration of exposure, show exposure-related patterns, or indicate biological significance.

2. Clinical chemistry

Selected serum chemistry values are summarized in Tables 4 (male rats) and 5 (female rats). Exposure-related changes in serum chemistry values were not observed in male or female groups exposed to 5 or 20 ppm sulfuryl fluoride, but changes were observed in both sexes exposed to 80 ppm of sulfuryl fluoride. Some changes were evident after 12 months of exposure, whereas other did not become evident until 19 months.

Serum levels of the following parameters were significantly ($p<0.05$) elevated in male rats after exposure to 80 ppm of sulfuryl fluoride for 12, 19 and 21 months of exposure: urea nitrogen (131-488% of control values), cholesterol (134-244%), triglycerides (174-346%), and creatinine (117-400%); phosphorus levels were significantly elevated at 19 and 21 months (168 and 207%, respectively). Total serum protein (91-96% of control values) and albumin (74-90%) were significantly decreased at 12, 19, and 21 months. Serum chloride levels were slightly, but significantly decreased (95% of control) at 19 and 21 months. Serum creatine kinase levels showed concentration-related decreases in male rats throughout the study, except at 12 months; statistically significant decreases were observed at 19 and 21 months for 80-ppm (33 and 52%) and 20-ppm group males (44 and 58%).

A similar serum chemistry pattern was seen in female rats exposed to sulfuryl fluoride. Urea nitrogen levels were significantly elevated (113-555% of control values) in the 80-ppm group at 12, 19, and 21 months; creatinine (211 and 371%) and phosphorus (174 and 231%) levels were significantly elevated at 19 and 21 months. Total protein (86 and 85%), albumin (74 and 72%), and chloride (95 and 94%) levels were significantly decreased at 19 and 21 months. Creatine kinase levels did not show a consistent change relative to control rats; the level was significantly

decreased in all groups at 19 months, increased in the 80-ppm group at 21 months (219%, not significant), and decreased in the 5- and 20-ppm groups at 24 months. Triglyceride levels were elevated (215 and 199%, respectively) in females exposed to 80-ppm of sulfuryl fluoride for 19 and 21 months; statistical significance was not achieved because of the very high level in one control animal with advanced chronic renal disease and other pathologic lesions. If the value of the outlier is omitted from the analysis, the mean triglyceride levels in controls would be 139 ±50 mg/dL at 19 months and 69±9 mg/dL at 21 months compared with 428±186 and 267±229 mg/dL ($p<0.05$ for both), respectively, for 80-ppm group females. All other changes noted in male and female rats did not show consistent increases or decreases with exposure duration or concentration.

TABLE 4. Serum chemistry values in male rats exposed to sulfuryl fluoride by inhalation for 2 years

Group	U. nitrogen (mg/dL)	T. protein (g/dL)	Albumin (g/dL)	Chol. (mg/dL)	Trig. (mg/dL)	C. kinase (mU/mL)	Creat. (mg/dL)	Phos. (mg/dL)	Chloride (mmol/L)
6 months									
control	17±2	9.0±0.2	4.2±0.1	67±6	65±16	121±59	0.7±0.1	7.4±0.2	118±3
5 ppm	16±1	8.8±0.2	4.1±0.1	67±7	61±16	67±24*	0.7±0.1	7.6±0.3	120±2
20 ppm	18±2	8.9±0.3	4.2±0.1	70±8	63±7	79±34	0.8±0.0*	7.3±0.4	118±2
80 ppm	18±2	8.9±0.2	4.1±0.1	65±6	63±9	73±29*	0.8±0.1	7.2±0.5	118±3
12 months									
control	13±2	7.1±0.4	3.1±0.1	76±13	57±9	131±47	0.6±0.1	6.6±0.7	107±3
5 ppm	14±2	7.1±0.2	3.1±0.1	78±8	72±10*	168±51	0.6±0.1	6.5±0.5	107±2
20 ppm	14±1	7.1±0.3	3.1±0.1	75±13	52±10	152±80	0.7±0.1	6.5±0.5	107±2
80 ppm	17±2*	6.8±0.2*	2.8±0.1*	102±16*	99±38*	88±29	0.7±0.1*	7.0±0.7	105±2
19 months									
control	18±2	8.0±0.3	3.5±0.2	139±32	131±30	143±40	0.8±0.1	5.7±0.3	114±3
5 ppm	20±2	7.4±1.7	3.3±0.2	125±22	164±79	91±25*	0.8±0.1	5.8±0.4	114±4
20 ppm	20±3	7.9±0.3	3.4±0.2	152±49	157±64	63±17*	0.8±0.1	5.9±0.2	115±3
80 ppm	62±52*	7.4±0.4*	2.6±0.2*	319±67*	396±130*	47±26*	2.4±1.6*	9.6±3.8*	108±4*

TABLE 4. Continued

Group	U. nitrogen (mg/dL)	T. protein (g/dL)	Albumin (g/dL)	Chol. (mg/dL)	Trig. (mg/dL)	C. kinase (mU/mL)	Creat. (mg/dL)	Phos. (mg/dL)	Chloride (mmol/L)
21 months									
control	16±1	7.8±0.2	3.4±0.1	143±21	125±23	101±33	0.7±0.0	5.7±0.2	117±5
5 ppm	18±4	7.9±0.6	3.4±0.4	153±27	148±76	75±46	0.7±0.1	5.6±0.4	118±3
20 ppm	20±6	7.7±0.4	3.1±0.6	159±52	198±138	59±21*	0.8±0.2	5.9±0.4	117±3
80 ppm	78±37*	7.1±0.3*	2.6±0.1*	349±57*	432±128*	53±24*	2.8±1.5*	11.8±4.0*	111±5*
24 months[a]									
control	22±3	7.8±0.4	3.1±0.6	206±52	169±64	204±103	0.8±0.1	6.3±0.5	113±3
5 ppm	23±7	7.7±0.5	3.2±0.3	221±94	192±126	187±100	0.8±0.1	6.6±0.6	114±3
20 ppm	26±12	7.5±0.6	3.1±0.3	218±61	224±141	130±121	0.9±0.3	6.3±1.5	114±2

