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

009479

MAY 4 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 062719-00004, Two Generation Reproduction and Unscheduled DNA Synthesis Studies with Sulfuryl Fluoride and misc. Data Reviews for The RED

Tox. Chem. No.: 816A
Project No.: 2-1201
Record No. : S410846

FROM: Melba S. Morrow, D.V.M. MSM 4/17/92
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Acting Section Head, Review Section II KB 4/24/92
Toxicology Branch I
Health Effects Division (H7509C)

Sponsor: DowElanco

CONCLUSIONS:

The two generation reproduction study and the mutagenicity study (unscheduled DNA synthesis) have been reviewed and Tox Branch has reached the following conclusions:

Two Generation Reproduction- Rats (83-4)

The administration of sulfuryl fluoride to male and female Sprague Dawley rats at concentrations of 0, 5, 20 and 150 ppm over two generations resulted in a parental NOEL of 5 ppm and a LOEL of 20 ppm based on gross observations of pale foci and histologic observations of aggregates of alveolar macrophages in the lung. Additionally, at 150 ppm, significant decreases in body weights were reported and there was an increased incidence of dental fluorosis and lung pathology characterized by increased numbers of alveolar macrophages. Chronic pulmonary inflammation was increased and vacuolation of the caudate putamen fiber tracts in the cerebrum was also observed at the high dose level, with females being affected more than males.

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The reproductive NOEL was 20 ppm and the LOEL was 150 ppm based on decreased pup weights that were probably secondary to maternal weight loss.

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The study was classified as core guideline.

Mutagenicity- Unscheduled DNA Synthesis (84-4)

Sulfuryl fluoride did not produce increased DNA synthesis in primary rat hepatocytes when tested up to cytotoxic levels. Doses tested in this assay were 0, 102, 204, 408, 612, 816 and 1020 ppm. At doses of 1530 ppm and higher cytotoxicity was too high to assay for unscheduled DNA synthesis. Cultures were exposed to sulfuryl fluoride for 18 to 19 hours.

The study was classified as acceptable.

Copies of the DERs are attached for your reference.

MISCELLANEOUS DATA

The following studies were reviewed for the Reregistration Eligibility Document (RED) (see attached DERs for details).

90-day rat inhalation study - MRID 4080909-02
Classified core-minimum

90-day rabbit inhalation study - MRID 4080909-01 4080909-01
Classified core-minimum

90-day neurotoxicity rat inhalation study - MRID 4080399-02
Classified core-supplementary

CASWELL FILE

Reviewed by: Melba S. Morrow, D.V.M. 4/10/92 009479
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D. 4/17/92
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 2 Generation Inhalation Reproduction - Rats

GUIDELINE #: 83-4

TOX. CHEM. #: 816A

MRID #: 421798-01

TEST MATERIAL: Sulfuryl Fluoride

SYNONYMS: Vikane

STUDY NUMBERS: K-016399-042

SPONSOR: DowElanco
Indianapolis, Indiana

TESTING FACILITY: Toxicology Research and Health and Environmental Sciences
Dow Chemical Company
Midland, Michigan

TITLE OF REPORT: Two Generation Inhalation Reproduction Inhalation Study in Sprague Dawley Rats

AUTHORS: Breslin, Liberacki, Kirk, Bradley and Crissman

REPORT ISSUED: January 7, 1992

CONCLUSIONS: Administration of sulfuryl fluoride to male and female Sprague Dawley rats at concentrations of 0, 5, 20 and 150 ppm for two generations resulted in a parental NOEL of 5 ppm and a LOEL of 20 ppm based on gross observations of pale foci and histologic observations of aggregates of alveolar macrophages in the lungs. Additionally, at 150 ppm, decreased body weights were reported and there was an increased incidence of dental fluorosis and lung pathology characterized by increased numbers of alveolar macrophages. Chronic pulmonary inflammation was also increased at the high dose level. Vacuolation of the caudate putamen fiber tracts in the cerebrum was also observed at the highest dose tested, with females being affected more than males.

The reproductive NOEL was 20 ppm and the LOEL was 150 ppm based on decreased pup weights that was probably secondary to maternal weight loss.

CLASSIFICATION: Guideline

MATERIALS:

Sulfuryl fluoride, with a purity of 99.8%, was the test material. The test compound was described as a colorless gas with a molecular weight of 102 daltons. Three lots of the test material were used in this study.

The test animals were male and female Sprague Dawley rats that were approximately 4 weeks of age at receipt. Thirty male and thirty female rats per group were used as the parental animals (F_0).

METHODS:

Following an acclimation period of approximately 2 weeks, the parental (F_0) rats were randomly assigned to the following treatment groups:

GROUP (ppm)	pre-mating exposure duration	mating, gestation and laction exposure duration
0	6h/d;5d/wk	6h/d; 7d/wk
5	"	"
20	"	"
150	"	"

The F_0 animals were exposed to the test material for 10 weeks prior to mating. Maternal animals were not exposed to sulfuryl fluoride from day 20 of gestation to day 4 post-partum. During lactation, pups were separated from their dams for the exposure periods (6 hours per day) on days 5 to 21.

Breeding Procedure:

For the F_0 and F_1 animals, breeding was accomplished by three seven-day cohabitation periods. Males and females from respective treatment groups were mated at a ratio of 1:1. Brother-sister matings were avoided for the F_1 animals. Successful mating was indicated by a sperm positive vaginal washing. Females which did not mate during the first seven-day cohabitation period were exposed to alternate males for a second and, if necessary, a third mating.

F_1 and F_2 litters were culled to a total of 8 pups (4M, 4F) on day 4 of lactation. Litters with less than 8 pups were not culled. All pups were weaned at 3 weeks of age. Thirty pups per sex per group were selected for the next generation (F_1). Those that were not selected were examined grossly then euthanized.

Animals selected from the F_1 offspring were exposed to sulfuryl fluoride for 6 hours per day for 5 days a week for the 12 weeks prior to mating. During mating, gestation and lactation, the animals were exposed for 6 hours a day for 7 days a week.

Observations:

Physical examinations were conducted daily. Dead or moribund animals were examined grossly. Body weights of the F_0 animals were recorded weekly for males and for females prior to breeding. Mated females were weighed on days 1, 7, 14 and 21 of gestation and on days 1, 4, 7, 14 and 21 of lactation. Food consumption was not measured because the experimental design required that food be withheld during exposure periods.

For F_1 and F_2 litters, the date of parturition, litter size, number of live and dead pups on days 0 (birth), 1, 4, 7, 14 and 21, sex, weights of pups on days 1, 4 (pre and post culling), 7, 14 and 21, physical abnormalities and behavioral abnormalities were recorded during lactation.

Pathology:

Necropsies were scheduled for maternal animals after the respective generations were weaned. Males were necropsied before the offspring were weaned. All animals were fasted and anesthetized with methoxyflurane. Eyes were examined in situ. Tissues were collected and preserved in 10% Neutral Buffered Formalin for control and high dose adults. Lungs were infused with formalin and the nasal cavities were flushed with formalin. Tissues from the low and mid dose groups were examined if there were suspected treatment related lesions in the high dose group. (See Table II for the tissues that were collected and examined histologically).

Ten pups per sex per group were selected from the F_1 and F_2 litters for complete necropsy. These animals were anesthetized with methoxyflurane and euthanized. Gross examinations were conducted on weanlings; tissues were collected and preserved but were not examined microscopically. Terminal body weights were not recorded.

Experimental conditions:

Concentrations of sulfuryl fluoride were selected based on the results of a previous study in rats. The distribution of the test material in the breathing zone was determined for 8 sample points prior to the start of the multigenerational study. Exposure chambers were 14.5 m^3 with a pyramidal top. The airflow was 2900 L/min. The concentration of sulfuryl fluoride was generated

using glass J tubes. The test material was metered through the tube along with compressed air. Airflow was measured at 60 minute intervals. Chamber temperature and relative humidity were measured at 60 minute intervals and were maintained at 22° C and 50%, respectively. Analytical concentrations of sulfuryl fluoride were determined at 30 minute intervals during exposure.

STATISTICAL ANALYSIS:

Body weights were analyzed using Bartlett's test and ANOVA. If ANOVA was significant, Dunnett's test or Wilcoxon Rank-Sum test with Bonferroni's correction was performed. ANOVA was used to evaluate gestation length. Fertility indices were analyzed using Fisher's exact test. Binomial distribution was performed on neonatal sex ratios and Wilcoxon test was performed on survival indices. Significance was at $p = 0.01$ and $p = 0.05$.

QUALITY ASSURANCE:

A statement of quality assurance and a statement of compliance with GLPs are included in the submission and are both dated January 7, 1992.

RESULTS:

Exposure Data:

For the F_0 adults, the temperature in the exposure chamber ranged from 22.4° to 23.0° C, the relative humidity ranged from 49.4 to 56% and the air flow was 2840 to 2924 L/minute. For the F_1 generation, the chamber temperature ranged from 21.9 to 23.0° C, the relative humidity ranged from 52.9 to 57.5% and the air flow was 2851 to 2946 L/minute.

Average analytical concentrations of sulfuryl fluoride were 5.0, 20.9 and 149.1 ppm for low, mid and high dose groups, respectively for the F_0 generation. Average concentrations for the F_1 generation were 5.2, 20.4 and 150.1 ppm for the low, mid and high dose groups, respectively. (See Table I for analytical data).

F_0 Adults

Five deaths and one case of euthansia were reported in the animals in this generation. One control female was euthanized when she was found moribund. On necropsy the animal was reported to have a mass on the ventral side and on histopathologic examination a diagnosis of adenocarcinoma of the mammary gland was made. In the low dose group, one male was found dead on day

73 and was diagnosed as having congestive heart failure; in the mid dose group, 2 deaths were reported in males and one female. One male in the mid dose group died of asphyxiation, one male died of undetermined causes and the female died of possible starvation. The female reportedly had no incisors at the time of death. In the high dose group, one male died of an undetermined cause after 100 days on the study.

The compound did not appear to have an effect on the body weights of male and female rats in the low and mid dose groups. Statistically significant differences were reported in body weights for both sexes at the highest concentration tested when compared to controls at $p = 0.05$. In males, weight differences were first observed on day 14 of the study and continued to day 133. The percent difference from controls ranged from 4 to 10.2%, with the greatest difference between groups being reported on days 42 and 49 of the study. Weight decrements in the high dose group appeared to be related to the administration of the test material.

In high dose females, decreases in body weights when compared to controls were reported during premating, gestation and lactation. During lactation, body weights began to increase and by the end of lactation, body weights were similar for controls and high dose animals. (See Table III for body weight data for F_1 and F_2 adults).

During gestation, decreases in weight gain were reported on days 7-14 and on days 1-21.

Clinically, there were no symptoms observed that could be attributed to the test material. In high dose males clinical observations of excessive chromodacryorrhea, rough hair coats and thin appearances were reported in 3/30 high dose males.

