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

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MAY 21 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID No. 078003. Sulfuryl fluoride, technical.
Evaluation (Validation of Original Review) of Rat and
Rabbit Developmental Toxicity Studies Submitted to
Support Reregistration of Sulfuryl Fluoride.

Tox. Chem. No.: 816A
PC No.: 078003
Barcode No.: D177002
Submission No.: S416088

FROM: Linnea J. Hansen, Ph.D.
Section IV, Tox. Branch I
Health Effects Division (H7509C) *Linnea J. Hansen* 5-12-92

TO: Larry Schnaubelt, Manager, PM Team 72
Don Mackey, Reviewer, PM Team 72
Special Review and Registration Division (H7508W)

THRU: Marion P. Copley, D.V.M., D.A.B.T., Section Head
Section IV, Tox. Branch I
Health Effects Division (H7509C) *Marion P. Copley* 5/13/92

KPB
5/10/92

CONCLUSIONS:

TB-I reevaluated the two developmental toxicity studies (DERs attached to this memo) and found them both to be acceptable for regulatory purposes. TB-I agreed with the conclusions of the original reviewer. The HED Mini-Peer Review Committee for developmental toxicity also evaluated both studies and agreed with the conclusions. It was determined that sulfuryl fluoride was not a concern for developmental toxicity and that a full developmental toxicity peer review should not be initiated.

Classification: Both rat and rabbit, Core-minimum

Study results are summarized as follows:

(1) Rat (83-3a): Doses administered were 0, 25, 75 and 225 ppm by inhalation, Fischer 344 rats, 6 hr/day during Days 6 - 15 of gestation. Maternal and developmental NOELs were \geq 225 ppm. Although an LEL was not achieved, 225 ppm was believed to be approaching an LEL based on the results of the

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preliminary range-finding study (significant maternal toxicity at 300 ppm) and a probable threshold decrease in maternal weight gain at 225 ppm.

(2) Rabbit (83-3b): Doses administered were 0, 25, 75 and 225 ppm by inhalation, New Zealand White rabbits, 6 hr/day during Days 6 - 18 of gestation. Maternal and developmental NOELS were 75 ppm. Maternal LEL of 225 ppm was based on decreased body weight/weight gain during treatment. Developmental NOEL of 225 was based upon decreased fetal weight, decreased crown-rump length and possible slightly increased incidence of fetal liver pathology (pale liver).

ACTION REQUESTED:

DowElanco resubmitted legible copies of a rat and rabbit developmental toxicity study (Guideline 83-3a and b) of sulfuryl fluoride (MRID No. 00090015) for review by TB-I (validation of original evaluation of study, HED Doc. No. 001421)

HANSEN/PC-4/4SUFLDTX.MEM/SULFURYL FLUORIDE DEV. TOX RAT AND
RABBIT/5-92/D177002

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CASWELL FILE

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GUIDELINE: 83-3

Primary Review: Linnea J. Hansen, Ph.D. *Linnea J. Hansen 4/30/92*
Review Section IV, Tox. Branch I
Secondary Review: Marion P. Copley, D.V.M., D.A.B.T. *Marion Copley 4/30/92*
Review Section IV, Tox. Branch I

DATA EVALUATION RECORD

STUDY TYPE: Teratology-Developmental Toxicity *Follow up To NED doc #1421 (attached)*
Species: Rabbit
Guideline: 83-3 (b)

TOX. CHEM. NO.: 816A

PC NO.: 078003

ACCESSION NO.: 246489
MRID # 00090015

TEST MATERIAL: Sulfuryl fluoride, technical

SYNONYMS: Vikane®; CAS No. 2699-79-8

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

STUDY NUMBER: HET K-16399-(15)

TESTING FACILITY: Dow Toxicology Research Laboratory - Health
and Environmental Sciences

TITLE OF REPORT: Vikane®: Inhalation Teratology Study in Rats
and Rabbits

AUTHORS: T.R. Hanley, Jr., L.L. Calhoun, R.J. Kociba,
S.R. Cobel-Geard, W.C. Hayes, J.H. Quellette,
L.M. Scherbarth, B.N. Sutter and J.A. John

REPORT ISSUED: October 26, 1981

CONCLUSIONS:

Doses tested: 0, 25, 75 and 225 ppm via inhalation to
female New Zealand White rabbits, treated 6 hr/day during
Days 6 - 18 of gestation, inclusive.

Maternal NOEL: 75 ppm. LEL: 225 ppm, based upon decreased
body weight/body weight gain during treatment.

Developmental NOEL: 75 ppm. LEL: 225 ppm, based upon
decreased fetal weight, decreased crown-rump length and
possible slightly increased incidence (non-statistically
significant) of fetal liver pathology (pale liver).

This study satisfies the guideline requirements for a rabbit developmental study (83-3b)
TB-I agrees with the conclusions of the original review of

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this study. In addition to the fetal effects noted in the original review, TB-I considers the slight increase in pale livers at high dose a possible threshold effect, given that similar liver effects have been observed in adult animals in other studies with sulfuryl fluoride.

Core Classification: Minimum

A signed Quality Assurance Statement was not present.

A. VALIDATION OF REVIEW:

This DER is intended to validate and to supplement details of a previous review (HED Document No. 001421) of this study. The study is evaluated here according to the Guidelines for developmental toxicity studies (83-3) and the report prepared in the standard manner. A copy of the original review is appended to this document (Appendix 1).