Data taken from Tables 37-41, pp 275-284, MRID 43354902.
[a]No 80-ppm group males survived to study termination.
* $p \leq 0.05$, compared with controls, Dunnett's or Wilcoxon's tests.
U. Nitrogen = urea nitrogen; T. protein = total protein; Chol. = cholesterol; Trig. = triglycerides; C. kinase = creatine kinase; Creat. = creatinine; Phos. = phosphorus

TABLE 5. Serum chemistry values in female rats exposed to sulfuryl fluoride by inhalation for 2 years

Group	U. nitrogen (mg/dL)	T. protein (g/dL)	Albumin (g/dL)	Chol. (mg/dL)	Trig. (mg/dL)	C. kinase (mU/mL)	Creat. (mg/dL)	Phos. (mg/dL)	Chloride (mmol/L)
6 months									
control	21±2	8.5±0.5	4.3±0.2	101±17	42±4	158±77	0.7±0.1	6.2±0.5	118±2
5 ppm	20±3	8.7±0.4	4.2±0.2	102±13	46±4	114±29	0.8±0.1	6.6±0.5	120±2
20 ppm	24±3*	8.7±0.4	4.3±0.2	99±8	44±6	122±35	0.7±0.0	6.9±0.6*	119±2
80 ppm	20±2	8.9±0.4	4.3±0.2	105±11	46±5	109±33	0.7±0.0	6.5±0.5	119±2
12 months									
control	15±2	7.4±0.5	3.6±0.2	107±15	47±13	124±47	0.6±0.0	6.3±0.4	108±2
5 ppm	14±2	7.4±0.4	3.5±0.1	99±11	48±17	141±56	0.6±0.0	6.1±0.4	107±1
20 ppm	14±2	7.4±0.5	3.6±0.2	105±11	44±8	111±56	0.6±0.0	6.1±0.6	107±1
80 ppm	17±1*	7.6±0.3	3.6±0.1	114±16	46±11	98±57	0.6±0.0	6.3±0.4	107±2
19 months									
control	24±3	9.1±0.8	3.9±0.5	202±103	199±196	161±56	0.9±0.1	5.3±0.5	115±4
5 ppm	23±3	8.9±0.5	4.1±0.1	165±28	117±30	102±35*	0.8±0.0	4.9±0.5	116±6
20 ppm	29±3*	8.4±0.3	3.9±0.2	151±17	127±32	77±17*	0.8±0.1	5.6±0.4	116±4
80 ppm	69±58*	7.8±0.9*	2.9±0.3*	362±78*	428±186	117±191*	1.9±1.2*	9.2±4.0*	109±1*

TABLE 5. Continued

Group	U. nitrogen (mg/dL)	T. protein (g/dL)	Albumin (g/dL)	Chol. (mg/dL)	Trig. (mg/dL)	C. kinase (mU/mL)	Creat. (mg/dL)	Phos. (mg/dL)	Chloride (mmol/L)
21 months									
control	20±4	7.9±0.5	3.6±0.5	193±119	134±212	68±22	0.7±0.1	5.2±0.4	109±2
5 ppm	20±3	8.1±0.5	3.8±0.2	156±41	80±26	61±20	0.7±0.1	5.0±0.4	109±2
20 ppm	23±3	8.0±0.4	3.8±0.2	148±13	74±17	54±35	0.7±0.0	5.1±0.5	110±2
80 ppm	111±70*	6.7±0.8*	2.6±0.1	310±90*	267±229	149±168	2.6±1.1*	12.0±4.4*	102±5*
24 months[a]									
control	22±5	8.2±0.7	3.7±0.4	185±51	117±57	198±133	0.7±0.1	5.9±0.9	109±2
5 ppm	20±3	8.4±0.4	3.9±0.2*	168±44	132±47	133±33*	0.6±0.1*	5.6±0.7	110±1
20 ppm	21±2	8.0±0.5	3.8±0.2	166±28	110±39	101±44*	0.6±0.1*	5.8±0.6	110±2

Data taken from Tables 42-46, pp 285-294, MRID 43354902.
[a]No 80-ppm group females survived to study termination.
* $p \leq 0.05$, compared with controls, Dunnett's or Wilcoxon's tests.
U. Nitrogen = urea nitrogen; T. protein = total protein; Chol. = cholesterol; Trig. = triglycerides; C. kinase = creatine kinase; Creat. = creatinine; Phos. = phosphorus

F. URINALYSIS

Data for urine specific gravity are summarized in Table 6. The sporadic and inconsistent increases or decreases in specific gravity of urine observed during the first year of the study in the 20- and 80-ppm groups and the increase in the 20-ppm male group at 24 months are not considered to be related to exposure to sulfuryl fluoride. The statistically significant ($p<0.05$) decreases in specific gravity in the 80-ppm male and female groups at 19 and 21 months are probably related to advanced chronic renal disease. Urine protein levels in urine samples were higher at 6 months in rats exposed to 80-ppm of sulfuryl fluoride, but not at later time points. There were occasional samples containing erythrocytes that may have been due to renal disease and not directly to exposure to sulfuryl fluoride.