At necropsy dental fluorosis was observed in 27/30 high dose males and in 29/30 high dose females. Fluorosis was described as dark discoloration of the incisors. In the lungs, pale or gray foci were reported in both sexes of high dose animals. All males were affected and 18/30 females were affected at the highest dose tested. At the mid dose of 30 ppm, five males were reported to have similar pale, gray foci in the lungs; however none of the females at this dose level were affected.

Histologically, aggregates of alveolar macrophages were observed in the lungs of animals receiving sulfuryl fluoride at concentrations of 30 ppm and greater. These lesions could be correlated to the pale foci reported on gross observation of the lungs of the high dose male animals. In females in the high dose group, aggregates of alveolar macrophages were present to some degree in all animals in the high dose group. At 30 ppm, aggregates of alveolar macrophages were present in 10/30 males

and in 19/19 females, but were described as slight. When compared to controls, there was also an increase in the incidence of chronic inflammation in the lungs at the highest dose tested. A total of 14/30 males and 15/30 females in the high dose group had some degree of chronic pulmonary inflammation, compared to two animals of each sex in the control groups.

In the brains of animals in the high dose group, bilateral, symmetrical vacuolation of the caudate putamen of the cerebrum was reported in 11/30 males and 14/30 females. In males, the degree of vacuolation was described as very slight in affected males and slight in affected females. This observation was not made in any of the animals receiving concentrations of sulfuryl fluoride less than 150 ppm.

F_1 Generation

The compound did not affect male and female fertility, the length of gestation or the time to mating. Additionally, no effect was reported on the litter size, pup survival and pup sex ratios. For female pups in the high dose group, pup weights were significantly decreased throughout the lactation period when compared to controls and for high dose male pups, weights were significantly decreased from day four of lactation ($p = 0.05$). An increased number of pups in the high dose group had stomachs that were void of milk at necropsy.

In the adult F1 generation, no treatment related clinical signs were observed. Two deaths and two cases of euthanasia were reported. Lymphosarcomas were diagnosed in one male and one female control that were sacrificed. The causes of death in the one animal in the low dose group and the one animal in the high dose group were not determined.

No treatment related effects on body weights were reported in males and females in the low and mid dose groups. In high dose males, significant decreases in body weight were reported beginning at day 9 of the study and continuing through day 238. Body weights in this dose group were 11 to 16% lower than those reported for males in the control group. Increases in body weights were reported at several intervals in the low and mid dose groups but were not related to the administration of sulfuryl fluoride.

In high dose females, from days 30 to 82, body weights were approximately 8 to 10% lower than body weights for controls. Throughout gestation, there were significant differences ($p = 0.05$) in body weights of control and high dose females. Significant differences in body weights of control and high dose females were also reported on days 4 and 14 of lactation.

Grossly, the observations in the F_1 animals were similar to those observed in the F_0 animals. Dental fluorosis was present in 22/30 males and 28/30 females in the high dose group. There were pale foci present in the lungs of 12/30 males and 24/30 females in the high dose group.

Histologically, aggregates of alveolar macrophages were observed in the lungs of animals in all groups. Twelve males and twelve females in the control group had aggregates of alveolar macrophages in the lungs compared to 29/30 males and 30/30 females in the high dose group. At 20 ppm, 21/30 males and 19/30 females had alveolar aggregates of macrophages. Eleven males and twelve females in the 5 ppm group also had this lesion , but the frequency of occurence was similar to that reported for controls.

Vacuolation of the caudate putamen fiber tracts in the cerebrum in high dose animals was also observed microscopically. Two males in the high dose group and seven females in the high dose group were reported to have this lesion.

(See Table IV for distribution of microscopic findings in F_0 and F_1 adults).

F_2

There was no effect on litter survival, litter size, sex ratios, gestation length or time to mating (parental animals). Additionally, the compound did not have an effect on viability of the offspring. During lactation, significantly lower body weight was reported for female pups in the high dose group when compared to controls on days 14 and 21 ($p=0.05$). No similar weight decrements were reported for males during lactation. At 20 ppm and at 150 ppm there were more pups that did not have milk in their stomachs than there were in the control and low dose groups; however, there were more pups and more litters observed for the highest concentration group than for the control group. At necropsy, there were no treatment related gross abnormalities reported.

DISCUSSION:

Based on the results of this study, the parental NOEL of sulfuryl fluoride was 5 ppm and LOEL was was 20 ppm based on the increased incidence of lung pathology characterized grossly by pale foci microscopically by aggregates of alveolar macrophages. At 150 ppm, adult animals in both the F_0 and the F_1 generations had decreased body weights, increased incidence of of dental

fluorosis, increased incidence of lung pathology which included aggregates of alveolar macrophages and chronic pulmonary inflammation, and increased incidence of vacuolation of the caudate putamen fiber tracts of the cerebrum. The reproductive NOEL was 20 ppm and the LOEL was 150 ppm based on decreased pup weights that was secondary to maternal weight loss.

The observed increase in the presence of alveolar macrophages in the lung are usually indicative of injury to the lung. In laboratory rats, aggregates of alveolar macrophages have also been reported to occur spontaneously. In this study, the increase in the number of animals affected and in the degree to which the lungs were affected (very slight for controls vs slight to moderate for animals receiving sulfuryl fluoride) suggest that there is an association between the presence of aggregates of alveolar macrophages and the administration of the test material. Additionally, in the high dose groups, damage to the lungs was supported by the finding of chronic inflammation in 14/30 males and in 25/30 females in the F_0 generation and 18/30 females in the F_1 generation.

In mammalian species, the most sensitive indicator of fluoride toxicity is the effect observed in permanent teeth formed during the time of excessive fluoride exposure. In this study, the gross changes observed in the incisors of the animals in the high dose groups are indicative of fluorosis.

The administration of sulfuryl fluoride at doses of 150 ppm was also associated with bilateral, symmetrical vacuolation in the brain. There were no clinical manifestations of this microscopic finding and the significance of this lesion is unknown. Animals in the F_0 generation were affected to a greater degree than were animals in the F_1 generation which were exposed to the test material in utero, during lactation and for a longer period prior to mating (12 weeks for F_0 and 10 weeks for F_1).

This study meets the guideline requirements for a multigeneration reproduction study as set forth in the Subdivision F Guidelines (83-4).

TABLE I
Analytical Data

F_0 Generation	Chamber concentrations (ppm)		
	5	20	150
Avg. analyt. conc.(ppm)	5.03	20.9	149.1
Range	3.1-5.9	16.7-24.1	117.8-161.8
Ratio Analyt/nomin.	ND	78.5-135.6	ND
F_1 Generation			
Avg. analyt. conc. (ppm)	5.15	20.35	150.08
Range	4.0-10.4	15.9-27.6	135.1-164.4
Ratio Analyt/nomin.	ND	71 -117.1	ND

TABLE II
Tissues collected at Necropsy

The following CHECKED (x) tissues were collected for histological examination for all dose groups.

Digestive system	Cardiovasc./Hemat.	Neurologic
Tongue	Aorta	x Brain
Salivary glands	Heart	Periph. nerves
Esophagus	Bone marrow	Spinal cord
Stomach	Lymph nodes	
Duodenum	x Spleen	
Jejunum	Thymus	Glandular
Ileum		Parathyroids
Cecum		Adrenals
Colon	Urogenital	Thyroid
Rectum	x Kidneys	x Pituitary
	x Urinary bladder	
x Liver	x Testes	
Gall bladder	x Epididymides	Other
Pancreas	x Prostate	Bone
	x Seminal vesicle	Skin
Respiratory	x Ovaries/oviducts	Skel. muscle
Trachea	x Uterus	x All gross lesions
x Lung	x Vagina	
Nose	X Cervix	
Pharynx	x Coagulating glands	
Larynx		

TABLE III
Body Weights (g)

F_0	Sulfuryl Fluoride Concentration (ppm)	
Males	0	150
Day		
-1	236.4	237.1
14	341.4	329.1*
28	421.8	390.3*
42	469.1	422.4*
49	485.2	435.9*
70	538.6	504.1*
91	573.8	534.8*
112	611.1	560.8*
133	639.0	589.7*
Females		
Premating day		
-1	166.0	165.6
14	226.2	214.6*
28	272.2	257.8*
42	292.2	274.5*
49	300.8	283.2*
70	323.9	309.1
Gestation day		
1	329.9	322.8
7	360.9	349.1
14	397.0	376.0
21	475.5	444.4*
Lactation day		
1	354.4	316.8*
4	366.3	320.8*
7	383.6	341.6*
21	379.4	374.8*

TABLE III (continued)

F_1 Generation	Sulfuryl Flouride Concentration (ppm)	
Males	0	150
Day		
1	106.3	109.1
12	219.9	191.4*
20	296.8	248.7*
26	346.5	294.7*
47	436.4	373.3*
61	484.1	419.6*
82	529.3	468.8*
117	573.8	516.1*
138	610.0	545.8*
Females		
Premating day		
1	95.8	97.9
12	173.8	157.9
20	216.9	195.4*
47	282.6	259.3*
61	304.9	278.7*
82	325.3	297.0*
Gestation day		
1	338.4	307.4*
7	367.8	333.5*
14	397.9	364.3*
21	476.1	441.7*
Lactation day		
1	352.0	328.1
4	367.6	329.4*
14	395.8	367.2*
21	392.2	373.3

* $p = 0.05$

Data extracted from tables 7-10 and 22-25 of the report.
Effects observed at lower dose levels were similar to controls.

