Conc. (ppm)	lbs/1000 ft^2	Exposure Duration (hours)	Sex	No. Died/ No. Exposued	Response - Remarks
8000	**2.1**	0.5	M	18/18	Tremors started after about 8 minutes of exposure. Rats were still able to crawl around after 15 minutes of exposure. All became convulsant and died in less than 2.5 hours.
			F	10/10	
8000	**2.1**	0.2	M	1/18	Slight weight loss. Rapid recovery.
			F	1/10	
4000	**1.05**	1.0	M	19/20	All animals were weak on removal but appeared to be conscious. Half were dead in less than 2 hours. Three rats died before morning. One rat died 4 days after exposure.
			F	10/10	
4000	**1.05**	0.5	M	5/20	Essentially no weight loss.
			F	7/10	
2000	**0.52**	2.0	M	12/18	All alive but drowsy on removal. Two hours after exposure, rats were very sick, slow moving and had slight tremors. One rat died 2 hours after exposure. Recovered appreciably in 2 days.
			F	10/10	
2000	**0.52**	1.0	M	1/18	Rats were only slightly effected, including only a slight weight loss.
			F	0/10	
1000	**0.26**	6.0	M	4/8	No unusual response 1.5 hours after exposure started. After 4 hours of exposure, tremors and slight convulsions occurred. The tremors and convulsions increased in frequency and intensity during the remainder of the exposure. The first death occurred after 5 hours of exposure. Four rats were dead at the end of the exposure. Survivors were sick and trembling but lost only a small amount of weight.
		5.6	M	2/10	
		5.6	F	2/10	
		4.0	M	4/10	
		4.0	F	2/10	
1000	**0.26**	3.0	M	0/8	Possibly slight tremors after exposure. Only slight weight loss occurred.
		2.0	M	0/10	
		2.0	F	1/10	

Conclusions: **The 4-hour LC_{50} for rats was ca. 1000 ppm (4.7 mg/L). Based on this result, sulfuryl fluoride is classified as Xn; R20 "Harmful by inhalation"**

Section A.6.1.3/01 Annex point IIA, VI. 6.1.3	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2004
Materials and methods	Applicant's version adopted. The study has several deviations from the EC guideline Method B.2. and the report is not extensive enough to fully compare it.
Results and discussion	The acute inhalation toxicity in rats was both concentration- and time-dependent. Shorter exposure time decreased the mortality to a great extent as seen in the rats exposed to equal concentration of sulfuryl fluoride but in different exposure time lengths. Also, better recovery was observed when the exposure time was shorter. The acute inhalation LC_{50} value was 1000 ppm at six hours' exposure. No (0/8, male) or low number (1/10, female) of deaths occurred at 1000 ppm level when exposure time was < 4 hours. The observed symptoms included drowsiness, laboured breathing, quietness and weakness, slow moving, tremor and convulsions followed by death. Slight body weight losses were recorded without further detailed information.
Conclusion	Due to the variation in number of rats in each group and exposure duration for different concentrations, no reliable LC_{50}-value can be calculated.
Reliability	Reliability indicator 3: Study with major methodological and/or reporting deficiencies.
Acceptability	The study is considered acceptable (see Remarks).
Remarks	The study was considered as a dose-range finding study.

Section A6.1.3/02 **Acute inhalation toxicity**

Annex Point IIA, VI.6.1.3

Acute inhalation toxicity (Rat, IIA5.2.3/02, B03)

Report: [redacted] (1980)
Sulfuryl Fluoride (Vikane Fumigant): An LC50 Determination
[redacted]
K-016399-013, dated August 25, 1980; study began 8/5/79.

Guidelines: US EPA 81-3.
Deviations from EC guideline Method B.2. Acute Toxicity (Inhalation): None.

GLP: Yes

Methodology: Test material: Sulfuryl fluoride (Lot # 141, from The Agricultural Products R&D department, Dow, Midland, Michigan; contained 99.7% SO_2F_2).

Groups of 10 Fischer 344 rats/sex/exposure level were given a single 4 hr exposure to Vikane vapours. The initial target concentrations selected were 0, 500, 1000, 1500 and 2000 ppm. The two higher concentrations caused the death of all animals. Then additional lower exposures were included: 1250 (males) and 250, 750, 900 and 1200 (females).

Rats were observed closely up to 4 hours and then twice daily for up to 14 days. Body weights were recorded prior to initiation of exposure and on days 2, 3, 4, 7 and 14. Final fasted body weights were recorded just prior to necropsy. All animals were subjected to a complete gross pathologic examination. Histopathology was conducted on rats from the control and 1250 ppm (male) and 1200 ppm (female) exposure groups. The liver, kidneys, brain, lungs and testes were weighed at necropsy.