TABLE 6. Urine specific gravity in male and female rats exposed by inhalation to sulfuryl fluoride for 2 years

Concentration (ppm)	Duration of exposure (months)					
	6	6[a] (repeat)	12	19	21	24
Males						
0	1.059	1.055	1.054	1.047	1.044	1.037
5	1.052	0.051	1.062	1.042	1.044	1.039
20	1.051	1.035*	1.050	1.042	1.050	1.044*
80	1.048	1.038*	0.051	1.027*	1.028*	-
Females						
0	1.022	1.043	1.041	1.044	1.046	1.042
5	1.029	1.038	1.036	1.045	1.046	1.040
20	1.052*	1.048	1.043	1.045	1.048	1.038
80	1.046*	1.041	1.057*	1.025*	1.025*	-

Data taken from Tables 25-36, pp. 251-274, MRID 43354902.
[a]The 6-month sampling was repeated 2 weeks after the first sampling.
*$p<0.05$ compared with controls, Dunnett's test.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

The relative weight of the kidneys and liver were slightly elevated (+7%, $p<0.05$ for both organs compared with controls) in male rats exposed to 80-ppm of sulfuryl fluoride for 12 months. The effect on kidney weight was probably exposure related; however, organ weights were not available for 24 months as no animals survived. At study termination, the absolute (76% of control) and relative weights (78%) of the testes were significantly ($p<0.05$) decreased in male rats exposed to 5 ppm; no significant effect was seen at 20 ppm and no 80-ppm group rats survived until study termination. There were no exposure-related effects on organ weight in female rats exposed to sulfuryl fluoride.

2. Gross pathology

Notable gross lesions in animals assigned to the satellite group are summarized in Table 7, and gross lesions in animals assigned to the main study are summarized in Tables 8 (males) and 9 (females). In satellite animals, exposure-related gross lesions were seen in the lungs of all ten 80-ppm group male and female rats examined; no lung lesions were seen in control, 5-ppm, or 20-ppm groups.

In the main study, the incidence of pale foci in the lungs of male rats exposed to 20 ppm of sulfuryl fluoride was increased; all other statistically significant increased incidences of gross findings (compared with controls) occurred only in rats exposed to 80 ppm. The incidences of gross findings in the aorta, kidney, lungs, oral tissue (teeth), stomach, and testes of animals in the 80-ppm groups were increased. Pale foci were seen in almost all male and female rats at the 80-ppm exposure level and in 16% of males at the 20-ppm level, but not in controls of either sex. The surface of the kidney was rough in 66% of male controls, but the incidence was increased significantly at the 80-ppm exposure level (90%). In female rats, the roughened surface of the kidneys was observed in only 8% of controls compared with 80% of females exposed to 80-ppm of sulfuryl fluoride. Mineralization of the aorta and the stomach occurred at the high exposure level in both sexes but was absent in control animals. In addition, the aorta was firm in males, and erosion or ulcers were seen in the

stomach mucosa of female rats. Sulfuryl fluoride also caused a noticeable mottling of the teeth in 24% of the males and 16% of the females exposed to 80 ppm of sulfuryl fluoride. The amount of body fat and the contents of the digestive tract were decreased in a large percentage of male and female rats in the 80-ppm groups compared with controls; gas was present in the digestive tract of a large percentage of the male rats.

TABLE 7. Gross and histopathologic lesions in male and female rats exposed to sulfuryl fluoride for 12 months - satellite animals				
Organ/Lesion	Concentration (ppm)			
	0	5	20	80
Males				
Number of animals examined	9/10[a]	10	10	10
Lungs Focus, pale, bilateral, multifocal[b] Alveolar macrophages, aggregates, multifocal[c]	 0 0	 0 0	 0 0	 10 10
Kidney Chronic progressive glomerulonephropathy[c]	9 (1.00)	10 (1.00)	10 (1.00)	10 (2.00)
Oral tissue Dental fluorosis, bilateral[c]	0	0	3	10
Females				
Number of animals examined	10	10	10	10
Lungs Focus, pale, bilateral, multifocal[b] Alveolar macrophages, aggregates, multifocal[c]	 0 0	 0 0	 0 0	 10* 10*
Kidney Chronic progressive glomerulonephropathy[c]	3 (1.00)	1 (1.00)	1 (1.00)	10 (1.10)
Oral tissue Dental fluorosis, bilateral[c]	0	0	0	9*

Data taken from Table 51, 52, and 53, pp. 301-315, MRID 43354902.
[a]9 animals examined grossly and 10 examined microscopically.
[b]Gross lesion
[c]Microscopic lesion; numbers in parentheses are the average severity grade: 1 = very slight, 2 = slight.
*$p \leq 0.05$; Fisher exact test for pairwise comparison of treated animals with controls; calculated by the reviewer.