TABLE IV
Gross and Microscopic Lesions in F_0 and F_1 Adults

F_0 Generation	Sulfuryl Fluoride Concentration (ppm)			
Males	0	5	20	150
Gross Lesions				
Lungs/grey multifocal	0/30	0/30	5/30	30/30
Teeth/dark	0/30	0/30	0/30	27/30
Microscopic Lesions				
Brain/vacuolation	0/30	0/30	0/30	11/30
Lungs/inflammation	2/30	2/30	1/30	14/30
Lungs/alveolar macrophages	3/30	5/30	11/30	30/30
Females				
Gross Lesions				
Lungs/grey multifocal	0/30	0/30	0/30	18/30
Teeth/dark	0/30	0/30	0/30	29/30
Microscopic Lesions				
Brain/vacuolation	0/30	0/30	0/30	14/30
Lungs/inflammation	2/30	3/30	1/30	25/30
Lungs/alveolar macrophages	7/30	10/30	19/30	30/30
F_1 Generation				
Males				
Gross Lesions				
Lungs/pale multifocal	1/30	0/30	5/30	12/30
Teeth/dark	0/30	0/30	0/30	22/30
Microscopic Lesions				
Brain/vacuolation	0/30	0/30	0/30	2/30
Lungs/inflammation	2/30	1/30	0/30	7/30
Lungs/alveolar macrophages	12/30	12/30	21/30	29/30
Females				
Gross Lesions				
Lungs/pale multifocal	0/30	0/30	5/30	24/30
Teeth/dark	0/30	0/30	0/30	20/30
Microscopic Lesions				
Brain/vacuolation	0/30	0/30	0/30	7/30
Lungs/inflammation	1/30	3/30	4/30	18/30
Lungs/alveolar macrophages	12/30	11/30	19/30	30/30

Guideline Series 84: **MUTAGENICITY**

Reviewed by: Linnea J. Hansen, Ph.D. Linnea J. Hansen 4/8/92
Section IV, Tox. Branch I (H7509C)
Secondary reviewer: Irving Mauer, Ph.D. Irving Mauer (4-8-92)
Tox Branch I (H7509C)

DATA EVALUATION REPORT

CHEMICAL: Sulfuryl Fluoride TOX. CHEM. NO.: 816A

SHAUGHNESSEY NO.: 078003

STUDY TYPE: Mutagenicity (84-4): Unscheduled DNA Synthesis in Primary Male Rat Hepatocytes

MRID NUMBER: 421798-02

SYNONYMS/CAS NO.: Vikane® gas fumigant, CAS No. 2699-79-8

SPONSOR: DowElanco, Indianapolis, IN

TESTING FACILITY: Health and Environmental Sciences-Texas, Lake Jackson Research Center, The Dow Chemical Company, Freeport, TX 77541

TITLE OF REPORT: Evaluation of Sulfuryl Fluoride in the Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay

AUTHORS: B. Bashkar Gollapudi, Mary L. McClintock and John A. Zempel

STUDY NUMBER: K-016399-043

REPORT ISSUED: October 7, 1991

CONCLUSION - Executive Summary:

Doses tested: 0, 102, 204, 408, 612, 816, 1020 and 1530 ppm (0, 0.43, 0.85, 1.70, 2.65, 3.40, 4.25 and 6.37 mg/l) sulfuryl fluoride gas injected into primary male rat hepatocyte cultures and exposed for 18 - 19 hr. Doses of 1530 and higher were too cytotoxic to assay for UDS.

No evidence of increased unscheduled DNA synthesis over negative controls was observed up to 1020 ppm.

This study appeared to have been performed properly. It was submitted voluntarily by the registrant but would be

considered acceptable for regulatory purposes if it were required.

Core-Classification: Acceptable

A signed Quality Assurance Statement was present.

A. MATERIALS

1. Test Material: Sulfuryl Fluoride (technical)
   Description: Colorless gas (MW 102)
   Batch #: 874
   Purity: 97.4%
   Contaminants: not reported

   Solvent used: none

2. Control Materials:
   Negative: Air equivalent to largest volume of test material used

   Positive: 2-acetylaminofluorene (2-AAF)
   Solvent/Final concentration: DMSO (1% of culture media volume)/2.233 µg/ml 2-AAF

3. Test Cells: Primary Male Rat Hepatocytes

   Preparation: For each experiment performed in this report, one acclimated male Sprague Dawley rat (outbred Crl:CD BR strain, Charles River, Portage, MI) was used. Rats were anesthetized with methoxyfluorane and livers perfused in situ with Ca^{+2}, Mg^{+2}-free Hank's balanced salt solution buffered with 10 mM HEPES, pH 7.3, and containing 0.5 mM EGTA (4 - 5 min at 10 - 40 ml/min). Collagenase solution (Williams Medium E supplemented with 2 mM L-glutamine, 50 µg/ml gentamycin, buffered with 10 mM HEPES, pH 7.3 and containing 0.5 mg/ml type IV collagenase, 395 units/mg) was perfused through the liver for 15 min at 20 ml/min. Livers were excised and incubated in 50 ml of the collagenase solution until cells were dispersed. Dissociated hepatocytes were sedimented by low speed centrifugation and resuspended in WME supplemented with 10% fetal bovine serum. Viability of hepatocytes was assessed by trypan blue exclusion. Viable cells were counted and diluted to 1.7×10^5 cells/ml in WE/10% FBS.

   Maintenance: Dissociated liver cells were plated in WME at 5×10^5 cells per 16 x 93 mm clear polystyrene Leighton tube containing a removable cell attachment surface (RCAS) of polymethylpentene coated with Vitrogen 100 to facilitate attachment of cells (5 cm^2 growth area). All cultures were incubated at 37°C in humidified 95% air:5% CO_2 for 3 hrs to allow attachment of cells. Quadruplicate cultures were used

for each dose.
Cultures appeared to have been properly maintained.

4. Test compound concentrations used:

Basis for concentrations chosen: To provide a wide dose range including cytotoxic doses.

Cytotoxicity evaluation (Trial 1): 610, 1020, 3060, 6120, 10,000, 31,000 and 61,000 ppm (2.65, 4.25, 12.8, 25.5, 41.6, 129 and 254 mg/l)

UDS evaluations (Trials 2, 3): 102, 204, 408, 612, 816, 1020 and 1530 ppm (0.43, 0.85, 1.70, 2.65, 3.40, 4.25 and 6.37 mg/l)

B. TEST PERFORMANCE

1. Cell treatment

Following the 3 hr attachment period, media was replaced with 1 ml WME (serum-free) containing 10 μCi/ml ^{3}-thymidine (spec. activity 20 Ci/mmol). Caps were replaced with caps containing a small hole and a teflon-faced silicone septum through which gas could be injected or withdrawn and were kept tightly capped during treatment.

A known volume of air equivalent to the volume of sulfuryl fluoride gas to be added was removed from the culture tubes using a gas-tight syringe and needle, then replaced with the appropriate amount of sulfuryl fluoride gas. For negative controls, air equivalent to the maximum volume replaced in the sulfuryl fluoride treatments was withdrawn and replaced with air.

Culture tubes were then placed on a tube rocker (American Tube Rocker) perpendicular to rocking motion for 18-19 hr at 37°C. Each half of the cell cultures was exposed directly to air or air plus sulfuryl fluoride as the rocker moved from side to side.

2. Cytotoxicity Assay

The first trial was used as a cytotoxicity assay (treatment levels described above). Treatment levels at which excessive toxicity occurred were noted and the second and third assays were repeated at lower doses for evaluation of UDS. Cell culture and treatment for the cytotoxicity assay were performed as described above.

UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES

Labelled treated and control cells attached to the RCAS were washed in 3 changes of PBS and one plate from each dose group was stained with trypan blue. The other 3 plates were swollen in 1.4% sodium citrate for 10 min, then fixed in 3:1 ethanol:acetic acid (2 x 10 min) and cleared with 70% ethanol (2 x 10 min). RCAS were dried and mounted on glass microscope slides using Gel/Mount (Biomeda Corp.).

Each slide was coated with NTB-2 photographic emulsion (Eastman Kodak), dried and exposed at 4°C for 9 days. Slides were developed with D-19 developer (Eastman Kodak), fixed with Kodak fixer and stained with Giemsa. Two slides per treatment level were chosen for UDS evaluation.

4. Scoring:

Slides were coded and nuclear and cytoplasmic counts measured by projection onto a television screen with a high resolution camera mounted on the microscope. Nuclear and cytoplasmic counts (counts over an adjacent area of cytoplasm the same size as the nucleus visually determined to have the highest number of silver grains) were determined manually. At least 25 cells per slide (total of 50 cells/treatment) were counted. Net nuclear counts were determined by subtracting cytoplasmic from nuclear grain counts. Pyknotic or darkly labelled cells (S-phase) were not counted. Means and standard deviations were calculated for each treatment group.

5. Evaluation of Results:

A treated culture with mean net nuclear grain counts of 5 or more and also significantly ($p < 0.05$) greater than the negative control value was considered a positive response. Statistical analysis of data was only performed if net nuclear grain counts exceeded 5, using a computer program that performed a non-parametric Kruskal-Wallis one-way analysis of variance followed by Wilcoxon's Rank Sum test with Bonferroni's Correction.

C. <u>RESULTS</u>:

Cytotoxicity evaluation: Virtually complete cytotoxicity was observed in cells treated with sulfuryl fluoride at 3060 ppm or higher. At 1020 ppm, slight cytotoxicity was observed (% viability relative to controls was not quantitated) and all cells survived at 612 ppm. The positive controls showed no cytotoxicity. UDS assays were repeated at 7 doses from 102 to 1530 ppm.

Unscheduled DNA Synthesis: Sulfuryl fluoride did not appear

to cause an increase in unscheduled DNA synthesis above negative controls at any of the dose levels tested and none of the test cultures contained cells in repair (tables from study showing results of UDS assay are appended to this evaluation). The highest dose tested in the UDS experiments, 1530 ppm, was too cytotoxic and UDS could not be quantitated. Cells treated at 1020 ppm sulfuryl fluoride showed moderate toxicity in the second UDS experiment but not in the first. This variability may have been due to variability in the condition of the individual hepatocyte cultures prepared for each experiment. Mean nuclear counts were lower than cytoplasmic counts for all doses tested. The positive control, 2-AAF, produced a marked increase in nuclear grain count over negative controls and 100% of these cells were in repair. A DMSO negative solvent control was not performed.

D. REVIEWER'S DISCUSSION/CONCLUSIONS:

Under the test conditions and up to cytotoxic doses (1020 - 1530 ppm), sulfuryl fluoride did not appear to cause increased unscheduled DNA synthesis in male rat primary hepatocytes. Negative and positive controls gave appropriate responses. Although cytotoxicity was evaluated, no quantitation of viability relative to negative controls was performed.

The actual volume of sulfuryl fluoride injected into each culture tube per dose was not given. The exact amount of sulfuryl fluoride that the cells were exposed is difficult to determine from the information provided in this study. However, since the assays were negative up to doses that were apparently cytotoxic, TB-I believes that this chemical was adequately tested for UDS in this assay. This study was submitted voluntarily by the registrant but would be considered acceptable for regulatory purposes if it were required.

Study Deficiencies: % relative survival not quantitated in cultures with partial toxicity, volume of sulfuryl fluoride injected at each dose not specified, no negative solvent control for positive control.

UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES

APPENDIX

Table 2

Mean Net Nuclear Grain Counts (MNNGC) - Trial 2, UDS Assay #1

Test Material: Sulfuryl Fluoride

Exposure Level (ppm)	Slide 1[a]	Slide 2[a]	Slide 1 & 2[b]
Negative Control	-7.8 ± 4.8	-5.8 ± 4.6	-6.8 ± 4.8
204	-8.3 ± 6.0	-9.3 ± 5.8	-8.8 ± 5.9
408	-8.0 ± 6.8	-8.3 ± 5.3	-8.2 ± 6.1
612	-8.7 ± 4.2	-8.4 ± 5.1	-8.5 ± 4.6
816	-9.2 ± 6.0	-6.7 ± 4.7	-8.0 ± 5.5
1020	-5.2 ± 3.5	-5.5 ± 4.5	-5.3 ± 4.0
Positive Control	76.1 ± 6.7	70.9 ± 5.8	73.5 ± 6.7[c]

a Fifty cells were evaluated per slide except in positive controls where only 25 cells were evaluated/slide.
b The values are based upon a total of 100 cells except positive controls where the total is 50 cells.
c Positive UDS response.

UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES

Table 3

Mean Net Nuclear Grain Counts (NNGC) - Trial 3, UDS Assay #2

Test Material: Sulfuryl Fluoride

Exposure Level (ppm)	Slide 1[a]	Slide 2[a]	Slide 1 & 2[b]
Negative Control	-8.3 ± 4.4	-8.6 ± 4.5	-8.4 ± 4.4
204	-8.9 ± 5.2	-7.6 ± 4.8	-8.2 ± 5.0
408	-8.2 ± 4.5	-7.6 ± 3.9	-7.9 ± 4.2
612	-6.0 ± 4.1	-6.0 ± 4.1	-6.0 ± 4.1
816	-6.9 ± 4.4	-7.3 ± 4.1	-7.1 ± 4.2
1020	-5.5 ± 4.3	-5.8 ± 4.4	-5.7 ± 4.4
Positive Control	76.5 ± 5.9	75.5 ± 8.0	76.0 ± 7.0[c]

[a] Fifty cells were evaluated per slide except in positive controls where only 25 cells were evaluated/slide.
[b] The values are based upon a total of 100 cells except positive controls where the total is 50 cells.
[c] Positive UDS response.

CASWELL FILE

009479

Reviewed by: Joycelyn E. Stewart, Acting Head, Section II, Toxicology Branch I, HED

Secondary Review: Marion Copley 11/10/92

DATA EVALUATION REPORT ADDENDUM

STUDY TYPE: 90 day rat inhalation study (82-4)

MRID NO: 4080909-02 TOX CHEM NO 816A

SYNONYM: Vikane Gas Fumigant

SPONSOR: Dow Chemical Company

TESTING FACILITY: Dow Chemical Company

STUDY NO: K-016399-023R

REPORT TITLE: Sulfuryl Fluoride (Vikane Gas Fumigant): 13 week Inhalation Toxicity Study with Rats.

AUTHOR(S): K.D. Nitschke, D.A. Dittenber, and D.L. Eisenbrandt

REPORT ISSUED: November 16, 1987.

CONCLUSIONS: The data submitted support a NOEL of 30 ppm and a LEL of 100 ppm in male and female Fisher 344 rats when the animals were administered inhalation doses of 0, 30, 100 and 300 ppm of sulfuryl fluoride for 6 hours/day, five days/week for 13 weeks. The LEL of 100 ppm was based on fluorosis of the teeth in both sexes. In addition, at the high dose level there were significant body weight decrements, inflammation of the nasal passage, alveolar histiocytosis, and microscopic vacuolation of the caudate-putamen nucleus and white fiber tracts of the internal capsule of the brain of all animals of both sexes, and very slight hyperplasia of the collecting ducts of the kidney in 9/10 females. Serum fluoride was increased, but not significantly so, in all dosage groups.

COMMENTS

It should be noted that inflammation of the nasal passages was reported in rats in this study. The incidences were 0, 2, 1 and 10 out of 10 in males, and 4, 3, 6, and 10 out of 10 in females of the control, low, mid and high dose groups. The inflammation observed in the treated animals is consistent with sulfuryl fluoride exposure. With respect to lung pathology, all of the male rats and 8, 9, and 8 of the female rats at the control, low, and mid dose groups were reported to be normal. All of the high dose rats were reported to have histiocytosis of the lungs. Vacuolation was present in the brains of all animals of both sexes. It was minimal in the area of the caudate-putamen nucleus and more prominent in the white fiber tracts of the internal capsule than in the adjacent neuropil. Very slight atrophy was reported in individual nephrons in the kidney across all dosage groups. This is not likely to be a consequence of administration of the chemical.

CASWELL FILE

009479

Primary reviewer: Stanley B. Gross, PhD, DABT, CIH. Stanley Gross 3/25/92
Secondary Reviewer: Joycelyn E. Stewart, PhD. Harrison Cagley for J Stewart 4/15/92
Section II, Tox. Branch I (H7509C).

DATA EVALUATION REPORT

STUDY TYPE: 90 Day rat inhalation toxicity study.

TOX. CHEM. No.: 816A See attached addendum (Same Document)

MRID No.:408909-02

TEST MATERIAL: Sulfuryl Fluoride

SYNONYMS: Vikane Gas Fumigant (Dow Chemical Company)

STRUCTURE: SO2F2

SPONSOR:Dow Chemical Company

TESTING FACILITY: Dow Chemical Company

STUDY NO.: K-016399-023R

REPORT TITLE: Sulfuryl Fluoride (Vikane® Gas Fumigant): 13-week Inhalation Toxicity Study with Rats.

AUTHOR(S): K. D. Nitschke, D.A. Dittenber, and D. L. Eisenbrandt.

REPORT ISSUED: November 16, 1987.

CONCLUSIONS: Rats (10 per sex/level) were exposed to SF vapor concentrations of 0, 29.8, 100, and 297 ppm 6 hr/day, 5 days per wk for 13 weeks. The reported NOEL was 30 ppm and LEL = 100 ppm. The rats exposed to mid dose and high dose ranges showed decreased body weights, mottled teeth (fluorosis), decreased specific gravity of the urine; increased serum fluoride; increased relative testicular weight in the HD males; vacuoles of the caudate--putamen nuclei; nasal exudate, hyperplasia and hypertrophy. Liver function (increased alkaline phosphatase and decreased blood proteins) was seen in the HD animals.

CORE GRADE: Core Minimum.

1. Previous Lower NOEL? Previous Dow studies have shown adverse effects in the rat, guinea pig, and mice exposed to 20 ppm or less for approximately one year (reported by Dow on October 22, 1959). The organs involved included the lung (pneumonitis); teeth (fluorosis); elevated blood F and increased F excretion in the urine; liver and kidney damage. The present study had a number of deficiencies which would tend to obscured adverse effects on target organs in the LD group:

a) Pulmonary System. The lung weights were not obtained. A number of pathological conditions were found in all test groups, including the controls, which may have obscured any effects due to SF.

b) Renal System. Renal disease permeated all test animals.

c) Possible Fluorosis. All exposure groups showed an increase in F exposures even though mottled teeth were noted only in the MD and HD groups.

d) Nasal Irritation. Nasal irritation was seen in all dose groups including the LD group assumed to be the NOEL group.

e) Food Consumption. Since there were significant decreases in BW in the MD and HD groups, it might be informative to determine if the animals were ingesting less food or the decrease was due to general toxicity.

STUDY DESIGN:

Groups of 10 rats/sex were exposed to target concentrations of 0, 30, 100 and 300 ppm sulfury Fluoride (SF) for 6 hours/day, 5 days/week for 13 weeks. The animals were observed daily and body weights were recorded at weekly intervals. Routine hematology, clinical chemistry, urinalysis, necropsy and histology was performed at the end of the study.

MATERIALS AND METHODS

Material and Methods section from the report is attached as Appendix A.

1. Test compound: Vikane gas fumigant. Description: colorless, odorless gas. Batch #TWP-83019-408. Purity 99.8%.

2. Test animals: Male and female albino rats. Strain: Fischer 344. Age: 7 weeks. Source: Charles River Breeding Laboratories, Kingston, NY.

3. Exposure Chambers: 4100 Liter stainless steel chamber (5' cube; with pyramidal top inlet, exhaust duct at the bottom). Airstream at approximately 800 L/min. (12 air changes/hr.); temp. 22 degree C. and relative humidity, 50%.

4. Generating System: Metered into the chamber air stream from SARAN bags using a RPG pump (Fluid Metering Inc., Oyster Bay, NY). The chamber concentrations used in this study were based on the concentrations used in a previous two week

inhalation range finding study in rats and rabbits (MRID #460086-006) carried out by Eisenbrandt et al. (1985). This study utilized concentrations of 0, 100, 300 and 600 ppm. The 600 ppm caused server toxicity and was therefore not used in the present study.

5. Chamber Monitoring: SF concentrations were determined 1-2 times/hour with a Miran 1A infrared spectrophotometer (Foxboro/Wilks, South Norwalk, CT), calibrated at least once per month with standards made by diluting measured volumes of SF gas with measured volume of filtered compressed air. The analytical system was evaluated with a least one standard prior to each day exposure. The daily time-weighted average concentration was determined using interpolation of the signal from the Miran using a microprocessor (Instrument Applications and Communications, Dow Chemical Company, Midland, MI).

6. Observations and Clinical Analyses. Clinical observations were made daily. Body weights were obtained on a weekly basis. Routine hematology, urinalyses, clinical chemistries, gross necropsies and histopathology were performed at the end of the study.

7. Quality Assurance was carried out as noted in the report.

RESULTS:

1. Exposures Concentrations/Conditions. The rats received 64 exposures over the 90 day period with averaged analytical concentrations as follows:

	Target Nominal	Analytical Concentrations
Control	0	0
LDG mg/L	30	29.8 (23.7-33.6)
MDG	100	100 (81-120)
HDG	300	297 (231-314)

The temperature ranged from 23 to 25 degrees C. and the relative humidity about 45 to 50%. When not in exposure chambers, the rats were maintained in animal rooms under unspecified controlled conditions.

2. Toxicity: There were no mortalities during the study. There also were no signs of toxicity except for mottling (fluorosis) of the teeth in the MD and HD groups.

3. Body Weight - BW of the LD and MD groups were similar to controls. The BWs of the males in the HD group decreased significantly from day 45 to the end of the exposure; the BW of

the females decreased significantly from day 24 to the end of the study; resulting in decreases of 16.8 % of the controls for males and -14.7% of the controls for the females at day 87 of the study.

4. Food consumption. Food consumption was determined not determined.

5. Hematology. Blood was collected by orbital sinus puncture from rats anesthetized with carbon dioxide immediately prior to necropsy. The CHECKED (X) parameters were examined.

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB conc.(MCHC)
X Mean corpuscular volume (MCV)

Abnormalities were observed only in the HD group and these were not consistent: RBC decreased significantly in the males and slightly in the females. The platelets were elevated in the HD males and females but achieve statistical significance only in the males.

The other parameters were not affected.

6. Urinalysis - Urine was collected (method not specified) from rats after 11 weeks of exposure to the test material. The CHECKED (X) parameters were examined (NR, not reported):

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

The specific gravity of the HD males (significantly) and females (not significantly). Trace amounts of protein, blood and bilirubin and urobilinogen were seen in several animals from each of the test groups.