B. MATERIALS

Test Compound: Sulfuryl fluoride, technical. Purity: 99.8%
Description: colorless, odorless gas
Lot No.: 217 (Dow Chemical)
Contaminant: Not described

Test Animal: Species: Rabbit
Strain: New Zealand White
Source: Langshaw Rabbitry, Augusta, MI
Age: Young adult (age not indicated)
Weight: 3.5 - 4.5 kg

C. STUDY DESIGN

This study was designed to assess the developmental toxicity potential of sulfuryl fluoride when administered by inhalation to pregnant female New Zealand White rabbits on gestation Days 6 through 18, inclusive.

Group Arrangement:

Female rabbits were assigned to the following treatment groups:

TABLE 1: ASSIGNMENT OF ANIMALS

Test Group	Dose Level (ppm)	Number Assigned (at mating)
Control	0	28
Low Dose	25	29
Mid Dose	75	28
High Dose	225	29

D. METHODS**Mating:**

Animals were acclimated at least 2 weeks prior to initiation of experiments. Females were artificially inseminated according to the method of Gibson et al. (Toxicol. Appl. Pharmacol. 9: 398, 1966) and were randomly assigned to gestational exposure groups. The day of artificial insemination was designated Day 0 of gestation. Females were then placed in the exposure chambers for 6 hr per day. Food (Purina Certified Laboratory Animal chow or Purina Rabbit Chow, non-certified) and tap water were supplied ad libitum throughout the study except during each 6 hr exposure period.

Dosing:

Rationale for Dosing: A developmental range-finding study of sulfuryl fluoride in rabbits (Study No. HET K-16399-(14), Dow) showed that at exposure (6 hrs/day) to 300 ppm, does showed significantly decreased body weight/body weight gain, decreased food consumption, decreased absolute/relative liver weight and slightly increased incidence of pale liver. Does treated at 100 ppm or lower showed no apparent treatment-related effects.

Administration of Compound: Animals were exposed to the test compound in 4.3 m³ Rochester-type stainless steel and glass chambers. Airflow was approximately 800 liters/min. Temperature was maintained at about 22°C and humidity at about 50%. Separate chambers were used for each dose and control animals were placed in chambers and exposed only to filtered air.

Test compound was admitted into the chambers via the air inlet at controlled rates using a precision pump to obtain the appropriate concentration and was mixed by turbulence with the incoming filtered air. Concentrations of the test compound were measured once or twice each hour during all exposure periods using infrared spectrophotometry (Miran I Variable Filter Infrared analyzer; 11.5μ). Temperature and relative humidity of the chambers were also measured daily during the exposure periods.

Mean time-weighted average concentrations were within 2% of target concentrations at each dose (individual measurement variation up to 4% of target).

Observations:

The animals were checked daily for mortality or abnormal

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condition throughout the study. Body weights were measured on Days 6, 9, 12, 15, 19 and 29 of gestation. Dams were sacrificed on Day 29 of gestation. Examinations at sacrifice by carbon dioxide consisted of: examination for gross abnormalities of internal organs, weighing of liver and kidneys, removal and examination of uteri and ovaries (corpora lutea, implantation sites and resorptions determined), preservation of ovaries, uterus, liver, lungs, nasal turbinates and kidneys in 10% neutral buffered formalin.

Fetuses were examined in the following manner: uteri were opened and fetuses were identified as live or dead. Each fetus was weighed, sexed and crown-rump length measured. Fetuses were examined under a stereomicroscope for external abnormalities and half were randomly chosen for soft tissue examination by dissection and examination by serial sectioning of heads fixed in Bouin's fluid (Wilson, 1965, Teratology Principles and Techniques, U. of Chicago Press). All fetuses were eviscerated, fixed in alcohol and stained with Alizarin Red S for skeletal examination (Dawson, 1926, Stain Tech. 1:123) under low power magnification.

Historical control data were not provided to allow comparison with concurrent controls.

Statistical analysis:

The following statistical analysis methods were employed: the Wilcoxon Test modified by Haseman and Hoel was performed on frequency of alterations, pre-implantation loss, resorptions and fetal population. Fisher Exact Probability Test was used to analyze the percentage of pregnancy and other incidence data. Fetal sex ratio was analyzed using the Steel and Torrie binomial distribution test. Other data was analyzed using parametric or nonparametric analysis of variance with either Dunnett's Test or Wilcoxon's Test. Statistical outliers for food and water consumption data were identified and deleted using a sequential outlier test (Grubbs, 1969).

E. RESULTS

The results of this study are summarized in the previous evaluation. Additional details and data tables are added below.

Maternal Toxicity

Mortality:

A total of 5 animals died during this study. Two does in

the 25 ppm group died on Days 10 and 27 of gestation and 3 in the 225 ppm group on Days 14, 18 and 29 of gestation as a result of pulmonary inflammation consistent with pneumonia, probably Pasteurellosis. Cause of a single death in the 75 ppm group on Day 24 was undetermined. Although these animals were pregnant they were not included in the mean maternal or cesarean data calculations.

Clinical Observations:

Except for those animals developing pulmonary inflammation, no clinical observations of significance were reported by the study authors.

Body Weight:

Mean maternal body weight and body weight gain during gestation is shown below in Table 2:

TABLE 2: MEAN MATERNAL BODY WEIGHTS AND WEIGHT GAIN (GRAMS)¹

Days of Gestation	Exposure Level (ppm)			
	0	25	75	225
No. Does	26	22	21	21
Body Weight:				
Day 6	4036	4161	3945	4025
9	4036	4169	3946	4065
12	4031	4187	3952	4036
15	4100	4256	3997	4016
19	4116	4285	4065	3974
29	4276	4360	4109	3965

Weight Gain:

Days 6-8	0	8	1	40
9-11	-5	18	6	-29
12-14	69	69	46	-21
15-18	16	29	67	-41
19-28	160	75	44	-60
6-28	240	199	163	-60

¹ Data taken from Table 6 of study

* p < 0.05

TB-I agrees with the original review that the decrease in maternal body weight and body weight gain observed at high dose was treatment-related. The pregnant females that died

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of pneumonia were not included in calculations of mean body weight/weight gain. These results are consistent with those observed in a range finding study (HET K-16377-(14)).

