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SULFURYL FLUORIDE

2-WEEK INHALATION STUDY, RABBITS

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DATA EVALUATION RECORD

STUDY TYPE: 2-Week Subchronic Inhalation Study, Rabbits OPPTS: None

DP BARCODE:
P.C. CODE: 078003

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

TEST MATERIAL: Sulfuryl fluoride

SYNONYMS: VIKANE™ gas fumigant, SO_2F_2

CITATION: Eisenbrandt, D.L., Nitschke, K.D., Streeter, C.M., and Wolfe, E.L. (1985) Sulfuryl Fluoride (Vikane* gas fumigant): 2-Week inhalation toxicity probe with rats and rabbits. Mammalian and Environmental Toxicology Research Laboratory, Health and Environmental Sciences U.S.A., Dow Chemical U.S.A., Midland, Michigan 48640. Laboratory project identification: none. April 2, 1985. MRID 148956. Unpublished.

SPONSOR: DowElanco, 9330 Zionsville, Indianapolis, IN 46268

EXECUTIVE SUMMARY: In a 2-week subchronic inhalation toxicity study (MRID 148956), three New Zealand white rabbits/sex/exposure group were exposed to sulfuryl fluoride gas (Vikane Gas Fumigant, lot #TWP 830919-408, 99.8% active ingredient) at exposure concentrations of 0, 100, 300, or 600 ppm for 6 hours/day, 5 days/week for a total of 9 exposures (calculated to be equivalent to 0, 30, 90, or 180 mg/kg/day for both males and females). The animals were sacrificed and examined the day after the last exposure. Mortality, clinical signs, body weights, hematology, clinical chemistries and organ weights were evaluated and gross and microscopic examinations were performed.

At 600 ppm, one female rabbit convulsed after 5 exposures, which caused a fractured tibia, and another female rabbit may have convulsed after 6 exposures because it had a fractured vertebrum (a convulsion was not actually observed). Both animals were euthanized. The surviving rabbits at 600 ppm were noted to be slightly hyperactive during the treatment period. At necropsy, treatment-related focal malacia (necrosis) was observed in the cerebrum of all surviving 600 ppm animals and in 1 male and 1 female animal at 300 ppm. In addition, vacuolation of the same part of the cerebrum was observed in all surviving 600 ppm animals and all 300 ppm animals. The

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lesion in the cerebrum was restricted to the globus pallidus and putamen (basal nuclei) and external and internal capsules (myelinated tracts). Most rabbits at 600 ppm and 300 ppm had moderate inflammation of the mucosa of the nasal tissues and most rabbits at 600 ppm also had inflammation of the mucosa of the trachea and larynx. One female at 600 ppm had inflammation of the bronchi and bronchioles also. Males at 600 ppm also had statistically significant decreased body weight at the terminal sacrifice (at 12 days) and possibly decreased absolute brain weights. An altered cytoplasmic homogeneity of the liver hepatocytes was also noted in all 600 ppm animals and in three 300 ppm animals. No exposure-related changes were observed in rabbits in the 100 ppm exposure group.

The LOAEL is 300 ppm (90 mg/kg/day) based on focal malacia (necrosis) in the cerebrum in 1 male and 1 female, vacuolation in the cerebrum in all male and females, moderate inflammation of nasal tissues in most animals and an altered cytoplasmic homogeneity of the liver hepatocytes in some animals. The NOAEL is 100 ppm (30 mg/kg/day).

This subchronic inhalation toxicity study is classified as an **Acceptable/Non-Guideline** study. Only 3 rabbits/sex/dose level were used and the duration of the study was only 2 weeks (9 exposures). The study was well conducted and reported.

COMPLIANCE: A signed and dated Quality Assurance statement was provided.

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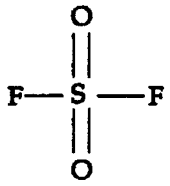
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Sulfuryl fluoride

Description: colorless, odorless gas
CAS No.: 2699-79-8
Lot No.: #TWP 830919-408
Purity: 99.8% a.i.
Structure:



2. Vehicle and/or positive control

Not applicable

3. Test animals

Species: Rabbits
Strain: New Zealand White
Age and weight at study initiation: approximately four months old;
males: 2926-3345 g, females: 2981-3637 g
Source: Hazelton Dutchland, Inc., Denver, Pennsylvania
Diet: Certified Purina Chow 5322, ad libitum except during exposure
Water: Municipal tap water, ad libitum except during exposure
Environmental conditions:
Temperature: approximately 72°F
Humidity: approximately 50%
Photoperiod: 12 hr light/12 hr dark
Acclimation period: at least 14 days

B. STUDY DESIGN

1. In life dates

Start: November 27, 1983; end: December 7, 1983

2. Animal assignment

The number of animals assigned to the exposure groups is listed in Table 1.