7. Clinical Chemistry. Blood samples were collected at the terminal sacrifice from severed cervical blood vessels. The following CHECKED (X) analyses were carried out:

Electrolytes:

X Calcium*
- Chloride*
- Magnesium*

Other:

X Albumin*
X Blood creatinine*
X Blood urea nitrogen*

X Phosphorus*
- Potassium*
- Sodium*
- Cholesterol*
X Globulins
X Glucose*

ENZYMES:
X Alkaline Phosphatase (AP)
- Cholinesterase (CHE)
- Creatinine phosphokinase* (CP)
- Lactic acid dehydrogenase (LDH)
- Serum alanine aminotransferase (also SGPT)
X Serum aspartate aminotransferase (also SGOT)
- Total bilirubin*
X Total plasma protein*
- Triglycerides (TG)

Alkaline phosphatase was significantly elevated in the HD groups of both sexes. Calcium was decreased slightly (but significantly) in HD females (not males) but there was no relationship to SF level. Total protein and albumin was decreased significantly in the HD females (only) and globulins were decreased in the HD males (only) no consistent changes in blood proteins otherwise.

8. Serum fluoride was measured in blood at the termination of the study using a fluoride specific electrode (Orion Research, Cambridge).

The F levels in the males were comparable in all exposures groups ranging from 0.7 to 1.12 ug/ml. The F levels in the females were comparable for the control, LD and MD groups (ranging from 0.6 to 0.74 ug/ml) however, the HD female group was about twice the level (1.37 ug/ml) but did not achieve statistical significance.

9. Ophthalmological examinations were not performed on live animals. The corneal integrity was measured during necropsy using a slide and fluorescent dye technique. All eyes were considered to be normal.

10. Sacrifice and Pathology - Terminal sacrifice involved the gross and histological examination of the following organs.

DIGESTIVE SYSTEM	CARDIVASC./HEMAT.	NEUROLOGIC
Tongue	Aorta*	W Brain*
Salivary glands*	W Heart*	Periph nerve*
Esophagus*	Bone marrow*	Spinal cord (3 levels)
Stomach*	Lymph nodes*	Pituitary*
Duodenum*	Spleen*	Eyes(optic nerve)
Jejunum*	Thymus*	GLANDULAR
Ileum*	UROGENITAL	Adrenal*
Cecum*	W Kidneys*	Lacrimal gland*
Colon*	Urinary bladder*	Mammary gland*
Rectum*	W Testes*	Parathyroids*

W Liver*	Epididymides*	Thyroids*
Gall bladder*	Prostate	OTHER
Pancreas*	Seminal Vesicle	Bone*
RESPIRATORY	Ovaries	Skeletal musc.*
Trachea*	Uterus*	All gross lesions & masses.
Lungs*		

The above organs marked with W (brain, heart, liver, kidneys and testes) were weighed and compared to body weight changes.

a. Organ weights and body ratios. All of the organs showed changes in absolute and/or relative weights in the HD groups. As noted above, the BW of the HD animals was significantly decreased compared to the control animals.

The brain weights of the MD and HD females were significantly reduced. The brain body ratio was increased significantly in the HD females. The decrease in absolute weight and increase in relative weight was also seen in the HD males but did not achieve significance.

Kidneys. The absolute wt of the kidneys of HD males and females were decreased (without stat. significance) but the relative weights were increased significantly in both HD sexes.

The liver was reduced by weight and ratio in the HD females and in the absolute weight of the liver in the males of the MD group.

Testes. The testes of the HD males showed a decreased without achieving stat. significance but the testis/body weight ratio was increase significantly.

Thymus. The thymus was decreased statistically in the HD groups and was accompanied by decreased relative wts. which did not achieve significance.

b. Gross pathology - There were a minimal number of gross abnormalities noted. Lungs. Almost all of the HD animals had multifocal pleural paleness. Teeth. All of the animals from the HD and MD groups showed mottled upper and lower incisors.

c. Microscopic pathology Only the following organs showed abnormalities:

Brain: all animals in the HD groups showed bilateral cerebral, focal vacuolation.

Kidneys: Very few of the males (0-3 of 10) in all dose groups and only 1 of 10 in the control and HD females were normal. Multifocal tubular mineralization was seen in a number of females throughout all experimental groups. Nine of 10 HD females had hyperplasia of the collecting ducts. All 10 HD males had decreased protein droplets in the cortex.

the significance of this change can not be determined.

Lungs: Lung pathology was seen throughout in all dose groups with the females being effected more than the males. Pathological changes included alveolar histiocytosis, fibrosis, hemorrhage, and inflammation. Subpleural alveolar histiocytosis was seen in all animals of both HD groups. ~~(Diagnosis?)~~

Nasal Tissues All of the SF treated animals were adversely affected in a dose response fashion. Only 6 of the 10 male rats in the control group were considered normal. The females were more adversely effected than the females: 10, 8, 9, 0 for control, LD, MD and HD respectively, compared to 6, 6, 4, and 0 for the females, respectively. Chronic inflammation of the submucosa was seen distributed in all groups. Chronic and subchronic inflamation was seen in all experimental groups including the LD group (except the control males), with most of the pathology centered in the HD animals.

D. DISCUSSION:

1. Authors Comments. The authors concluded that the NOEL for this study was 30 ppm (LD group). They assumed a number of the changes in organ weight and clinical chemistries were due to the decrease in body weights seen in the MD and HD groups. They concluded that the mottled teeth was due to the increase in body fluoride derived from the absorbed SF. They noted that the respiratory and renal changes seen here were also seen in a previously run 2 week inhalation study carried out by Dow (Eisenbrandt et al , 1985).

2. Reviewers Comments. From Dow studies and others it is clear that the target organs for SF toxicity are the naso-pharyngeal tract; the pulmonary system; the liver and kidney and teeth. These effects are probably due to the conversion of SF to F in the body. Short--term exposures to SF have resulted in neurological (shaking and convulsions) and breathing difficulties. Humans exposed to SF and an irritant gas (CP?) Not specified but probably experienced nausea, vomiting, reddening of the eyes, nose, throat and lungs. (Occupational Health Guideline for SF, NIOSHs, 1978).

Previously reported studies by Dow (October 22, 1959) indicated adverse effects in several species exposed to 20 ppm or less over a one year period. The species included rats, mice and Guinea pigs. It was clear that a significant amount of F was found in the blood, teeth and urine; the lungs (pneumonitis), kidneys and livers were damaged.

The present studies had intercurrent pathology of the nasal, pulmonary and renal systems which were seen in all test groups makes it difficult to conclude that the LD group (30 ppm) represented the NOEL for 13 weeks of SF inhalation exposure. The

lack of lung weights is important.

The studies point to the need to fine tune the current study and probably to extend the exposure time to 6 months or more. It should be possible to use animals which have less intercurrent pathology in the test groups and to expand the analyses of organ changes. Beside the lung weights, it should be desirable to analyze for blood gas changes, pH changes.

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* Recommended by Subdivision F (Oct. 1982) guidelines for subchronic studies.

SF90INH.Rat 4-1-92

VIKANE (Sulfuryl fluoride) TH Review 009479

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PAGE

TOXCHEM NO. 816 A Sulfuryl fluoride

FILE LAST PRINTED:

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CORE GRADE/ DOCUMENT#
[illegible]-day inhalation - rat [illegible] K-016377-013R [illegible]/10/1987	Vikane Gas Fumigant 99% lot # TSP 8-3017-40	408909-02	NOEL = 30 ppm. Toxicity discolored kidneys, fluorosis teeth, increased serum fluoride, vacuoles in caudate-putamen nuclei. Doses tested 0, 30, 100, 300 ppm in [illegible] Fischer 344 rats		Minimum
[illegible]-day inhalation - rabbit [illegible] K-016377-025B [illegible]/10/1987	Vikane Gas Fumigant 99% TSP 8-3017-40	408909-[illegible]	NOEL = 30 ppm LEL=100 Toxicity: discolored [illegible], [illegible], vacuolation of caudate-putamen nuclei. Doses tested 0, 30, 100, 300 ppm in New Zealand White rabbits 6 hr/day 5 days/week.		Minimum

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009479

Reviewed by: Joycelyn E. Stewart, Acting Section Head KS 4/24/92
Section II, Toxicology Branch I
Secondary reviewer: Marion Copley Marion Copley 4/29/92

DATA EVALUATION REPORT ADDENDUM

STUDY TYPE: 90 day rabbit inhalation study (82-4)

MRID NO: 4080909-01 TOX CHEM NO 816A

SYNONYM: Vikane Gas Fumigant

SPONSOR: Dow Chemical Company

TESTING FACILITY: Dow Chemical Company

STUDY NO: K-016399-023R

REPORT TITLE: Sulfuryl Fluoride (Vikane Gas Fumigant): 13 week Inhalation Toxicity Study with Rabbits.

AUTHOR(S): K.D. Nitschke, D.A. Dittenber, and D.L. Eisenbrandt

REPORT ISSUED: November 16, 1987.

CONCLUSIONS: The data submitted support a **NOEL of 30 ppm** and a **LEL of 100 ppm** in male and female New Zealand White rabbits when the animals were administered inhalation doses of 0, 30, 100 and 300 ppm of sulfuryl fluoride for 6 hours/day, five days/week for 13 weeks based on decreased body weights, decreased liver weight and mottling of the teeth in both sexes and microscopic vacuolation of the white matter of the brain in females. In addition, at the high dose level there were significant body weight decrements, alveolar histiocytosis, histologic changes in the nasal epithelium and microscopic malacia to vacuolation of the internal and external capsules, putamen and globus pallidus of the brain in both sexes. Serum fluoride was significantly increased at all dose levels.

COMMENTS

Liver weight was decreased at the mid and high dose levels. In males, absolute but not relative liver weight was significantly reduced. In females, both the absolute and relative liver weights were significantly reduced in high dose animals.

Histological changes seen which were attributable to compound administration were nasal exudate, olfactory epithelial degeneration, and hypertrophy and hyperplasia of the nasal turbinates in high dose male and female rabbits, and in one mid dose male. Additionally, brain lesions, including vacuolation of the white matter (3/7 M, 5/7 F); malacia of the internal and external capsules, putamen and globus pallidus (3/7M, 5/7F);

gliosis of the cerebrum (2/7F) were reported in high dose rabbits. One out of seven mid dose females also had vacuolation of the cerebral white matter.

Histopathological changes seen in all treatment groups, including the controls, were histiocytosis in the lungs, and mineralization of the kidneys. None of these changes were consistent enough to be attributable to administration of the chemical.

CASWELL FILE

009479

Primary reviewer: Stanley B. Gross, PhD, DABT, CIH. 3/22/92
Secondary Reviewer: Joycelyn E. Stewart, PhD. 4/8/92
Section II, Toxicology Branch I (H7509C).

DATA EVALUATION REPORT

See attached addendum (same document)

STUDY TYPE: 90 Day rabbit inhalation toxicity study.