TABLE 8. Gross lesions in male rats exposed to sulfuryl fluoride for 24 months

Organ/Lesion	Concentration (ppm)			
	0	5	20	80
Number of animals examined	50	50	50	50
General				
Fat, decreased amount	5	2	5	26**
Aorta				
Mineralization	0	0	1	18**
Firm	0	0	0	7**
Digestive tract				
Decreased Ingesta	2	3	2	21**
Gas	2	3	1	18**
Kidney				
Pale, bilateral	0	2	3	18**
Roughened surface, bilateral	33	28	31	45**
Lungs				
Focus, pale, multifocal	0	1	8**	46**
Oral tissue				
Mottled tooth	0	0	0	12**
Stomach				
Mineralization, nonglandular	0	1	0	7**
Mineralization, wall	0	0	0	9**
Testes				
Decreased size, unilat./bilateral	3	4	1	15**
Flaccid, unilateral/bilateral	0	0	0	7**

Data taken from Tables 54, pp. 316-339, MRID 43354902.
*p<0.05, **p<0.01 pairwise comparison with controls calculated by the reviewer using the Fisher exact test.

TABLE 9. Gross lesions in female rats exposed to sulfuryl fluoride for 24 months

Organ/Lesion	Concentration (ppm)			
	0	5	20	80
Number of animals examined	50	50	50	50
General Fat, decreased amount	5	1	1	37**
Aorta Mineralization	0	0	0	6*
Digestive tract Decreased Ingesta	0	0	1	11**
Kidney Pale, bilateral Roughened surface, bilateral	1 4	0 0	0 1	10** 40**
Lungs Focus, dark, multifocal Focus, pale, multifocal	2 0	3 0	1 1	14** 46**
Oral tissue Mottled tooth	0	0	0	8**
Stomach Mineralization, wall Erosion and/or ulcers, glandular mucosa, multifocal	0 0	0 1	0 2	7** 6*

Data taken from Tables 54, pp. 316-339, MRID 43354902.
*p<0.05, **p<0.01 pairwise comparison with controls calculated by the reviewer using the Fisher exact test.

3. Microscopic pathology

a) Non-neoplastic - Notable microscopic lesions are summarized in Table 7 for animals assigned to the satellite group and in Tables 10 (males) and 11 (females) for animals assigned to the main study. The incidences of a large number of lesions show statistically significant reductions at the high exposure level, probably due the early death of animals in this group. These lesions are not listed in the table and will not be discussed in the text of this DER.

At the 12-month evaluation, exposure-related effects were observed in the lungs, kidneys, and teeth. Chronic glomerulonephropathy was seen in all 10 males and 10 females ($p<0.01$) exposed to 80-ppm of sulfuryl fluoride compared with 9 males and 3 females in the control groups. The lesion was more severe in exposed males than in the controls. Aggregates of alveolar macrophages were observed in the lungs of all ten 80- ppm male and female rats and in none of the control or other exposure groups. Dental fluorosis of the upper incisors was observed in all males and 9 of 10 females exposed to 80 ppm, 3 males exposed to 20 ppm, and in none of the controls.

In the main study, microscopic findings directly or indirectly related to exposure to sulfuryl fluoride occurred in the adrenal gland, bone, eyes, heart, kidney, respiratory tract, oral tissue, parathyroid, stomach, and lymphoid organs. Although a clear concentration-response relationship was not evident for all lesions, the pronounced increases in incidences or severity in the high exposure group indicate that the effects were due to sulfuryl fluoride exposure. The low exposure level (5 ppm) caused no effects. The only exposure-related effect occurring at 20 ppm was fluorosis of the upper incisors in 20% ($p<0.01$) of male rats and in 4% (not significant) of female rats. Fluorosis was observed in all 50 male and female rats in the high-exposure groups. All other lesions showed statistically significant increases only in the 80-ppm groups.

The primary effect due to sulfuryl fluoride exposure was exacerbation of chronic progressive glomerulonephropathy (advanced chronic renal disease), which occurred in almost all animals in

the study and was the cause of death of almost all rats in the 80-ppm exposure group. Although chronic renal disease occurred in 100% of male controls and 96% of female controls, the lesion was significantly more severe in animals exposed to 80 ppm of sulfuryl fluoride than in the control or lower exposure groups. Renal lesions were rated very severe (involved >75% of each kidney) in 43/50 males ($p<0.01$) and 40/50 females ($p<0.01$) exposed to 80 ppm compared with only 1/50 male controls and 1/50 female controls. Mineralization, which is a secondary effect of advanced chronic renal disease, was clearly evident in the following organs and tissues of 80-ppm group animals: aorta, heart (muscle fibers and blood vessels), kidney, larynx, lungs (alveoli), mediastinal tissue, mesenteric tissue, mammary gland, stomach, tongue, and trachea. The incidences ranged from 25/50 for the aorta to 45/50 for the kidney in males and 7/50 for the aorta to 39/50 for the tongue in females. The incidences of tissue mineralization did not exceed 4% in the control, 5-ppm or 20-ppm groups. Fibrous osteodystrophy and hyperplasia of the parathyroid gland, which occurred at high incidences in both sexes, were considered to be secondary to renal disease.

The incidences of respiratory tract lesions were significantly increased in males and females exposed to the 80-ppm group compared with incidences in controls. Increases were noted for hyperplasia and inflammation of the respiratory epithelium of nasal tissue in both sexes, acute inflammation of the larynx in females, chronic active inflammation of the larynx in males, acute tracheal inflammation in males, chronic passive congestion in females, and aggregates of alveolar macrophages in both sexes.