Food Consumption:

There were no significant differences in food consumption among any of the treatment groups before or during gestation.

Gross and Histological Pathological Observations:

No treatment-related effects on absolute and relative liver and kidney weights were observed. No treatment-related gross pathological lesions were noted and further histological examination was not done.

Cesarean Section Observations:

Cesarean Section Observations are presented in Appendix 2 in the table from the study. One dam in each group exposed to sulfuryl fluoride had uterine bacterial infections and these animals were not included in the summarized data. No treatment-related effects on implantations, resorptions, live fetuses or sex ratio were observed. A statistically significant decrease in fetal body weight was noted at high dose only (-14%). A small, non-statistically significant decrease in crown-rump length was also noted (-4%) at high dose only.

TB-I agrees with the original review that these observations represented signs of developmental toxicity at 225 ppm.

2. Developmental Toxicity

External, Soft Tissue and Skeletal Observations:

No statistically significant, clearly dose-related effects were observed among the fetuses in the treatment groups. Pale liver was observed only at 225 ppm in 3 fetuses from 2 litters (fetal % incidence = 3%; litter incidence 10%). While this is very low incidence and not statistically significantly increased over controls, TB-I believes that it is a possible threshold effect since this is a common finding among adult animals treated with sulfuryl fluoride, including does treated at 300 ppm in the range-finding study.

Other abnormalities noted among fetuses but which did not appear to be treatment-related are summarized in the original review.

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F. DISCUSSION/CONCLUSIONS

In summary, TB-I agrees with the conclusions of the original review of this study. No maternal or developmental effects were noted at doses of 75 ppm and lower. At 225 ppm, maternal toxicity was evident as statistically significant decreases in body weight/body weight gain.

Developmental toxicity was also evident at 225 ppm as statistically significant decreases in mean fetal weight and crown-rump length. TB-I considers the slightly increased incidence (not statistically significant) of pale liver among high dose fetuses to be a possible threshold effect, based on effects previously described for adult animals treated with sulfuryl fluoride (including the range-finding study included in this report). There was no evidence of teratogenicity at any dose level. The HED Mini-Peer Review Committee for developmental toxicity also evaluated this study and did not consider these effects to be a serious developmental risk.

Study Deficiencies: Historical control data not included, individual daily clinical evaluations of does not included (eg., when did does that died begin to look ill), signed Quality Assurance Statement not included.

Core Classification: Core-Minimum.



PENDIX 1

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

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7/3/82
Trans. P. 1
for 1/10/81

83-310 Rabbit Dev.
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

FEB 8 1982

Doc No. 001421

MEMORANDUM

DATE: February 5, 1982

SUBJECT: Review of Rat and Rabbit Inhalation Teratology
Studies of VIKANE (Sulfuryl fluoride)
Acc. No. 246489 Tox. Chem. 816A

FROM: Gary J. Burin, Toxicologist *Gary J. Burin*
Toxicology Branch/HED (TS-769) *2/5/82*

TO: W. Miller (16)
Registration Division (TS-767)

THRU: Orville E. Paynter, Chief
Toxicology Branch/HED (TS-769) *dp/rp*

Registrant: Dow Chemical USA
Midland, MI 48640

Chemical Name: VIKANE, sulfuryl fluoride (SO_2F_2)

Registration Number: 464-236

Physical State: Odorless, colorless gas

Recommendations: The inhalation teratology study of VIKANE in rabbits is classified as Core-Minimum. The NOEL for maternal and fetotoxicity is 75 ppm.

The inhalation teratology study in rats is also classified as Core-Minimum. The NOEL for maternal and fetotoxicity is 225 ppm.

Neither study suggests a teratogenic potential for VIKANE.

Review of Data

- 1) Inhalation Teratology, Rabbits. Conducted and submitted by Dow Chemical USA, October 26, 1981.

New Zealand white rabbits were artificially inseminated using the method of Gibson et al*. Groups of 28 or 29 inseminated rabbits were then exposed to 0, 25, 75 or 225 ppm of test compound, via inhalation, on days 6-18 of gestation. Exposure was for 6 hours a day under dynamic conditions in a 4.3 cubic meter Rochester-type chamber with an airflow of approximately 800 liters per minute. Temperature was maintained at approximately 22°C and relative humidity was maintained between 55-70%. Test material concentration was determined 1-2 times per hour by infrared spectrophotometry. Animals were observed daily for indications of toxicity. Body weights were recorded on days 6, 9, 12, 15, 19 and 29 of gestation.

Animals were sacrificed on day 29 of gestation by carbon dioxide asphyxiation. The uterine horns were removed and the following data was recorded: number and position of fetuses, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea; sex, body weight and length of fetuses and external alterations. The uteri of animals not showing evidence of pregnancy were stained with a 10% solution of sodium sulfide and examined for evidence of implantation sites. One half of each litter was randomly examined for soft tissue alterations, using the technique of Staples**. The heads of these fetuses were removed, fixed and serial sectioned. All fetuses were then eviscerated, preserved in alcohol, cleared and stained with alizarin-S. All fetuses were examined for skeletal alterations.