TOX. CHEM. No.: 816A

MRID No.:408909-01

TEST MATERIAL: Sulfuryl Fluoride

SYNONYMS: Vikane Gas Fumigant (Dow Chemical Company)

STRUCTURE: SO2F2

SPONSOR:Dow Chemical Company

TESTING FACILITY: Dow Chemical Company

STUDY NO.: K-016399-025B

REPORT TITLE: Sulfuryl Fluoride (Vikane* Gas Fumigant): 13-week Inhalation Toxicity Study with Rabbits.

AUTHOR(S): K. D. Nitschke, M.A. Zimmer and D. L. Eisenbrandt.

REPORT ISSUED: November 16, 1987.

CONCLUSIONS: Groups of rabbits (7 per sex) were exposed to SF vapor concentrations of 0, 29.8, 100 or 337 ppm, 6 hr/day, 5 days per wk for 13 weeks. Initially the HD groups were exposed to 600 ppm for 9 exposure periods. When one male and one female developed convulsions and a second female developed hind leg paralysis, the concentrations were reduced to 300 ppm for the remaining 11 weeks.

The HD female with the paralysis from the 600 ppm exposures had to be euthanized. No other animals died due to exposures. The only other toxic signs that were due to SF exposures were the mottling of the teeth seen in the MD and HD groups.

The authors concluded that the LD (30 ppm) was the NOEL and the MD group (100 ppm) represented the LEL. However, the serum fluoride was substantially elevated in all the treatment animals. The HD and MD exposure animals experienced significant body weight reductions, liver weight reductions, microscopic vacuoles in caudate-putamen nuclei; respiratory irritation, inflammation of nasal tissues and hyperplastic changes in the renal collecting ducts.

CORE GRADE: : Core minimum:

1. Previous Lower NOELs? Previous Dow studies have shown adverse effects in the rat, guinea pig, and mice exposed to 20 ppm or less for approximately one year (reported by Dow on October 22, 1959). The target organs involved included the lung (pneumonitis); teeth (fluorosis); elevated blood F and increased F excretion in the urine; brain, liver and kidney damage. The present study had a number of deficiencies which may have obscured adverse effects of SF in the LD group:.

a) Pulmonary System. The lung weights were not obtained. A number of pathological conditions were found in all test groups, including the LD groups.

b) Renal System. Urinalyses was not performed on these animals. One to two females of all of the each of the test group had renal lesions.

c). Possible Fluorosis. All exposure groups showed an increase in serum F even though mottled teeth were noted only in the MD and HD groups. In previous studies elevated blood F was associated with increased urinary F and increased F deposition in various tissues.

d). Food Consumption. Since there were significant decreases in BW in the MD and HD groups, it might be informative to determine if the animals were ingesting less food or the decrease was due to general toxicity.

e). Clinical Chemistry. There was a wide random variability in the clinical chemistry results which point to the need to increase the number of animals or the number of samples taken.

2. Choice of Rabbits? It is not clear why the rabbit was chosen for this study.

3. Expansion Study. Since previous older studies have shown significant effects at or below 20 ppm in several species, it would seem important to extend the duration of the subchronic study (using rats) and to expand the study to include better analyses of the effects on the target organs.

STUDY DESIGN:

Groups of 7 rabbits/sex were exposed to target concentrations of 0, 30, 100 and 300 ppm sulfury Fluoride (SF) for 6 hours/day, 5 days/week for 13 weeks. The animals were observed daily and body weights were recorded at weekly intervals. Routine hematology, clinical chemistry, urinalysis,

necropsy and histology was performed at the end of the study.

MATERIALS AND METHODS

Materials and Methods section from the report is attached as Appendix A.

1. Test compound: Vikane gas fumigant. Description: colorless, odorless gas. Batch #TWP-83019-408. Purity 99.8%.

2. Test animals: Male and female white rabbits. Strain: New Zealand. Age: 5 months. Weight: 3000 gm. Source: Hazleton Dutchland, Denver, PA.

3. Exposure Chambers: 4100 Liter stainless steel chamber (5' cube; with pyramidal top inlet, exhaust duct at the bottom). Airstream at approximately 800 L/min. (12 air changes/hr.); temp. 22 degree C. and relative humidity, 50%.

4. Generating System: SF vapor was metered into the chamber air stream from SARAN bags using a RPG pump (Fluid Metering Inc., Oyster Bay, NY). The chamber concentrations used in this study were based on the concentrations used in a previous two week inhalation range finding study in rats and rabbits (MRID #460086-006) carried out by Eisenbrandt et al. (1985). These studies used concentrations of 0, 100, 300 and 600 ppm. The 600 ppm level caused convulsions in one rabbit and focal necrosis of the brain.

5. Chamber Monitoring: SF concentrations were determined 1-2 times/hour with a Miran 1A infrared spectrophotometer (Foxboro/Wilks, South Norwalk, CT), calibrated at least once per month with standards made by diluting measured volumes of SF gas with measured volume of filtered compressed air. The analytical system was evaluated with a least one standard prior to each day exposure. The daily time-weighted average concentration was determined using interpolation of the signal from the Miran using a microprocessor (Instrument Applications and Communications, Dow Chemical Company, Midland, MI).

6. Observations and Clinical Analyses. Clinical observations were made daily. Body weights were obtained on a weekly basis. Routine hematology, urinalyses, clinical chemistries, gross necropsies and histopathology were performed at the end of the study.

7. Quality Assurance was carried out as noted in the report.

RESULTS:

1. Exposures Concentrations/Conditions. The rabbits received 66 exposures over the 90 day period.

	Target Nominal	Analytical Concentrations Mean (Range)
Control	0	0
LDG mg/L	30	29.8 (23.7-33.6)
MDG	100	100 (81-120)
HDG	300	297 (231-616)*

* The initial concentration of 600 ppm was reduced to 300 ppm after 9 exposures because of severe toxicity in a number of animals.

The temperature ranged from 23 to 25 degrees C. and the relative humidity about 45 to 50%.

2. Toxicity: One male and one female rabbit had convulsions following the ninth exposure to 600 ppm. A third animal (a female) developed hind leg paralysis after the 8th 600 ppm exposure, suffered a broken back and was euthanized because of her inability to get to food. There were no other signs of toxicity except for tooth mottling (fluorosis) seen the MD and HD exposure groups.

3. Body Weight. The BWs of the HD and MD groups were depressed from week 11 to the end of the study, however, only the depression in the HD males achieved statistical significance. (It is likely that an application of ANOVA, instead of Dunnet's test would show these weight reduction to all be significant). The LD group was similar to controls.

4. Food consumption. Food consumption was estimated to be reduced by observation only.

5. Hematology. Blood was collected by venipuncture from an ear vein immediately prior to necropsy. The CHECKED (X) parameters were examined.

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB conc.(MCHC)
X Mean corpuscular volume (MCV)

The only statistically significant alteration was an increase in WBCs in HD males. There was a decrease in platelets in HD males and females but they were not statistically significant. None of these changes were attributable to the

exposures to SF. :

6. Urinalysis - No urinalyses were carried out in this study.

7. Clinical Chemistry. Blood samples were collected at the terminal sacrifice from severed cervical blood vessels. The following CHECKED (X) analyses were carried out:

Electrolytes:	Other:
X Calcium*	X Albumin*
- Chloride*	X Blood creatinine*
- Magnesium*	X Blood urea nitrogen*
X Phosphorus*	- Cholesterol*
- Potassium*	X Globulins
- Sodium*	X Glucose*
ENZYMES:	- Total bilirubin*
X Alkaline Phosphatase (AP)	X Total plasma protein*
- Cholinesterase (CHE)	- Triglycerides (TG)
- Creatinine phosphokinase* (CP)	
- Lactic acid dehydrogenase (LDH)	
- Serum alanine aminotransferase (also SGPT)	
X Serum aspartate aminotransferase (also SGOT)	

There were no alterations in clinical chemistry which were attributable to SF exposures. There wide variability in the results through out the test groups: for alkaline phosphatase, the standard deviations ranged from 20 to 165 hu/ml and for glucose SD from 12 to 87 mg/dl.

8. Serum Fluoride as also measured with the above clinical chemistry using a fluoride specific electrode (Orion Research, Cambridge).

The fluoride level increased consistently with increased exposures in all exposure groups.

9. Ophthalmological examinations were not performed on live animals. The corneal surfaces were examined post mortem using the slide and fluorescent staining techniques. The eyes were normal except in the case of one HD F who had unilateral opacity of the lens.

10. Sacrifice and Pathology - Terminal sacrifice involved the gross and histological examination of the following organs.

DIGESTIVE SYSTEM	CARDIVASC./HEMAT.	NEUROLOGIC
Tongue	Aorta*	W Brain*
Salivary glands*	W Heart*	Periph nerve*
Esophagus*	Bone marrow*	Spinal cord

		(3 levels)
Stomach*	Lymph nodes*	Pituitary*
Duodenum*	Spleen*	Eyes(optic nerve)
Jejunum*	Thymus*	GLANDULAR
Ileum*	UROGENITAL	Adrenal*
Cecum*	W Kidneys*	Lacrimal gland*
Colon*	Urinary bladder*	Mammary gland*
Rectum*	W Testes*	Parathyroids*
W Liver*	Epididymides*	Thyroids*
Gall bladder*	Prostate	OTHER
Pancreas*	Seminal Vesicle	Bone*
RESPIRATORY	Ovaries	Skeletal musc.*
Trachea*	Uterus*	All gross lesions & masses.
Lungs*		

The above organs marked with W (brain, heart, liver, kidneys and testes) were weighed and compared to body weight changes.

a. Organ weights Of the liver of the MD and HD animals showed both absolute and relative reductions in weight. Statistical significance for the liver changes was seen only in the HD males and MD females. The rest of the organ weights were randomly distributed among the experimental groups.

b. Gross pathology - There were a minimal number of gross abnormalities noted. None were related to the SF exposures except for the fractured vertebrae of the HD female which was paralyzed during the initial 600 ppm exposures.

c. Microscopic pathology

Brain: A series of lesions were seen in the HD males and females such that only 1 male and 1 female was considered to have a normal brain. The brain lesions included gliosis, endothelial hypertrophy, cerebral malacia and vacuolation. Except for on MD female, the other LD and MD animals had normal brains.

Kidneys: Multifocal tubular mineralization was seen in 1 to 2 females throughout all experimental groups. Only two males in the MD group had this lesion. None of the lesions were relatable to the SF exposures.

Lungs: The majority of animals in all dose groups had abnormal lungs which included alveolar histiocytosis, fibrosis, hemorrhage, and inflammation. There were no dose related incidence to these lesions.

Nasal Tissues In all of the experimental groups, only 0 to 2 of the 7 animals were considered normal. Chronic inflammation of the submucosa was seen distributed in all groups. Exudate,

hyperplasia and hypertrophy was seen primarily in the males and females of the high dose groups.