The incidence of degenerative lesions in the heart was not increased in either male or female rats, but the severity increased in the high-exposure group. The lesion was moderate in 27 male ($p<0.05$) and 27 females ($p<0.01$) compared with 16 male controls and 2 female controls. Other lesions showing statistically significant increases at 80 ppm included atrophy of the mesenteric lymph nodes and spleen, which occurred in 74 and 44% , respectively, of the females and 64 and 58%, respectively, of the males. Erosion

and ulcers of the glandular stomach occurred in eight female rats compared with only one control. Adrenal cortical hemorrhage was observed in 30% of the male rats and 66% of the female rats in the 80-ppm group; only one female control and no male controls displayed this lesion. In addition, necrosis of adrenal cortical cells was observed in 44% of the 80-ppm group females compared with none of the controls. The incidence of corneal lesions of the eye was slightly increased in both male and female rats. Females showed evidence of inflammation and mineralization, whereas males showed evidence only of mineralization. The incidence of mineralized mammary gland ducts was increased in both male and female rats exposed to 80-ppm compared with the corresponding controls.

Additional lesions showing statistically significant increased incidences at the 80-ppm exposure level included hepatocellular atrophy in male and female rats and periportal vacuolation in a few male rats. Very slight vacuolation in two regions of the brain (cerebral cortex, thalamus, and hypothalamus) was observed in 44% of female rats, compared with 0 to 6% in the other groups of female rats and in all groups of male rats.

TABLE 10. Histopathologic lesions in male rats exposed to sulfuryl fluoride for 24 months

Organ/Lesion	Concentration (ppm)			
	0	5	20	80
No. of organs or tissues examined	50	50	50	50
Adrenal cortex Hemorrhage, M/D	0	0	0	15**
Bone Fibrous osteodystrophy[a]	0	2	1	44*
Eyes, cornea Mineralization, U/B	28* (1.00)[b]	29 (1.00)	36 (1.00)	39* (1.00)
Heart Degeneration, w/wo inflammation Atrial thrombus, acute or recent	48 (2.06) 1	48 (1.77) 2	50 (1.74) 0	49 (2.51) 11**
Kidney Chronic progressive glomerulonephropathy, bilateral Mineralization[a]	50 (2.54) 1	49 (2.43) 2	50 (2.44) 1	50 (4.72) 45**
Liver Hepatocellular atrophy[e] Periportal vacuolation, M/D	12 0	7 1	13 1	32** 5*
No. of organs or tissues examined	50	50	50	50
Nasal tissues Hyperplasia, reactive, respiratory epithelium Inflam. subacute/chronic, respiratory epithelium	3 (1.33) 12 (1.25)	2 (2.50) 7 (1.43)	2 (2.00) 8 (1.25)	31** (1.19) 33** (1.19)
Larynx Inflammation, chronic active	6*	6	7	18**[b]
Lungs Aggregates, alveolar macrophages, multifocal	5 (1.60)	1 (1.00)	2 (1.50)	49** (2.69)
Trachea Inflammation, acute	1	0	0	9**
Mammary gland Ducts, mineralization, F/M	1[b]	3	0[b]	11**[d]
Mesenteric tissue Atrophy, adipose tissue Periarteritis	4 4	5 7	8 6	45* 15**

TABLE 10. Continued

Organ/Lesion	Concentration (ppm)			
	0	5	20	80
No. of organs or tissues examined	50	50	50	50
Oral tissue Inflammation, acute, gingiva	1	3	3	7*
Teeth Fluorosis, upper incisors, bilateral	0	0	10** (1.00)	50** (1.76)
Parathyroid gland Hyperplasia[a]	2[b]	2	1	46**[c]
Lymph node, mesenteric Atrophy	2	2	2[c]	32**[d]
Spleen Atrophy	2	1	4	29**

Data taken from Tables 55 and 57, pp. 340-383 and 392-415, MRID 43354902.
[a]Lesions secondary to renal disease.
[b]Numbers in parentheses are the average severity grade: 1 = very slight, 2 = slight, 3 = moderate, 4 = severe, 5 = very severe.
[c]Only 49 animals examined in this group.
[d]Only 48 animals examined in this group.
[e]Lesion secondary to inanition.
*p≤0.05; **p≤0.01, pairwise comparison with controls by Yates's Chi-square test performed by study author or Fisher exact test performed by reviewer; Cochran-Armitage trend test under control column .
F/M = focal or multifocal; M/D = multifocal or diffuse; U/B = unilateral or bilateral

TABLE 11. Histopathologic lesions in female rats exposed to sulfuryl fluoride for 24 months

Organ/Lesion	Concentration (ppm)			
	0	5	20	80
No. organs or tissues examined	50	50	50	50
Adrenal cortex				
Hemorrhage, multifocal/diffuse	1	0	0	33**
Necrosis, individual cell, multifocal	0	0	0	22**
Bone				
Fibrous osteodystrophy[a]	1	0	0	45**
Brain, cerebral cortex				
Vacuolation, multifocal	1	3	3	22**
Brain, thalamus/hypothalamus vacuolation, multifocal	1	3	2	22*
Eyes, cornea				
Inflammation, chronic active, U/B	8 (1.50)[b]	6 (1.33)	4 (1.50)	17* (1.24)
Mineralization, U/B	28 (1.00)	24 (1.00)	28 (1.00)	38* (1.18)
Heart				
Degeneration, w/wo inflammation	49 (1.33)	46 (1.13)	40* (1.10)	48 (2.46)
Atrial thrombus, acute or recent	2	2	0	14**
Kidney				
Chronic progressive glomerulonephropathy, bilateral	48 (1.19)	47 (1.02)	50 (1.04)	50 (4.64)
Mineralization[a]	1	0	0	37**