*Gibson, J.P., Staples, R.E. and Newberne, J.W. (1966)

"Use of the Rabbit in Teratogenicity Studies", Tox. Appl. Pharmacol. 9:398-408.

**Staples, R.E. (1974) Detection of visceral alterations in mammalian fetuses. Teratology 9:37.

Results: (Time weighted average actual concentrations were found to be 0, 25 \pm 1, 76 \pm 1 and 225 \pm 2 ppm).

Two dams of 25 ppm group, one dam of the 75 ppm group and 3 dams of the 225 ppm groups died during the course of the study. One of the deaths at 25 ppm and two of the deaths at 225 were attributed to pneumonia (Pasteurellosis), the cause of the other deaths was not known.

Maternal body weight and maternal body weight gain were both significantly less than control values for the 225 ppm dose group.

Three females (one each from the 25, 75 and 250 ppm dose levels) were found to have litters which had completely aborted early in pregnancy and each of these animals also had mucopurulent exudates indicative of uterine bacterial infections. Evidence of early resorption sites were also found in one 25 ppm rabbit.

Three control animals and two high dose animals delivered litters prior to cesarian section.

No compound related effects on litter size, incidence of resorptions, incidence of major malformations or the incidence of most minor variations were apparent. The most common fetal alterations included satellite vessals off major arteries, pale livers, extra ribs, 8 lumbar vertebra and delayed ossification of the sternbrae, all of which are considered to be variations. Fetal body weight was significantly decreased in the 225 ppm dose group and crownrump length was slightly decreased in this group. No effects on these parameters were observed at 25 or 75 ppm.

Core Classification: Core-Minimum. A positive control group was not used in this study and historical control data was not submitted. The NOEL for maternal and fetotoxicity was found to be 75 ppm. Teratogenicity was not demonstrated at 225 ppm (highest dose tested).

2. ~~Inhalation Teratology, Rats. Conducted and submitted by, Dow Chemical USA, October 26, 1981.~~

~~Fischer 344 female rats were bred with males of the same strain. The finding of sperm in a vaginal smear was considered to be day 0 of gestation. Groups of 35 and 36 animals were exposed to 0, 25, 75 or 225 ppm of test material, via inhalation; on days 6 through 15 of gestation. Exposure was for 6 hours per day under dynamic conditions~~

APPENDIX 2

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TABLE -

TREATMENT: TERATOGENICITY STUDY IN RATS AND RABBITS

Observations Made at the Time of Cesarean Section of Rabbits Exposed to VIKANE^a

Exposure Levels	VIKANE, ppm			
	0	25	75	225
Number of females bred	28	29	28	29
Number of maternal deaths/number bred	0/28	2/29	1/28	3/29
Percent pregnant ^b	82(23/28)	90(26/29)	79(22/28)	90(26/29)
Pregnancies detected by stain ^c	0/5	2/3	1/6	1/3
Percent pregnant, total ^d	82(23/28)	97(28/29)	82(23/28)	93(27/29)
Number of litters	18	22	18	21
Corpora lutea/dam ^e	18±2	18±2	18±2	18±2
Implantation sites/dam ^e	9±2	9±3	8±3	8±2
Pre-implantation loss ^f	11±11	19±10	14±12	11±10
Fetuses/litter ^g	8±3	8±2	7±2	9±3
Resorptions/litter ^{h,i}	1.10±0.67	0.91±1.02	2.10±3.83	0.99±1.49
% Implantations resorbed ^j	13(22/173)	11(20/187)	16(20/173)	9(11/177)
% Litters with resorptions	45(9/20)	36(8/22)	57(12/21)	13
Litters totally resorbed	1	0	3	0
Resorptions/litters with resorptions ^j	2.4(22/9)	2.3(20/8)	2.3(20/12)	2.0(10/7)
% Dead fetuses	0	0	0	0
Sex ratio, M/F	90:90	92:88	42:98	44:96
Fetal body weight, g ^k	38.22±0.87	38.18±0.82	38.44±0.63	38.99±0.90 ^l
Fetal crown-rump length, mm ^k	93.6±0.3	94.9±0.6	93.8±0.3	99.7±0.8

^aUnanesthetized New Zealand white rabbits were exposed to 0, 25, 75, or 225 ppm VIKANE fumigant by inhalation for 6 hrs/day on days 6 through 18 of gestation.

^bNumber of females with visible implantations at the time of cesarean section or resorptions/total number bred. Three control animals, two animals at the 25 ppm level and one animal at the 225 ppm level which delivered prior to cesarean section are included.

^cNumber of females with implantation sites detected only after staining the uterus/total stained.

^dNumber of females pregnant by visual inspection of the uterus or by stain/total bred.

^eMean±S.D.

^fPercent per litter, mean±S.D.

^gResorptions detected only by staining were not included in these calculations.

^hMean of litter mean±S.D.

ⁱSignificantly different from control value using the appropriate statistical test, p<0.05.

See Table 4-9 for individual animal data.