Testes. Only the testes of the control and HD groups were examined. One of the HD animals had very severe atrophy of the seminiferous epithelium, unilaterally.

Trachea: Very slight chronic inflammation of the submucosa of one male of the MD group and one female of MD group.

Control and HD Tissues. The following organs were histologically examined for the control and HD animals: adrenal, aorta, appendix, none, bone marrow, cecum, epididymides, esophagus, eyes, gall bladder, heart, large intestine, larynx, mammary glands, lymph nodes, ovaries, oviducts, pancreas, parathyroids, peripheral nerves, pituitaries, prostate, sacculus rotundas, salivary glands, skeletal muscle, skin and subcutis, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, urinary bladder, uterus and vagina.

None of these organs indicated SF exposure relatable diseases.

DISCUSSION.

1. Author's Comments: The authors concluded that the NOEL for this study was 30 ppm (29.8 ppm) and the LEL was 100 ppm. The convulsions in the rabbits was in response to the 9 exposures at 600 ppm. The body weight decreases seen in the 100 and 300 ppm groups was dose related. Brain lesion were dose related and were consistent with a previous 2 week exposure study reported by Dow (Eisenbrandt et al., 1985).

The inflammation of the nasal tissues and the purulent exudate in the nasal passages and respiratory hypertrophy and hyperplasia was a response to the SF exposures. The WBC elevations were in response to these reactions.

Reviewers Comments. The reviewer agrees with much of investigator's comments, except for the conclusion that the NOEL= 30 ppm. From previous Dow studies (1959) and others, it is clear that the target organs for SF toxicity are the nasal pharyngeal tract; the pulmonary system; the brain, liver, kidney , bone and teeth. These effects are probably due to the conversion of SF to F in the body. Short--term exposures to SF have resulted in neurological (shaking and convulsions) and breathing difficulties. Humans exposed to SF and an irritant gas (CP) experienced nausea, vomiting, reddening of the eyes, nose, throat and lungs. (Occupational Health Guideline for SF, NIOSHs, 1978).

Previously reported studies by Dow (October 22, 1959) indicated adverse effects in several species exposed to 20 ppm or less over a one year period. The species included rats, mice and Guinea pigs. It was clear that a significant amount of F was found in the blood, teeth and urine; the lungs (pneumonitis), kideys and livers were damaged.

The present studies had intercurrent pathology of the nasal, pulmonary and renal systems which were seen in all test groups. These effects are frequently seen in these animals apparently due to the effects of cage bedding (consultation with pathologist, Lucas H. Brennecke, DVM, 3/18/93). The occurrence of these lesion in the control animals make it difficult to show clearly that the LD group (30 ppm) represented the NOEL for 13 weeks of SF inhalation exposure. The lack of lung weights is important.

The studies point to the need to fine tune the current study and probably to extend the exposure time to 6 months or more. It should be possible to use animals which have less intercurrent pathology in the test groups and to expand the analyses of organ changes. Beside the lung weights, it should be desirable carry out lung function studies and to analyze for blood gas changes, pH changes.

* Recommended by Subdivision F (Oct. 1982) guidelines for subchronic studies.

SF90DINH.RBT ~~3/19/92~~ 4/1/92 SBG

VIKANE (Sulfuryl fluoride) TOX Review 009479

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CASWELL FILE
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3/3/92 JW 3-3-92

Reviewed by: Nguyen B. Thoa & John E. Whalan
Section I, Tox. Branch I (H7509C)
Secondary reviewer: Roger L. Gardner
Section I, Tox. Branch I (H7509C)
3/3/92

GUIDELINE: 82-5

009479

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Neurotoxicity Study in Rats Dosed by Inhalation

MRID NO: 408399-02

TOX. CHEM. NO.: 816A

TEST MATERIAL: Sulfuryl fluoride (99.8 % pure; colorless, odorless gas; lot no. TWP 830919-408)

SYNONYMS: Vikane® Gas Fumigant

STUDY NUMBER(S): K-016399-026

SUBMITTED BY: Dow Chemical Company

TESTING FACILITY: Mammalian and Environmental Toxicology Research Laboratory, Dow Chemical Company

TITLE OF REPORT: Neurological Examination of Fischer 344 Rats Exposed to Sulfuryl Fluoride (Vikane® Gas Fumigant) for 13 Weeks

AUTHOR(S): J.L. Mattsson, R.R. Albee, D.L. Eisenbrandt, and K.D. Nitschke

REPORT ISSUED: November 21, 1986

CONCLUSIONS: Sulfuryl fluoride gas was administered by inhalation to rats at concentrations of 0, 30, 100, and 300 ppm for 6 hours/day, 5 days/week for 13 weeks. At the conclusion of dosing, each rat was subjected to a functional observational battery (FOB) and electrophysiological testing including a visual evoked response (VER), cortical flicker fusion (CFF), auditory brain stem response (ABR), somatosensory response (SER and CER), and caudal nerve evoked action potential (CNAP).

The NOEL for both systemic and neurotoxic effects was 30 ppm. The LOEL was 100 ppm for both systemic effects (fluorosis of teeth, pale foci in pleura) and neurotoxic effects (slowing of VER and SER waveforms in females, and ABR waveform in males). The high-dose produced in both sexes a significant decrease in terminal body weight, fluorosis of the

teeth, pale foci in the pleura, poor grooming, excessive salivation, and a slowing of all waveforms except the CNAP. There was also a decrease in the power of the ABR, and vacuolation of the caudate putamen white matter. These electrophysiological and neuropathological findings suggest that sulfuryl fluoride may have a generalized white matter degenerative effect on the brain. The decreases in ABR (III-V) power suggests that the upper brain stem may be affected more than the other structures.

STUDY CLASSIFICATION: This study is **Core Supplementary**, and thus does not satisfy data requirement 82-5 for a 90-Day Neurotoxicity Study (new neurotoxicity guidelines, EPA 540/09-91-123; PB 91-154617, March 1991). This study received Quality Assurance review.

Each dose group consisted of 7 rats/sex instead of 10 rats/sex. Only 2 rats/sex/group were used in the recovery period, which is too few for meaningful evaluation. Daily observations of clinical signs (including prior to surgery), weekly observations of forced motor activity, and functional observation batteries (required prior to dosing, and at weeks 4, 8, and 13) were absent from this study. The lack of this information made it difficult to find a correlation with the neurologic findings. Because brain lesions were found in the high-dose group, sequential histopathologic evaluations should have been conducted in the mid and low-dose groups as needed. Positive control and historical data should have been provided to demonstrate the sensitivity of the electrophysiological tests.

****

PROTOCOL: Groups of 7 male and 7 female Fischer 344 rats were individually housed in stainless steel cages with food and water available *ad libitum*. At study commencement, they were 7 weeks old. The rats were dynamically exposed in 4.1 m^3 stainless steel and glass chambers to sulfuryl fluoride nominal concentrations of approximately 0, 30, 100, or 300 ppm. Chamber concentrations were measured one or two times hourly with a Miran 1A infrared spectrophotometer.

Surgery:

Two months into the study, each rat was withheld from exposure for a day to permit surgical implantation of epidural electrodes. Each rat was anaesthetized (zylazine and ketamine I.M.), the cranial fur was clipped, and the head fixed in a stereotaxic apparatus. Epidural electrodes (# 0-80, stainless steel set screws, 7 mm in length) were inserted into the skull. The electrodes were held in place with dental acrylic, with a 3 mm length exposed above the acrylic for connection to recording equipment. The somatosensory electrode was located 1.5 mm posterior and 3 mm lateral left of the bregma (coronal and sagittal suture intersection). The visual cortex electrode was 6.8 mm posterior and 3 mm lateral right of the bregma. The cerebellar electrode was 12 mm posterior and 0 mm lateral of the bregma. The reference electrode was 7 mm anterior and 1 mm lateral left of the bregma. All the implantations were completed within 5 days.

Clinical Observations, Body Weights, and Pathology:

Body weights were measured weekly. The rats were observed for clincal signs only at the end of the dosing period.

Eight rats, 2 males and 2 females from the control and 300 ppm groups, constituted a recovery group. They were sacrificed two months after exposures ceased. All other rats were sacrificed immediately after the exposure period. Every rat was necropsied. An *in situ* examination of the eyes was performed using a glass slide technique with fluorescent illumination. The following tissues were preserved:

HISTOPATHOLOGY

Liver	Lacrimal glands	Sebaceous gland
Pancreas	Harderian glands	Spleen
Sciatic nerve	Oral tissues	Pituitary
Tibial nerve	Heart	Bone marrow
Caudal nerve	Brain	Stomach
Optic nerve	Spinal cord	Large intestine
Adrenals	Kidneys	Testes
Small intestine	Cecum	Coagulating glands
Mesenteric lymph node	Mesenteric tissues	Ovaries
Epididymides	Seminal vesicles	Vagina
Prostate	Uterus	Skeletal muscle
Oviducts	Cervix	Mediastinal tissues
Urinary bladder	Lungs (infused)	Esophagus
Salivary glands	Thymus	Trachea
Mediastinal lymph node	Aorta	Mammary gland
Thyroid	Parathyroid	Nasal tissues
Larynx	Skin	Bone
Eyes	Tongue	

Of these tissues, only the liver, kidneys, testes, ovaries, lungs, eyes, oral tissues, external skin, colon, and lymph nodes were evaluated histopathologically for all rats. Representative sections of middle ear tissues were prepared (6 males and 1 female from the control group, and seven 100 ppm males). Additional sections were prepared for 3 control males from a previous study - a standard 13-week study (Study No. K-016399-025). These animals did not have surgical implants. Cerebrum sections were prepared from a control male and a 300 ppm male that died during implant surgery.

The brains were histopathologically evaluated by Dr. Louis W. Chang of the University of Arkansas (3, 6, 7, and 4 males, and 5, 7, 7, and 5 females in the control, low-dose, mid-dose, and high-dose groups, respectively). The right hemisphere was bisected longitudinally, and the left hemisphere was cut into 5 equal cross-sections yielding 7 tissue specimens per brain.

Ten μm sections were stained with hematoxylin and eosin. Sections from the control and high-dose groups were also prepared with three special stains, including gallocyanine for detection of any changes in the neuronal Nissl substance, Luxol fast blue for detection of any changes in the myelin sheaths, and Bodian for detection of any axonal changes. Holzer stain was used on sections from the long-term recovery animals to detect any scarring in damaged brain areas.

Functional Observational Battery (FOB):

An FOB was administered to the rats once, 12 hours after the final exposure. The rats were evaluated for evasive behavior (reactivity to removal and handling), hair condition, salivation, lacrimation, urine and fecal staining, muscle tone, presence of tremors, open field locomotion (gait, pattern, and intensity), and responses to sensory stimuli (touch, tail pinch, and sharp noise). The evaluations were conducted in a blind fashion.