TABLE 11. Continued

Organ/Lesion	Concentration (ppm)			
	0	5	20	80
No. organs or tissues examined	50	50	50	50
Liver Hepatocellular atrophy[e]	14[c]	10	5*	37**
Nasal tissues Hyperplasia, reactive, respiratory epithelium Inflam. subacute/chronic, respiratory epithelium	4 (1.5) 22 (1.05)	1 (1.00) 20 (1.00)	2 (1.00) 17 (1.00)	26** (1.08) 32** (1.09)
Larynx Inflammation, acute	3	0	1	18**
Lungs Chronic passive congestion Aggregates, alveolar macrophages, multifocal	0 2 (1.0)	0 0	0 3 (1.0)	5* (2.00) 48** (2.88)
Lymph node, mesenteric Atrophy	2[c]	0	0	37**[c]
Mammary gland Ducts, mineralization, F/M	5	0*	1	33**
Mesenteric tissue Atrophy, adipose tissue	8[c]	4	1	46**
Teeth Fluorosis,, upper incisors, bilateral	0	0	2 (1.00)	50** (1.92)

TABLE 11. Continued

Organ/Lesion	Concentration (ppm)			
	0	5	20	80
No. organs or tissues examined	50	50	50	50
Parathyroid gland Hyperplasia[a]	1	0	0[d]	43**[c]
Spleen Atrophy	6	4	1	22**
Stomach, glandular Erosion/ulcers, focal/multifocal	1	2	2	8*

Data taken from Tables 55 and 58, pp. 340-383 and 416-440, MRID 43354902.
[a]Lesions secondary to renal disease.
[b]Numbers in parentheses are the average severity grade: 1 = very slight, 2 = slight, 3 = moderate, 4 = severe, 5 = very severe.
[c]Only 49 animals examined in this group.
[d]Only 48 animals examined in this group.
[e]Lesion secondary to inanition.
*p≤0.05; **p≤0.01, pairwise comparison with controls by Yates' s Chi-square test performed by study author or Fisher exact test performed by reviewer; Cochran-Armitage trend test under control column .
F/M = focal or multifocal; M/D = multifocal or diffuse; U/B = unilateral or bilateral

b) Neoplastic - None of the groups exposed to sulfuryl fluoride showed statistically significant increase in the incidences of neoplastic lesions. The incidence of several neoplastic lesions, however, showed statistically significant decreases at the high exposure level. Female rats in the 80-ppm exposure group showed significant decreases in the incidences of mammary adenocarcinoma, adenoma and fibroadenoma combined (1/50 vs 9/50 in controls, $p<0.01$), pituitary pars distalis adenomas/carcinomas (7/50 vs 23/48 in controls, $p<0.01$), benign endometrial stromal polyps (12/50 vs 23/50 in controls, $p<0.05$). The incidence of large granular lymphocytic leukemia (any site) was significantly decreased in females exposed to 20 ppm (3/50 vs 10/50 in controls, $p<0.05$) but not to 80 ppm (4/50). In male rats exposed to 80 ppm, the incidences of pituitary pars distalis adenomas/carcinomas (4/50 vs 17/49 in controls, $p<0.01$) and large granular lymphocytic leukemia (any site) (1/50 vs 16/50 in controls, $p<0.01$) were significantly decreased.

III. DISCUSSION

A. DISCUSSION

Groups of male and female rats were exposed by inhalation to 0, 5, 20, or 80 ppm sulfuryl fluoride 6 hours/day, 5 days/week for up to 2 years. Gross and histopathological examination of animals killed at 12 months identified the kidney, lungs, and teeth as targets for sulfuryl fluoride exposure. Gross examination of animals assigned to the main study, showed lesions in the same tissues with corresponding lesions identified by histopathological examination. The primary effect of sulfuryl fluoride is exacerbation of chronic glomerulonephropathy (advanced chronic renal disease). This lesion was a common lesion affecting at least 47 animals in the control and exposed groups. Exacerbation of chronic renal disease was evident at 12 months. The lesion was more severe in 80-ppm male rats than in controls and the incidence was higher in female rats than in controls. In addition, the mean kidney weight was slightly elevated at 12 months (+7%) in 80-ppm males; the increased weight was probably related to the increased severity of renal lesions. Kidney weights were not measured in 80-ppm animals from the main study as none survived until termination. In main study animals, the kidney lesion progressed to "very severe" (advanced chronic renal disease) in rats exposed to 80

ppm of sulfuryl fluoride compared with "very slight" in most of the control and 5- and 20-ppm groups. No male or female rats exposed to 80 ppm survived to study termination, and almost all deaths (>90%) at this exposure level were due to advanced chronic renal disease. Further, almost all findings in the 80-ppm groups were due directly or indirectly to renal failure in animals with advanced chronic renal disease. In addition to advanced chronic renal disease, secondary effects resulting from renal failure occurred in a variety of organs and tissues, including the parathyroid (hyperplasia), bone (fibrous osteodystrophy), and the kidney, heart, eyes, tongue, stomach, alveoli of the lungs, ducts of the mammary gland, and blood vessels (mineralization).

During the second year of the study, severe decreases in body weight gain and pronounced body weight loss was seen in male and female rats exposed to 80-ppm of sulfuryl fluoride. The effect on body weight gain was attributed to inanition in rats with advanced chronic renal disease. The nutritional state could not be assessed as food consumption was not measured. The study authors also attributed hepatocellular atrophy, periportal fatty vacuolation, and decreased mesenteric fat to inanition. The individual animal data showed that renal disease was very slight and body weights were within 5% of the group mean for the five 20-ppm females with hepatocellular atrophy, suggesting that neither inanition nor renal failure was associated with the hepatic lesion in these animals. The increased incidences of lymph node and splenic atrophy in males and females, and gastric erosion in females were attributed to stress of renal failure.