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GUIDELINE: 83-3

Primary Review: Linnea J. Hansen, Ph.D. *Linnea J. Hansen 5/1/92*
Review Section IV, Tox. Branch I
Secondary Review: Marion P. Copley, D.V.M., D.A.B.T.
Review Section IV, Tox. Branch I *Marion Copley 5/1/92*

DATA EVALUATION RECORD

(Follow up to HED Doc.# 001421)

STUDY TYPE: Teratology-Developmental Toxicity
Species: Rat
Guideline: 83-3 (a)

TOX. CHEM. NO.: 816A

PC NO.: 078003

ACCESSION NO.: 246489

MRID NO.: 00090015

TEST MATERIAL: Sulfuryl fluoride, technical

SYNONYMS: Vikane®; CAS No. 2699-79-8

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

STUDY NUMBER: HET K-16399-(15)

TESTING FACILITY: Dow Toxicology Research Laboratory - Health
and Environmental Sciences

TITLE OF REPORT: Vikane®: Inhalation Teratology Study in Rats
and Rabbits

AUTHORS: T.R. Hanley, Jr., L.L. Calhoun, R.J. Kociba,
S.R. Cobel-Geard, W.C. Hayes, J.H. Quелlette,
L.M. Scherbarth, B.N. Sutter and J.A. John

REPORT ISSUED: October 26, 1981

CONCLUSIONS:

Doses tested: 0, 25, 75 and 225 ppm via inhalation to
pregnant female Fischer 344 rats treated 6 hr/day during
Days 6 - 15 of gestation, inclusive.

Maternal NOEL: \geq 225 ppm. LEL: was not determined in this
study (no clear maternal toxicity was observed).

Developmental NOEL: \geq 225 ppm. LEL: was not determined in
this study (no significant developmental or teratogenic

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effects were observed).

TB-I agrees with the conclusions of the original review of this study. Although no maternal toxicity was observed at the high dose of 225 ppm, the study is considered Minimum because the preliminary range-finding study indicated significant maternal toxicity at 300 ppm and therefore 225 ppm is probably very close to the LEL for pregnant rats.

This study satisfied the guideline requirement for a rat developmental toxicity study (83-3a) and is considered acceptable for regulatory purposes.

Core Classification: Minimum

A signed Quality Assurance Statement was not present.

A. VALIDATION OF REVIEW:

This DER is intended to validate and to supplement details of a previous review (Document No. 1421) of this study. The study is evaluated here according to the Guidelines for developmental toxicity studies (83-3) and the report prepared in the standard manner. A copy of the original review is appended to this document (Appendix 1).

B. MATERIALS

Test Compound: Sulfuryl fluoride, technical. Purity: 99.8%
Description: colorless, odorless gas
Lot No.: 217 (Dow Chemical)
Contaminant: Not described

Test Animal: Species: Rat
Strain: Fischer 344
Source: Charles River Laboratories,
Portage, MI
Age: Young adult (age not indicated)
Weight: 175 - 220 g at breeding

C. STUDY DESIGN

This study was designed to assess the developmental toxicity potential of sulfuryl fluoride when administered by inhalation to pregnant female Fischer 344 rats on gestation Days 6 through 15, inclusive.

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Group Arrangement:

Female rats were assigned to the following treatment groups:

TABLE 1: ASSIGNMENT OF ANIMALS

Test Group	Dose Level (ppm)	Number Assigned (at mating)
Control	0	36
Low Dose	25	35
Mid Dose	75	35
High Dose	225	36

D. METHODSMating:

Animals were acclimated at least 2 weeks prior to initiation of experiments. Females were mated 1:1 with males of the same strain and matings were identified by vaginal smears. Day 0 of gestation was designated as the day on which sperm were identified in smears. Following mating, females were randomly assigned to gestational exposure groups. Females were then placed in the exposure chambers for 6 hr per day. Food (Purina Certified Laboratory Animal chow) and tap water were supplied ad libitum throughout the study except during each 6 hr exposure period.

Dosing:

Rationale for Dosing: A developmental range-finding study of sulfuranyl fluoride in rats (Study No. HET K-16399-(14), Dow) showed that at exposure (6 hrs/day) to 300 ppm, dams showed statistically significantly decreased body weight/body weight gain, decreased food consumption, increased water consumption, slightly increased absolute/relative kidney weight and slightly increased incidence (not statistically significant) of pale liver and kidney. Dams treated at 100 ppm or lower showed no apparent treatment-related effects. Based on these results, the doses used in this study were chosen.

Administration of Compound: Animals were exposed to the test compound in 4.3 m³ Rochester-type stainless steel and glass chambers. Airflow was approximately 800 liters/min. Temperature was maintained at about 22°C and humidity at about 50%. Separate chambers were used for each dose and control animals were placed in chambers and exposed only to filtered air.

Test compound was admitted into the chambers via the air inlet at controlled rates using a precision pump to obtain

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the appropriate concentration and was mixed by turbulence with the incoming filtered air. Concentrations of the test compound were measured once or twice each hour during all exposure periods using infrared spectrophotometry (Miran I Variable Filter Infrared analyzer; 11.5μ). Temperature and relative humidity of the chambers were also measured daily during the exposure periods.

Mean time-weighted average concentrations were within 2% of target concentrations at each dose (individual measurement variation up to 4% of target).

Observations:

The animals were checked daily for mortality or abnormal condition throughout the study. Body weights were measured on Days 6, 9, 12, 16 and 21 of gestation. Dams were sacrificed on Day 21 of gestation. Examinations at sacrifice by carbon dioxide consisted of: examination for gross abnormalities of internal organs, weighing of liver and kidneys, removal and examination of uteri and ovaries (corpora lutea, implantation sites and resorptions determined), preservation of ovaries, uterus, liver, lungs, nasal turbinates and kidneys in 10% neutral buffered formalin.

Fetuses were examined in the following manner: uteri were opened and fetuses were identified as live or dead. Each fetus was weighed, sexed and crown-rump length measured. Fetuses were examined under a stereomicroscope for external abnormalities and half were randomly chosen for soft tissue examination by dissection and examination by serial sectioning of heads fixed in Bouin's fluid (Wilson, 1965, Teratology Principles and Techniques, U. of Chicago Press). All fetuses were eviscerated, fixed in alcohol and stained with Alizarin Red S for skeletal examination (Dawson, 1926, Stain Tech. 1:123) under low power magnification.