Electrophysiological Tests:

The following electrophysiological tests were conducted after the 13-week dosing period:

Visual evoked response (VER) - Low intensity strobe flashes (0.300+/-0.003 cd-s/m^2) provided the stimuli for the VER at a rate of 0.9 flashes/sec.

Cortical flicker fusion (CFF) - The maximum rate of flashes that will produce a synchronized cortical response (CFF) was determined by increasing or decreasing the nominal flash rate of 48 Hz in 2 Hz steps.

Auditory brain stem responses (ABRs) - For testing of cochlear function, the tone pips were 4 kHz (low frequency) and 16 kHz (high frequency). The pips (1.2 msec. rise/fall, no plateau, 2.4 msec. total duration) were delivered at a rate of 19.1 pips/sec (recording at 60 and 80 dB SPL). For testing of postcochlear auditory pathway, clicks were delivered at the same rate.

Somatosensory evoked response (SER) - Stimuli for the SER were electrical pulses (3 mA, 50 usec., 1.1 pulses/sec) which were delivered to the ventrolateral caudal nerves at the base of the tail via a small needle electrode.

Cerebellar evoked response (CER) - Response to stimulation of the ventrolateral caudal nerves was also recorded with the cerebellar electrode.

Caudal nerve evoked action potential (CNAP) - Stimuli for the CNAP were electrical pulses (3 mA, 20 usec., 10.1 pulses/sec) which were delivered consecutively as a single and a pair of pulses (2 msec interval) via a small needle electrode inserted into the tip of the tail.

The rats were restrained, but were fully conscious during the 35-minute battery of tests. Their rectal temperatures were recorded prior to each test. The electrophysiological system used to record and analyze the data was a Nicolet Path finder II (Nicolet Biomedical Instruments, Madison, Wi).

Statistical Analyses:

The following were stated regarding the statistical analyses of the data :

> "Electrophysiology data (optimal correlation, latency, and power) were analyzed by a factorial multivariate analysis of variance (MANOVA), using the REG procedure of SAS (SAS Institute inc., Carey, NC). The factor was sex. The main effect (treatment), sex, and the sex by treatment interaction were assessed by an F-test based on the Hotelling-Lawley trace statistics (alpha = 0.05). If no meaningful interaction patterns were found, multivariate contrasts of each treatment level versus control (using the overall pooled variance) were further examined. The statistical procedure also provided analogous univariate analyses for the 3 dependent variables of the MANOVA: optimal correlation, latency, and power. Single variables measures (body weight, CNAP latency, grip strength, temperature) were similarly tested with the factorial univariate analysis of variance (ANOVA). Analysis of covariance (ANCOVA), with temperature as the covariate, were also used when temperatures were significantly elevated.
>
> Because numerous measurements were statistically compared in the same animals, the overall false positive rate (type I error) was greater than the cited alpha would suggest. For this reason, a firm statement of statistical probability cannot be made. To assist in data analysis, however, an alpha = 0.05 was regarded as statistically suggestive and an alpha = 0.01 was regarded as statistically significant".

RESULTS: The rats received 71 exposures to the test article over 90-days. Although chamber concentrations varied as much as ± 23%, the overall mean concentrations were very close to the nominal concentrations:

Nominal Concentration mg/l/day	Analytical Concentration mg/l/day
0	0
30	29.8 (23.7-33.6)
100	100 (81-120)
300	297 (231-314)

Four male rats died during the study - three due to anesthetic complications, and one (control) due to a cage transfer accident. These rats were necropsied, but their eyes were not examined.

None of the rats were observed for clinical signs at any point in the study, including prior to surgery. At the end of the dosing period, all rats appeared to be in good health, although some of the high-dose rats had unkempt fur and excessive lacrimation. High-dose body weights were significantly reduced compared to controls (♂ -15%; ♀ -22%). Body weights in the low and mid-dose groups were not significantly different from control values.

All rats in the mid and high-dose groups had tooth mottling, presumably due to fluorosis. Pale foci were seen on the pleura of all high-dose rats and one male and one female in the mid-dose group. All of the high-dose rats in the recovery group had pale foci on their pleura.

There was no mention of histopathologic lesions in any nervous tissues other than the brain. The only lesion found in the brain was vacuoles in the white fiber tracts of the caudate-putamen in all the high-dose rats (no other dosed groups were evaluated). There was no evidence of necrosis or neuronal destruction. The vacuolation was absent in the high-dose recovery group which suggests that the lesion was reversible.

Functional Observation Battery (FOB):

Most of the abnormal changes were observed in the high-dose group; 3 out of 6 males and 4 out of 7 females had unkempt hair coat and 3 rats of each sex had excessive lacrimation. One mid-dose male had decreased response to noise, but this was considered coincidental because of a lack of dose-effect relationship. All other FOB parameters examined were comparable between groups (See table 1 below).

Table 1
Observational Battery Summary

Observation	Exposure Concentration (ppm)			
	0	30	100	300
			MALES	
Evasion	5	6	7	6
Muscle tone	5	6	7	6
Tremor	5	6	7	6
Coat	5	6	7	3(3)
Salivation	5	6	7	6
Lacrimation	5	6	4(3)	3(3)
Urine staining	5	6	7	6
Fecal staining	5	6	7	5
Locomotion	5	6	7	6
Touch response	5	6	7	6
Noise response	5	6	6	6
Pinch response	5	6	7	6
			FEMALES	
Evasion	7	7	7	7
Muscle tone	7	7	7	7
Tremor	7	7	7	7
Coat	7	7	7	3(4)
Salivation	7	7	7	7
Lacrimation	7	6(1)	7	4(3)
Urine staining	7	7	7	5(2)
Fecal staining	7	7	7	7
Locomotion	7	7	7	7
Touch response	7	7	7	7
Noise response	7	7	7	7
Pinch response	7	7	7	7

Data excerpted from tables 5a and 5b of study.
Numbers represent the number of rats with normal responses.
( ) = number of animals with unkempt fur coat, excessive lacrimation, or urine staining.

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Electrophysiological Tests:

Most of the abnormal changes were observed in the high-dose group, including a statistically significant lengthening of the latencies (i.e. slowing) of the VER, ABR (peaks III-V), CER (4-25 msec and 25-70 msec), and SER (20-90 msec) waveforms, and a decrease in the CFF in both sexes. The VER and SER waveforms were also statistically significantly slowed in females of the mid-dose group, and the ABR waveform was slowed in the mid-dose males. In addition, the ABR (III-V) power was slightly, but statistically significantly, reduced in the high-dose male and female groups (see attached figures 1-4 of study and table 2 below).

All other electrophysiological parameters examined were comparable between groups. Rectal temperatures in the high-dose group were stated to be slightly lower than that of the control group during the VER, CFF, and ABR tests, but were back to control levels at the end of the 35 minute testing period. The authors stated that statistical differences in latency were still present after statistical analyses using body temperature as a covariate. The 4 recovery rats had control-like ABRs (see attached figure 5 of study).

Table 2
Electrophysiological Changes

Ppm	Sex		VER Lat.	CFF Hz	ABR (III-V) Lat.	ABR (III-V) Power	CER (Lat.) 4-25	CER (Lat.) 25-70	SER Lat.
0	♀	$\bar{x}$	0.00	47.47	0.00	1.15	0.25	0.51	0.17
		SD	6.08	2.14	0.09	0.17	0.89	2.79	3.18
		N	7	7	7	7	7	7	7
0	♂	$\bar{x}$	-0.18	45.26	0.02	1.20	-0.07	-0.54	-0.36
		SD	2.01	3.10	0.10	0.39	0.23	3.14	0.96
		N	5	5	5	5	5	5	5
100	♀	$\bar{x}$	11.9						4.44
		SD	8.99						3.32
		N	7						7
300	♀	$\bar{x}$	10.9	42.67	0.16	0.90	1.27	4.05	5.19
		SD	7.19	1.63	0.13	0.19	0.80	1.94	3.08
		N	6	6	7	7	7	7	7
300	♂	$\bar{x}$	5.91	42.67	0.18	0.88	0.89	2.75	3.90
		SD	7.08	1.63	0.11	0.26	0.48	1.41	2.61
		N	6	6	6	6	6	6	6

Data excerpted from table 4 of study. All values from treated groups are statistically significantly different from control group at $p < 0.01$.
Lat.= latency measured in msec.

Author's Discussion:

"The findings of the current study were milder but consistent with rabbit data in a 2-week probe study (Eisenbrandt et al., 1985). These rabbits had vacuolation and malacia in the basal ganglia, which was moderate at 300 ppm and severe at 600 ppm. Rats in the probe study, however, did not have brain lesions.

All of the evoked responses in rats exposed to 300 ppm for 13 weeks were affected, which suggested a widespread CNS effect. Vacuoles were limited to an area within the caudate putamen. Therefore mechanisms other than vacuolation were considered responsible for much of the electrophysiologic slowing. Differences in body temperature were excluded as a cause by analysis of covariance. Statistical differences in latency were still present after analyses using body temperature as a covariate. In addition, there were no significant differences in temperature when ABR's were recorded.

Minimal changes in evoked responses occurred at 100 ppm, and no treatment-related changes were seen at 30 ppm. The recovery of ABR and brain histopathology of 300 ppm rats indicated that the treatment effects were, to at least a great extent, reversible".

Reviewer's Discussion:

The reviewer agrees with the authors that sulfuryl fluoride may have a widespread CNS effect. The slowing of most of the evoked responses (VER, SER, CER, and ABR III-V) suggests a deleterious effect on the brain white matter (myelinated fibers) at the visual, auditory, somatosensory, and cerebellar cortices. Additionally, since the power of the ABR (III-V) waveform was also affected, and since ABR (III-V) are considered related to the upper brain stem, it would appear that this site was more affected than the other areas where the evoked responses were recorded. A decrease in power may be associated with damage to nerve cells or nerve connections.

The caudate putamen is concerned with unconscious movements of the skeletal muscles. It is curious that vacuolation in the white matter of the caudate putamen in the high-dose group was not accompanied by any increase in tremor incidence. This suggests that either the lesion was of insufficient severity to be expressed as tremors, or there was sufficient neuronal reduncancy to preclude tremor expression. Because brain lesions were found in the high-dose group, sequential histopathologic evaluations should have been conducted in the mid and low-dose groups as needed.

Considering that the electrophysiological battery for each rat took 35 minutes to complete, the testing must have been performed over the space of several days; the report failed to mention the duration of the testing. This delay could have allowed time for reversal of the brain lesions.

The recovery groups contained too few animals for meaningful interpretation. Considering that the only electrophysiological test performed on these rats was the ABR, it is inappropriate to claim that there was a complete recovery in these animals.

VIKANE (Sulfuryl fluoride) TOX Review 009479

Page____ is not included in this copy.

Pages 62 through 66 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

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____ Sales or other commercial/financial information.

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