Serum chemistry values, particularly in animals sampled during the second year of the study (months 19 and 21), were consistent with advanced chronic renal disease. Urea nitrogen and creatinine levels were markedly elevated in males and females; these parameters are measures of glomerular filtration and are indicators of substantially impaired renal function, i.e., advanced chronic renal disease. The other changes in clinical chemistry values considered to be indicators of renal disease and impaired renal function were decreased serum protein, increased serum cholesterol, triglycerides, phosphorus, and decreased serum chloride. Decreased total serum protein was due primarily to decreased levels of albumin. The statistical test performed by the study authors did not show a statistically significant increase in serum triglycerides in 80-ppm females because of high triglyceride levels in one

control rats with advanced chronic renal disease. However, if this rat is omitted from the analysis, the results show that triglycerides are also significantly elevated in female rats. Creatine kinase activity was decreased in male rats. There are no known disease states associated with decreased creatine kinase activity suggesting that the decreases are not toxicologically significant. The changes in creatine kinase activity in female rats showed no consistent pattern suggesting that the changes were not exposure related. Statistically significant changes in serum urea nitrogen, total protein, albumin, cholesterol, and triglycerides were also seen at 12 months in 80-ppm group male rats. These changes probably reflected the slight increase in severity of renal disease in the 80-ppm group males killed at 12 months compared with the control group. The specific gravity of urine was decreased in 80-ppm group rats during the second year; this effect is related to loss of functional capacity of the kidney. The occasional observation of erythrocytes in urine is probably related to renal damage.

Respiratory effects in animals exposed to sulfuryl fluoride were seen in the nasal tissue, larynx, trachea, and lungs. Effects in the lungs manifested grossly as pale foci and microscopically as aggregates of alveolar macrophages were observed in 80-ppm group male and female rats killed at 12 months. Almost all rats exposed to 80-ppm of sulfuryl fluoride in the main study had both the gross and microscopic lesions in the lungs; the microscopic lesion, however, was more severe in animals assigned to the main study than in those killed at 12 months showing progression of the lesion with continued exposure. There was a statistically significant increase in the incidence of pale foci in 20-ppm group male rats, but no corresponding increase in the incidence of aggregates of alveolar macrophage. Therefore, no biological significance can be associated with the pale foci in 20-ppm group males. The study authors suggested that aggregates of alveolar macrophages may have been due, in part, to secondary effects of renal disease. This lesion could have been caused also by irritant effects of sulfuryl fluoride. Likewise, the inflammatory reactions in the larynx and trachea may have been due to irritation, but mineralization in these organs may have contributed also. Another lung lesion, chronic passive congestion, in females exposed to 80 ppm of sulfuryl fluoride was considered a secondary effect of cardiac atrial thrombosis, which was attributed to renal failure. Other effects on the respiratory tract (excluding mineralization) included reactive hyperplasia and

84

inflammation of the respiratory epithelium of the nasal turbinates. The study authors noted that decalcification of the nasal tissue for microscopic examination prevented adequate evaluation of mineral deposits. They attributed the nasal lesions to renal failure, because this lesion was not observed at 12 months before the onset of advanced chronic renal disease. However, irritant effects of sulfuryl fluoride cannot be ruled out.

The upper incisors was identified as the third target of sulfuryl fluoride in animals killed at 12 months. Dental fluorosis was observed microscopically, but not grossly, in the upper incisors of all male and in 9/10 female rats killed at 12 months. Dental fluorosis was also observed microscopically in all 80-ppm group male and female rats and in a significantly increased number of the 20-ppm group males (20%) assigned to the main study. Fluorosis of the upper incisors probably developed because the teeth were continuously renewed throughout the life span of the animals. There was no associated damage seen as broken or fractured teeth. The study authors considered dental fluorosis as a biomarker rather than a toxic effect; it is likely due to the fluoride moiety of sulfuryl fluoride.

The incidence of vacuolation of the cerebrum and thalamus/hypothalamus was significantly increased in female rats exposed to 80-ppm of sulfuryl fluoride. The study authors noted that the lesion resulted from perivascular edema. They did not attribute this lesion to sulfuryl fluoride exposure, because it was not observed at 12 months, it was not found in the region identified as an affected area (caudate putamen in the region of the cerebrum) in a previous study, it was not found in both sexes, and it was seen in Sprague-Dawley rats in moribund condition or that died from renal disease. Hence the study authors concluded that vacuolation in the cerebrum and thalamus/hypothalamus was associated with advanced chronic renal disease. However, vacuolation was seen in the brain of one female control and three females in the 5-ppm group; all had "very slight" grade of chronic renal disease and not the "very severe" grade associated with secondary renal effects. Therefore, vacuolation of the brain in these rats is not obviously associated with advanced chronic renal disease or other lesions. The study authors also suggested that vacuolation may be associated with Fisher rat leukemia. However, the incidence of leukemia showed a significant concentration-related negative trend, but the incidence of vacuolation was significantly increased at the high exposure level. The data suggest that

vacuolation of the cerebrum and thalamus/hypothalamus should be considered a exposure-related effects of sulfuryl fluoride in female rats.

Chronic active inflammation of the cornea was attributed to decreased lacrimal secretion caused by general debility resulting from renal failure. However, irritant effects of sulfuryl fluoride cannot be ruled out.