Historical control data were not provided to allow comparison with concurrent controls.

Statistical analysis:

The following statistical analysis methods were employed: the Wilcoxon Test modified by Haseman and Hoel was performed on frequency of alterations, pre-implantation loss, resorptions and fetal population. Fisher Exact Probability Test was used to analyze the percentage of pregnancy and other incidence data. Fetal sex ratio was analyzed using the Steel and Torrie binomial distribution test. Other data was analyzed using parametric or nonparametric analysis of variance with either Dunnett's Test or Wilcoxon's Test.

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Statistical outliers for food and water consumption data were identified and deleted using a sequential outlier test (Grubbs, 1969).

E. RESULTS

The results of this study are summarized in the previous evaluation. Additional details and data tables are added below.

Maternal Toxicity

Mortality:

There was no mortality during this study.

Clinical Observations:

No remarkable clinical signs of toxicity were observed at any dose.

Body Weight:

Mean maternal body weight gain during gestation is shown below in Table 2:

TABLE 2: MEAN MATERNAL BODY WEIGHT GAIN (GRAMS)¹

Days of Gestation	Exposure Level (ppm)			
	0	25	75	225
No. Does	31	29	32	31
Weight Gain:				
Days 6-8	3.7	5.7	2.3	1.6
9-11	8.7	8.4	9.0	8.0
12-15	10.7	10.8	8.6	9.7
16-20	34.8	35.0	36.0	34.3
6-20	58.6	59.8	55.9	53.6(9%)

1 Data taken from Table 2 of study

A small decrease in mean body weight gain was observed at 225 ppm relative to controls. This decrease was not statistically significant and was not clearly treatment-related (although given the statistically significant decrease in body weight gain of 87% for Days 6 - 16 noted at 300 ppm in the range-finding study, it could be a threshold

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effect). TB-I agrees with the original review that there were no significant effects on maternal weight at this dose.

Food Consumption:

There were no significant differences in food consumption among any of the treatment groups before or during gestation.

Water Consumption:

Water consumption during gestation is presented below in Table 3:

TABLE 3: WATER CONSUMPTION DURING GESTATION (GRAMS)¹

	<u>Dose, ppm</u>			
	0	25	75	225
No. Dams	31	29	32	31
Days of Gestation:				
6-8	20	19	21	23*
9-11	22	21	23	26*
12-13	23	24	25	27*
15-17	28	27	28	31*
18-20	26	26	27	27

1 Data taken from Table 3 of study

* $p < 0.05$

Water consumption showed a statistically significant increase during treatment (11 - 18%) among dams treated at 225 ppm. No effects were noted at lower doses. This effect was also noted in the range-finding study at 300 ppm but is not considered a significant toxic effect when taken by itself.

Gross and Histological Pathological Observations:

No treatment-related effects on absolute and relative liver and kidney weights were observed. No treatment-related gross pathological lesions were noted and further histological examination was not done.

Cesarean Section Observations:

Cesarean Section Observations are summarized in Appendix 2 in the table from the study. No treatment-related effects on implantations, resorptions, live fetuses, fetal weight, fetal length or sex ratio were observed. A single complete litter resorption was found at 225 ppm but not at lower doses. No complete litter resorptions were noted at 300 ppm

in the preliminary range-finding study.

2. Developmental Toxicity

External, Soft Tissue and Skeletal Observations:

Statistically significant increases in the incidence of bilobed vertebral centra were noted at 25 ppm and 225 ppm (% fetuses affected 2.14 X and 2.33 X control, respectively) and increased incidence of unfused vertebral centra at 75 ppm. Incidence data is included in the original review for these observations. Skeletal evaluations were not performed in the range-finding study. Calculations of mean % fetal incidence, total % affected fetuses/dose and % litters affected for bilobed vertebral centra and combined bilobed/unfused vertebral centra are contained in the attached supplementary table (Appendix 3). TB-I does not consider these effects to be of biological significance and they are not considered to be indicative of developmental toxicity. Following a discussion with Dow (see attached ROC) it was determined that these 2 endpoints were a less severe stage and should also be combined with delayed ossification. When this is done there is no evidence of a treatment related effect.

F. DISCUSSION/CONCLUSIONS

In summary, TB-I agrees with the conclusions of the original review of this study. No significant maternal toxicity was noted: only increased water consumption at 225 ppm was noted. There was no evidence of significant developmental toxicity or teratogenicity up to 225 ppm. Calculations for incidences of bilobed and unfused vertebral centra are appended. The HED Mini-Peer Review Committee for developmental toxicity also evaluated this study and did not consider sulfuryl fluoride to be a developmental toxicant in rats.

Maternal and developmental NOELs for this study are ≥ 225 ppm. Although a maternal toxicity LEL could not be determined, the high dose of 225 ppm is considered adequate and probably approaching the LEL, based on the marked maternal toxicity that was observed in the range-finding study at 300 ppm.

Study Deficiencies: No obvious maternal toxicity at high dose, historical control data not included, no signed Quality Assurance Statement was included.

Core Classification: Core-Minimum.