TABLE 1: Animal assignment

Exposure Group	Target Exposure Concentration (ppm)	Mean Exposure Concentrations (ppm)	Animals	
			Male	Female
Control	0	0	3	3
Low	100	100 ± 6	3	3
Mid	300	293 ± 17	3	3
High	600	597 ± 13	3	3

Data taken from Table 1, p.18, MRID 148956.

3. Doses and exposures

In this two-week inhalation study, 3 New Zealand White rabbits/sex/exposure group was exposed to sulfuryl fluoride gas at target exposure concentrations of 0, 100, 300, or 600 ppm for 6 hours/day, 5 days/week for a total of 9 exposures.

4. Exposure chambers

The volume of the exposure chambers was 4.1 m^3. The airflow rate through the chambers was approximately 800 L/min. The temperature and relative humidity in the chambers were controlled by a system designed to maintain temperature and relative humidity at approximately 70°F and 50% humidity, respectively. No indication was given that the oxygen concentrations were measured.

5. Generation system

Sulfuryl fluoride gas was diluted with the main air stream prior to entering the exposure chamber.

6. Chamber monitoring

The concentration of sulfuryl fluoride gas in the exposure chamber was determined about 2 time/hour with a MIRAN 1A infrared spectrometer at a wavelength of 11.8 microns. The infrared spectrometer was calibrated with air standards made by diluting measured volumes of sulfuryl fluoride gas with a measured volume of filtered compressed air. The concentration of sulfuryl fluoride in the chamber was determined by interpolation from known standards. The analytical system was evaluated with at least one standard prior to each day of exposure.

7. Statistics (The following is quoted from page 8 of the study report, MRID 148956).

"Body weights, organ weights, hematology data, clinical chemistry data, and urinary specific gravity were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or non-parametric analysis of variance (ANOVA), followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons. Statistical outliers were identified by a sequential test and excluded from analysis only for documented, scientifically sound reasons unrelated to treatment.

The nominal alpha levels used and test references were:

Bartlett's test	$\alpha = 0.01$
Parametric ANOVA	$\alpha = 0.10$
Non-parametric ANOVA	$\alpha = 0.10$
Dunnett's test	$\alpha = 0.05$, two-sided
Wilcoxon Rank-Sum test	$\alpha = 0.05$, two-sided
Outlier test	$\alpha = 0.02$, two sided"

C. METHODS

1. Observations

Animals were observed after each exposure period and an additional one time each day for mortality, morbidity, signs of clinical toxicity, and availability of feed and water. On weekends and holidays, the animals were not observed for signs of clinical toxicity.

2. Body weight

Animals were weighed prior to the initial exposure, prior to the fifth and sixth exposures, and at termination of the study.

3. Food consumption

Food consumptions were not determined.

4. Ophthalmoscopic examination

Eyes of animals surviving to termination of the study were examined the day after the last exposure. Eye examinations were performed in situ by a glass-slide technique with fluorescent illuminiation.

5. Blood was collected for hematological examinations by venipuncture prior to the ninth exposure. Blood was also collected for clinical chemistries from cervical blood vessels at the terminal sacrifice. The CHECKED (X) parameters were examined.

a. Hematology

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

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical chemistry

	ELECTROLYTES		OTHER
	Calcium*	X	Albumin*
	Chloride*		Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*		Total Cholesterol
	Potassium*	X	Globulins
	Sodium*	X	Glucose*
			Total bilirubin*
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	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 subchronic studies based on Subdivision F Guidelines

6. Urinalysis

Not performed. (Not required for subchronic studies based on Subdivision F Guidelines)

7. Sacrifice and pathology

Gross necropsies were performed on all animals that were moribund or died during the study. Survivors were sacrificed and necropsied the day after the last exposure. The rabbits were not fasted prior to sacrifice. Each animal was euthanized with carbon dioxide, and then decapitated (after the trachea was clamped). The CHECKED (X) tissues listed below were collected from each animal for possible histological examination. Lungs were perfused with buffered formalin to approximate normal inspiratory volume and the nasal cavity was flushed with formalin via the pharyngeal duct to ensure rapid fixation. The (XX) organs, in addition, were weighed.