The increased incidence of adrenal cortical hemorrhage in the 80-ppm male and female groups does not appear to be related to renal failure and should be considered a direct effect of exposure to sulfuryl fluoride. The significant increase in the incidence of small testes in the 80-ppm group males is probably related to the smaller size of Leydig cell tumors, because growth of the Leydig cell tumors size was interrupted as the animals died early.

In conclusion, the lowest-observed-effect levels (LOEL) for systemic effects due to inhalation of sulfuryl fluoride are 20 ppm for male rats based on fluorosis of the teeth and 80 ppm for female rats based on primary and secondary renal effects; effects in the adrenal cortex, brain, eyes, liver, nasal tissue, respiratory tract; and dental fluorosis. The corresponding no-observed-effect levels (NOEL) are 5 ppm for male rats and 20 ppm for female rats.

No statistically significant increases in the incidences of neoplastic lesions occurred in male or female rats exposed to sulfuryl fluoride. The incidences of mammary neoplasms, pituitary neoplasms, benign endometrial polyps, and large granular lymphocytic leukemia were significantly decreased in the male and/or female rats exposed to 80 ppm of sulfuryl fluoride due to the early deaths of all animals at this exposure level. There were no statistically significant increases in hyperplastic lesion suggestive of neoplasia had the animals survived until study termination.

B. STUDY DEFICIENCIES

There were no major deficiencies in this inhalation study. Urine volume was not determined, but this does not affect the outcome of the study.

Sulfuryl Fluoride

MRID 43354402

83-5 Chronic ~~Feeding~~ Inhalation/Oncogenicity in the Rat

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 50 rats/sex/group (3 test groups and control group).
3. ✓ Dosing duration is at least 24 months.
4. ✓ Number of survivors in any group does not fall below 50% at 18 months or 25% at 24 months.
5. ‡ ✓ Doses tested include an MTD or limit dose if nontoxic (1000 mg/kg).
6. • ✓ Doses tested include a NOEL.
7. • ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
8. ✓ Individual daily observations.
9. ✓ Individual body weights.
10. ✓ Individual or cage food consumption.
11. • ✓ Opthalmoscopic examination (at least pertest and at term) control and high dose.
12. ✓ Clinical pathology data for at least 10 rats/group consisting of 13, 14 & 15
13. ✓ Hematology at 6 month intervals consisting of at least;
    - ✓ Erythrocyte count
    - ✓ Hemoglobin
    - ✓ Hematocrit
    - ✓ Leucocyte count
    - • ✓ Differential count
    - ✓ Platelet count (or clotting measure)
14. ✓ Clinical chemistry at 6 month intervals consisting of at least;
    - ✓ Alkaline phosphatase
    - ✓ Aspartate aminotransferase
    - • ✓ Creatinine kinase
    - ✓ Lactic dehydrogenase
    - ✓ Glucose
    - ✓ Bilirubin
    - ✓ Cholesterol
    - • ✓ Creatinine
    - ✓ Total Protein
    - ✓ Albumin
    - ✓ Urea
    - ✓ Inorganic phosphate
    - ✓ Calcium
    - • ✓ Potassium
    - ✓ Sodium
    - • ✓ Chloride
15. ✓ Urinalysis at 6 month intervals consisting of at least;
    - ✓ Blood
    - ✓ Protein
    - ✓ Ketone bodies
    - ✓ Appearance
    - ✓ Glucose
    - ✓ Total bilirubin
    - • ✓ Urobilirubin
    - ✓ Sediment
    - ✓ Specific gravity (osmolality)
    - • ✓ Volume
16. ✓ Individual necropsy of all animals.
17. ✓ Histopathology of the following tissues performed on all nonrodents and rodents, all control and high dose animals, all animals that died or were killed on study, all gross lesions on all animals, target organs on all animals and lungs, liver and kidneys on all other animals.
    - ✓ aorta
    - ✓ jejunum
    - ✓ peripheral nerve

Criteria marked with a • are supplemental and may not be required for every study.

✓ eyes	✓ bone marrow	✓ kidneys†
✓ caecum	✓ liver†	✓ esophagus
✓ colon	✓ lung†	✓ ovaries†
✓ duodenum	✓ lymph nodes	✓ oviduct
✓ brain†	✓ stomach	✓ pancreas
✓ skin	✓ mammary gland	✓ rectum
✓ heart†	✓ spleen†	✓ spinal cord (3x)
✓ testes†	✓ musculature	✓ thyroid / parathyroids
✓ pituitary	✓ epididymis	✓ salivary glands
✓ ileum	✓ adrenals†	✓ thymus
✓ trachea	✓ uterus	✓ urinary bladder

† organs to be weighed.

‡ The position document entitled "Selection of a Maximum Tolerated Dose (MTD) in Oncogenicity Studies (EPA No. 540/09-88-003) stated EPA's criteria for determining if an oncongenicity study has been adequately performed in terms of doses tested. However OPP is also aware that older oncogenicity studies, upon initial review or re-review. may have been tested at doses lower than the predicted MTD. In the event that such testing appears to be at doses less than the predicted MTD, the Office of Pesticides Program has been reviewing and considering the entire weight of the evidence to determine if retesting is necessary. Certain factors which affect the agency's decision to retest include but are not limited to the following: demonstrated oncogenicity in another species, nearness to the apparent MTD, genotoxic effects, structure-activity factors, absolute value of the highest dose tested and metabolic considerations.

Criteria marked with a • are supplemental and may not be required for every study.