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APPENDIX 1

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

CASWELL FILE

Tested -
rat / mice

83-3 a Rat develop
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

FEB 8 1982

Doc No. 001421

MEMORANDUM

DATE: February 5, 1982

SUBJECT: Review of Rat and Rabbit Inhalation Teratology
Studies of VIKANE (Sulfuryl fluoride)
Acc. No. 246489 Tox. Chem. 816A

FROM: Gary J. Burin, Toxicologist
Toxicology Branch/HED (TS-769)

TO: W. Miller (16)
Registration Division (TS-767)

THRU: Orville E. Paynter, Chief
Toxicology Branch/HED (TS-769)

Registrant: Dow Chemical USA
Midland, MI 48640

Chemical Name: VIKANE, sulfuryl fluoride (SO₂F₂)

Registration Number: 464-236

Physical State: Odorless, colorless gas

Recommendations: The inhalation teratology study of VIKANE in rabbits is classified as Core-Minimum. The NOEL for maternal and fetotoxicity is 75 ppm.

The inhalation teratology study in rats is also classified as Core-Minimum. The NOEL for maternal and fetotoxicity is 225 ppm.

Neither study suggests a teratogenic potential for VIKANE.

Results: (Time weighted average actual concentrations were found to be 0, 25 \pm 1, 76 \pm 1 and 225 \pm 2 ppm).

Two dams of 25 ppm group, one dam of the 75 ppm group and 3 dams of the 225 ppm groups died during the course of the study. One of the deaths at 25 ppm and two of the deaths at 225 were attributed to pneumonia (Pasteurellosis), the cause of the other deaths was not known.

Maternal body weight and maternal body weight gain were both significantly less than control values for the 225 ppm dose group.

Three females (one each from the 25, 75 and 250 ppm dose levels) were found to have litters which had completely aborted early in pregnancy and each of these animals also had mucopurulent exudates indicative of uterine bacterial infections. Evidence of early resorption sites were also found in one 25 ppm rabbit.

Three control animals and two high dose animals delivered litters prior to cesarian section.

No compound related effects on litter size, incidence of resorptions, incidence of major malformations or the incidence of most minor variations were apparent. The most common fetal alterations included satellite vessals off major arteries, pale livers, extra ribs, 8 lumbar vertebra and delayed ossification of the sternbrae, all of which are considered to be variations. Fetal body weight was significantly decreased in the 225 ppm dose group and crownrump length was slightly decreased in this group. No effects on these parameters were observed at 25 or 75 ppm.

Core Classification: Core-Minimum. A positive control group was not used in this study and historical control data was not submitted. The NOEL for maternal and fetotoxicity was found to be 75 ppm. Teratogenicity was not demonstrated at 225 ppm (highest dose tested).

2. Inhalation Teratology, Rats. Conducted and submitted by, Dow Chemical USA, October 26, 1981.

Fischer 344 female rats were bred with males of the same strain. The finding of sperm in a vaginal smear was considered to be day 0 of gestation. Groups of 35 and 36 animals were exposed to 0, 25, 75 or 225 ppm of test material, via inhalation; on days 6 through 15 of gestation. Exposure was for 6 hours per day under dynamic conditions

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in a 4.3 meter Rochester-type chamber with an airflow of approximately 800 liters per minute. Temperature was maintained at approximately 22°C and relative humidity was maintained between 55-70%. Test material concentration was determined 1-2 times per hour by infrared spectrophotometry. Animals were observed daily for indications of toxicity. Body weights were recorded on days 6, 9, 12, 16 and 21 of gestation. Maternal food and water consumption were recorded at 3 day intervals beginning on day 6 of gestation.

Animals were sacrificed on day 21 of gestation by carbon dioxide asphyxiation. The uterine horns were removed and the following data were recorded: number and position of fetuses, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea; sex, body weight and length of fetuses and the incidence of external alterations. The uteri of animals not showing evidence of pregnancy were stained with a 10% solution of sodium sulfide and examined for evidence of implantation sites. One half of each litter was randomly examined for soft tissue alterations using the technique of Staples*. The heads of these fetuses were then eviscerated, preserved in alcohol, cleared and stained with alizarin-S. All fetuses were examined for skeletal alterations.

Results: (Time weighted average actual concentrations were found to be 0, 25 \pm 1, 76 \pm 1 and 225 \pm 2 ppm.)

No mortalities occurred during the course of the study. No maternal signs of toxicity were noted at any dose level, although it is noted that maternal body weight and body weight gain were slightly less than controls in the high dose group. Although food consumption was not affected by treatment, water consumption was significantly increased in the high dose group on gestation days 6-17.

No compound-related effect on incidence of pregnancy, pre-implantation loss, mean litter size or incidence of resorptions was observed at any dose level. At 225 ppm, fetal body weight and crown-rump length were significantly increased compared to controls. No compound related effects on major malformations were apparent. A significant increased bilobed centra of the vertebrae was observed in the 25 and 225 ppm groups. However, the incidence did not appear to be dose-related and the magnitude of the increase was not large. A significant increase in unfused centra was observed in the 75 ppm group compared to controls. These variations are shown in the following table:

*Staples, R.E. (1974) Detection of visceral alterations in mammalian fetuses, *Teratology* 9:37.

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Table: Incidence of Bilobed or Unfused Sternebrae*

	<u>0 ppm</u>	<u>25 ppm</u>	<u>75 ppm</u>	<u>225 ppm</u>
Unfused Centra	2(2)	2(2)	7(1)**	2(2)
Bilobed Centra	1(10)	22(16)**	12(6)	24(17)**
TOTAL***	13(10)	24(18)	19(7)	26(17)

*Fetuses effected (litters effected)

**Significant at $p < .05$.

***Total fetuses effected either by variation (total litters effected by either variation)

The possibility that the increases in either unfused or bilobed centra were due to chance can not be eliminated. No other possible effects on skeletal development were apparent.