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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*
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL		Adrenal gland*
X	Large intestine*	XX	Kidneys*+	X	Lacrimal gland
	Rectum*	X	Urinary bladder*		Mammary gland*
XX	Liver*+	XX	Testes*+	X	Parathyroids*++
X	Gall bladder*	X	Epididymides	X	Thyroids*++
X	Pancreas*	X	Prostate		
X	Oral tissue		Seminal vesicle		OTHER
X	Appendix	X	Ovaries*+	X	Bone*
	RESPIRATORY	X	Uterus*	X	Skeletal muscle*
X	Trachea*	X	Cervix	X	Skin*
X	Lung*	X	Oviducts	X	All gross lesions and masses*
	Nose	X	Vagina	X	Mediastinal tissue
	Pharynx			X	Mesenteric tissue
X	Larynx				Tonsils
X	Nasal tissue				Lower jaw (one-half) with teeth**
					Femur**
				X	Sacculus rotundus

Data taken from Table 3, p.20, MRID 148956.
* Required for subchronic studies based on Subdivision F Guidelines.
+ Organ weight required in subchronic studies.
++ Organ weight required for non-rodent studies.

Tissues from all control and 600 ppm group animals were examined. In other groups, target organs and several other tissues were examined. Tissues were stained with H & E. In addition, the cerebrum was stained with luxol fast blue-PAS and Sevier-Munger stains.

II. RESULTS

A. CHAMBER CONDITIONS

The sulfuryl fluoride concentrations, temperatures, and relative humidities were within the target range for each parameter. The average mean analytical concentrations (+SD) of sulfuryl fluoride for the target concentrations of 0, 100, 300, or 600 ppm were 0, 100±6, 293±17, and 598±13 ppm, respectively. The mean temperatures and relative humidities (+SD) ranged from 21±1°C to 25±1°C and 42±5% to 51±4%, respectively.

B. OBSERVATIONS

1. Clinical Signs of Toxicity

One female rabbit in the 600 ppm group, after the fifth exposure, had convulsions which caused a fractured tibia. Another female rabbit in the 600 ppm group, after the sixth exposure, had a fractured vertebrum, although a convulsion was not observed in this animal. Both of these females were euthanized. The remaining 600 ppm female and 3 males survived to termination of the study, but appeared slightly hyperactive compared to the control animals. Rabbits in all the other groups appeared normal at all times.

2. Mortality

Two female rabbits in the 600 ppm group were euthanized during the treatment period. See above under "Clinical Signs of Toxicity".

C. BODY WEIGHT

Mean body weights (g + S.D.) for male and female rabbits exposed to sulfuryl fluoride gas are presented in Table 2. No statistically significant body weight differences were observed for any of the treated male or female groups compared to their respective control groups, except for the terminal mean body weights for the 600 ppm males. The statistically significant decreased mean body weight for the 600 ppm males at day 12 (termination) is considered to be most likely treatment-related.

TABLE 2: Mean body weight (g +S.D.) for rabbits exposed to sulfuryl fluoride

Study Day	Exposure Group (ppm) [a]			
	0	100	300	600
	Males			
1	3202 ± 150	3171 ± 135	3055 ± 139	3233 ± 75
5	3205 ± 145	3165 ± 126	3068 ± 117	3195 ± 82
8	3232 ± 145	3208 ± 134	3088 ± 172	3156 ± 80
12 (terminal)	3250 ± 159	3212 ± 130	3062 ± 146	2918 ± 109 *
	Females			
1	3424 ± 163	3421 ± 52	3298 ± 329	3391 ± 93
5	3483 ± 184	3508 ± 140	3433 ± 392	3178 ± 170
8	3537 ± 197	3548 ± 163	3419 ± 414	3090 ± 387 [b]
12 (terminal)	3503 ± 212	3594 ± 176	3384 ± 409	3315 ± 0 [c]

Data taken from Table 11, p.37, MRID 148956.
[a] N = 3 animals/group except where noted
[b] N = 2 animals
[c] N = 1 animal
* Statistically significant difference from control group mean; Dunnett's test, $\alpha = 0.05$.