Core Classification: Core-Minimum. Although maternal toxicity was not demonstrated, it is probable that the high dose level was approaching a level which would have demonstrated maternal toxicity based on the results of the "probe" study which demonstrated maternal toxicity (significantly decreased body weights and body weight gains) at 300 ppm. The NOEL for maternal and fetotoxicity is 225 ppm in this study.

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APPENDIX 2

TABLE 4

VIXANE: INHALATION TERATOLOGY STUDY IN RATS AND RABBITS

Observations Made at the Time of Cesarean Section of Rats Exposed to VIXANE^a

Exposure Levels	VIXANE, ppm			
	0	25	75	225
Number of females bred	36	35	39	36
Number of maternal deaths/number bred	0/36	0/35	0/39	0/36
Percent pregnant ^b	86(31/36)	83(29/35)	91(32/35)	86(31/36)
Pregnancies detected by stain ^c	0/5	0/6	0/3	0/5
Percent pregnant, total ^d	86(31/36)	83(29/35)	91(32/35)	86(31/36)
Number of litters	31	29	32	31
Corpora lutea/dam ^e	12±2	12±1	12±1	12±2
Implantation sites/dam ^e	10±3	10±2	10±3	10±3
Pre-implantation loss ^f	18±10	13±14	19±20	23±23
Fetuses/litter ^g	9±3	8±3	8±3	8±4
Resorptions/litter ^{h,i}	1.53±1.23	2.07±1.49	2.03±1.36	1.94±1.16
5 Implantations resorbed	15(67/311)	20(68/304)	21(69/310)	20(68/309)
5 Litters with resorptions	7(23/31)	9(27/29)	8(27/32)	8(25/31)
Litters totally resorbed	0	0	0	1
Resorptions/litters with resorptions ^j	2.0(47/23)	2.2(68/27)	2.6(69/27)	2.4(68/25)
5 Dead fetuses	0	0	0	0
Sex ratio, M:F	51:49	45:55	52:48	49:51
Fetal body weight, g ^k	4.32±0.25	4.28±0.16	4.45±0.22	4.46±0.25 ^l
Total crown-rump length, mm ^k	40.7±0.8	40.7±0.8	41.1±1.0	41.3±1.2 ^l

^aBred Fischer 344 rats were exposed to 0, 25, 75, or 225 ppm VIXANE fumigant by inhalation for 5 hrs/day on days 6 through 15 of gestation.

^bNumber of females with visible implantations at the time of cesarean section or cesareopy/total number bred.

^cNumber of females with implantation sites detected only after staining the uterus with sodium sulfide stain/total stained.

^dNumber of females pregnant by visual inspection of the uterus or by sodium sulfide stain/total bred.

^eMean±S.D.

^fPercent per litter, mean±S.D.

^gResorptions detected only by sodium sulfide staining were not included in these calculations.

^hMean of litter mean±S.D.

ⁱSignificantly different from control value using the appropriate statistical test, p<0.05.

See Table A-6 for individual animal data.

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APPENDIX 3

INCIDENCE CALCULATIONS FOR THORACIC VERTEBRAL CENTRA ALTERATIONS

CALCULATION AND VERTEBRAL CENTRA DEFECT:	SULFURYL FLUORIDE, PPM			
	0	25	75	225
$\left(\frac{\# \text{ Fetuses affected}}{\text{Total \# of Litters}} \right) \times 100$				
(1) bilobed + unfused	13/31 = 42%	24/29 = 83%	19/32 = 60%	26/30 = 86%
(2) bilobed only	11/31 = 36%	22/29 = 76%	12/32 = 38%	24/30 = 80%
$\left(\frac{\# \text{ Litters affected}}{\text{Total \# of Litters}} \right) \times 100$				
(1) bilobed + unfused	10/31 = 32%	18/29 = 62%	13/32 = 41%	17/30 = 57%
(2) bilobed only	10/31 = 32%	16/29 = 55%	9/32 = 28%	17/30 = 57%

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Attachment - Rat. Develop.

RECORD OF
COMMUNICATION
☒ PHONE CALL ☐ DISCUSSION ☐ FIELD TRIP ☐ CONFERENCE
☐ OTHER (SPECIFY)

(Record of item checked above)

 Mrs. W. Breslin & J. Quast
 Chemical
 636-0274

 FROM:
 Marion Copley

DATE

5/14/92

TIME

1:00 pm

ECT

furyl Fluoride-information about rat develop. study

SUMMARY OF COMMUNICATION

questions pertained to the rat developmental study completed with SFI 1981. There were 3 areas of concern discussed.

The sacrifice schedule at termination of the study was not clear in the report. Dr. Breslin explained that the start of the study was staggered with animals from all 4 groups starting at each time point. Although the sacrifice for each respective time was not random with respect to treatment group, one animal from each treatment group was sacrificed then the cycle was repeated (ie. 1 dam from Control, High, Mid, Low, Control, High, etc.). This eliminated a possible bias in development due to sacrifice time.

Dr. Breslin explained the use of the term delayed ossification of centra used in the report. He said it actually referred to unossified centra. This is the most severe appearance on the continuum including unfused centra and bilobed centra (least severe). He also said that as of about 4 years ago the above three variations have been listed together as delayed ossification rather than being separated. When the study is evaluated combining these 3 variations there does not appear to be a treatment repeated effect in delayed ossification of the centra.

Historical control data will be sent as soon as possible for the above 3 observations, however only 4 studies have them separated out. The remaining studies only list the variation as DO (delayed ossification of the centra).

CONCLUSIONS, ACTION TAKEN OR REQUIRED

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