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 treatment-related changes were observed in any animal.

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F. BLOOD

1. Hematology

No treatment-related differences in any of the mean hematological parameters were observed between any of the treated male or female groups compared to their respective control groups.

2. Clinical chemistry

No treatment-related differences in any of the mean clinical chemistry parameters were observed between any of the treated male or female groups compared to their respective control groups.

G. URINALYSIS

N/A

H. SACRIFICE AND PATHOLOGY

1. Organ weight

The mean absolute brain weight in the 600 ppm males (9.043 g $\pm$ 0.280) was significantly decreased ($p<0.05$) when compared to the control males (9.743 g $\pm$ 0.388). The mean relative brain weight for this group (0.310 $\pm$ 0.021), however, was similar to that of the control males (0.300 $\pm$ 0.030). Because of the treatment-related histopathological changes observed in the brains of all rabbits at 600 ppm (malacia and vacuolation in the cerebrum), this decrease in mean absolute brain weight in the 600 ppm males is considered to be possibly related to treatment with the test material.

An increased absolute and relative heart weight in the 600 ppm females was not attributed to treatment with the test material because only one female was examined at this concentration level. A statistically significant increase in mean absolute and relative liver weight in the 100 ppm females also was not attributed to treatment with the test material because it was clearly not dose-related. No other organ weight changes in any of the animals were considered to be related to the test material.

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2. Gross necropsy

No gross necropsy findings (other than the fractured tibia and vertebrum in the two 600 ppm females) were considered to be treatment-related.

3. Microscopic pathology

In this 2-week inhalation study on rabbits, the brain and the upper respiratory system were the primary target sites affected by treatment with sulfuryl fluoride. Selected microscopic lesions are presented in Table 3. At 600 ppm, treatment-related bilateral, focal malacia (necrosis) in the cerebrum was observed in all 3 males and all 3 females. In males, the severity of the lesion was moderate in all 3 rabbits; in females, it was moderate in 1 female (the survivor) and slight in the 2 females that were euthanized prior to termination of the study. The same lesion was also observed at 300 ppm in 1 male (moderate severity) and in 1 female (slight severity). Reactive gliosis and demyelination accompanied the malacia. Also at 600 ppm, treatment-related bilateral, focal vacuolation in the cerebrum was observed in all 3 males and all 3 females. In males, the severity of the lesion was slight in all 3 rabbits; in females, it was slight in 1 female (the survivor) and very slight in the 2 females that were euthanized prior to termination of the study. The same lesion was also observed at 300 ppm in 3 males (slight severity) and in 3 females (slight severity). For both the malacia and the vacuolation, the lesions in the cerebrum were restricted to the globus pallidus, putamen (basal nuclei), and external and internal capsules (myelinated tracts).

At 600 ppm, treatment-related acute, diffuse inflammation of the mucosa of the trachea was observed in 2 males (slight severity) and 2 females (slight severity). Also at 600 ppm, subacute to chronic multifocal inflammation of the mucosa of the nasal tissues with a mucopurulent exudate was observed in all 3 males (moderate severity) and all 3 females (moderate severity). The same lesion was also observed at 300 ppm in 3 males (moderate severity) and in 2 females (moderate severity in 1 and slight severity in 1). The same lesion, however, was also observed in 2 control males (slight severity) and in all 3 control females (slight severity). Also at 600 ppm, acute inflammation of the mucosa of the larynx was observed in all 3 males (slight severity in 2 and moderate severity in 1) and in 2 females (slight severity in 1 and moderate severity in 1). In females, however, the same lesion was also observed in 2 control animals (very slight severity in 1 and slight severity in the other). Although also observed in control animals, the severity of the inflammation in the nasal tissues was increased in the 600 ppm and 300 ppm rabbits and is

considered to be related to treatment with the test material. In addition, 1 female at 600 ppm also had inflammation of the bronchi and bronchioles.

In the liver, an altered cytoplasmic homogeneity of the hepatocellular cells was observed at 600 ppm in all 3 males and all 3 females, and at 300 ppm in 2 males and 1 female. This lesion was considered to be treatment-related.

In the 2 female rabbits that were euthanized prior to termination of the study because of fractured bones, several lesions were observed in these animals that were considered to be secondary to the fractured bones and not directly related to treatment with the test material. These lesions included malacia of the adjacent spinal cord and degeneration of the adjacent psoas muscle in the rabbit with the fractured vertebrum; and certain non-specific histopathology such as depletion of lymphoid tissue in the thymus, degeneration of the pancreas, and altered cytoplasmic homogeneity of renal tubules.

TABLE 3: Number of rabbits with selected microscopic lesions

	Males				Females			
Dose (ppm)	0	100	300	600	0	100	300	600
No. exam.	3	3	3	3	3	3	3	3
Lesion								
Brain								
Malacia, cerebrum, bilateral, focal sl	0	0	0	0	0	0	1	2
mod	0	0	1	3	0	0	0	1
Brain								
Vacuolation, cere- v. sl	0	0	0	0	0	0	0	2
brum, bilat., focal sl	0	0	3	3	0	0	3	1
Liver								
Altered cytoplasmic homogeneity, hepatocell	0	0	2	3	0	0	1	3
Trachea								
Inflammation, acute v.sl	0	0	0	0	2	0	0	0
mucosa, diffuse sl	0	0	0	2	0	0	0	2
Nasal Tissues								
Subacute to chronic sl	2	1	0	0	3	3	1	0
mucosa, multifocal mod	0	0	3	3	0	0	1	3
Larynx								
Inflammation, acute v.sl	0	0	0	0	1	0	0	0
mucosa sl	0	0	0	2	1	1	1	1
mod	0	1	0	1	0	0	0	1

Data taken from Table 16, pp.42-48, MRID 148956.

III. SUMMARY AND CONCLUSION

A. SUMMARY

In a 2-week subchronic inhalation toxicity study (MRID 148956), three New Zealand white rabbits/sex/exposure group were exposed to sulfuryl fluoride gas (Vikane Gas Fumigant, lot #TWP 830919-408, 99.8% active ingredient) at exposure concentrations of 0, 100, 300, or 600 ppm for 6 hours/day, 5 days/week for a total of 9 exposures (calculated to be equivalent to 0, 30, 90, or 180 mg/kg/day for both males and females). The animals were sacrificed and examined the day after the last exposure. Mortality, clinical signs, body weights, hematology, clinical chemistries and organ weights were evaluated and gross and microscopic examinations were performed.

At 600 ppm, one female rabbit convulsed after 5 exposures, which caused a fractured tibia, and another female rabbit may have convulsed after 6 exposures because it had a fractured vertebrum (a convulsion was not actually observed). Both animals were euthanized. The surviving rabbits at 600 ppm were noted to be slightly hyperactive during the treatment period. At necropsy, treatment-related focal malacia (necrosis) was observed in the cerebrum of all surviving 600 ppm animals and in 1 male and 1 female animal at 300 ppm. In addition, vacuolation of the same part of the cerebrum was observed in all surviving 600 ppm animals and all 300 ppm animals. The lesion in the cerebrum was restricted to the globus pallidus and putamen (basal nuclei) and external and internal capsules (myelinated tracts). Most rabbits at 600 ppm and 300 ppm had moderate inflammation of the mucosa of the nasal tissues and most rabbits at 600 ppm also had inflammation of the mucosa of the trachea and larynx. One female at 600 ppm had inflammation of the bronchi and bronchioles also. Males at 600 ppm also had statistically significant decreased body weight at the terminal sacrifice (at 12 days) and possibly decreased absolute brain weights. An altered cytoplasmic homogeneity of the liver hepatocytes was also noted in all 600 ppm animals and in three 300 ppm animals. No exposure-related changes were observed in rabbits in the 100 ppm exposure group.

B. CONCLUSION

The LOAEL is 300 ppm (90 mg/kg/day) based on focal malacia (necrosis) in the cerebrum in 1 male and 1 female, vacuolation in the cerebrum in all male and females, moderate inflammation of nasal tissues in most animals and an altered cytoplasmic homogeneity of the liver hepatocytes in some animals. The NOAEL is 100 ppm (30 mg/kg/day).

This subchronic inhalation toxicity study is classified as an **Acceptable/Non-Guideline** study. Only 3 rabbits/sex/dose level were used and the duration of the study was only 2 weeks (9 exposures). The study was well conducted and reported.

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