Findings: The LC_{50}'s were 1122 ppm for male Fischer 344 rats (1015-1232 ppm, 95% C.I.) and 991 ppm for females (905-1072 ppm, 95% C.I.).

An inhibition of growth was apparent for groups of animals which survived exposure to a concentration of approximately 750 ppm or higher, as shown in Table 5.2.3/02. If recovery occurred, it was generally apparent within the first few days after exposure and no spontaneous mortalities occurred after 6 days post-exposure.

Organ weights from animals which survived until the end of the 14 day observation period revealed no definite treatment-related abnormalities.

Gross pathologic examinations revealed lesions primarily in the upper and lower respiratory tract. Histopathologic examinations for animals which died as a result of 1200-1250 ppm revealed definite treatment effects in the liver and kidneys and possible effects in lung, heart and spleen. Kidney effects which were considered to be regenerative responses subsequent to previous toxicity were noted in animals which survived for two weeks following the exposure to 1200-1250 ppm.

The study indicates that a generalised sedation, possibly via central nervous system depression, should be anticipated within 20- 40 minutes of exposure to high concentrations (e.g. greater than 1500- 2000 ppm) of VIKANE. Continued exposure following sedation results in convulsions and adverse effects on the respiratory system, liver and kidneys, followed by death within 3-4 hours. Exposure concentrations below the LC_{50} (e.g. 700 ppm) may have had some slight short-term

effect as evidenced by the decreased growth of rodents during the first few days after exposure. However, there were no discernible treatment-related effects after 14 days of recovery in animals exposed to concentrations lower than 1000 ppm.

Table 5.2.3/02: Body Weight Gains for Male and Female Rats

Exposure Conc. (ppm)	Days after Exposure					
	2	3	4	8	14	Terminal
Male Rats						
0	6.4	8.4	2.8	13.5	24.4	22.1
450	2.9	-1.5	3.5	10.1	27.0	13.8
1000	-13.1*	-12.1*	-14.2*	-7.9*	1.1	-7.3*
1250	-22.6*	-11.6*	-5.4-	-0.7	11.2	7.8
Female Rats						
0	-0.1	3.5	-6.3	-6.7	10.5	3.6
320	8.8*	-3.8*	5.5*	11.5*	13.0	11.1*
450	10.5*	0.9	9.5*	16.0*	16.3*	14.5*
700	-9.2*	3.4	1.4*	8.8*	13.5	10.4*
790	-4.5	-3.7*	-4.5	1.2	N/A	3.4
1000	-24.0*	-28.2*	-43.8	--	--	
1020	-16.2*	-13.3*	-12.7*	-7.5	N/A	0.7
1200	-16.4*	-17.9*	-17.0*	-10.1	N/A	-13.7

*Statistically significant deviation from control group using Dunnett's test, $p<0.05$. N/A - not applicable

Conclusions: **The LC_{50}'s for male and female rats in this study were 1122 ppm (4.68 mg/L) and 991 ppm (4.13 mg/L), respectively. Under the conditions of this study, the classification for sulfuryl fluoride would be Xn; R20 'Harmful by inhalation'.**

Section A6.1.3/02 Annex point IIA, VI. 6.1.3.	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2004
Materials and methods	The applicant's version is adopted with the following amendments. The exposure was whole-body conducted in stainless steel and glass chambers under dynamic airflow conditions. The intended target concentrations described in the method differed slightly from the actual time-weighted average analytical concentrations (TWA) reported in the results. The TWA concentrations were: 0, 450, 1000, 1250, 1425 and 2025 ppm for males and 0, 320, 450, 700, 790, 1020, 1000, 1200, 1425 and 2025 ppm for females.
Results and discussion	The applicant's version is adopted with the following amendments. The mortality in the female group changed dramatically between 1000 ppm (9 /10) and 790 ppm (1/10) an interval where the LC_{50} value is calculated to be. The effects in lung (slight perivascular edema) was considered part of the agonal death, and the effects in spleen (cellular depletion of cells in the red pulp or atrophy) was considered to be stress-mediated effects of the exposure. One female rat showed mineralization and inflammation of the pleura and multifocal myocardial degeneration, inflammation and mineralization that may or may not be related to exposure. Other findings in this experiment were exsudation around the eyes in most dead rats during or after the exposure between 500 and 2000 ppm. In the original study, the phenomenon was described as "Exudative material around external nares and/or eyes". In the same experiment 1/4 of the survived rats hade unilateral focal corneal cloudiness at 1250 ppm. 1/10 of rats showed similar changes in control group. The possible mechanism for the exudation around the eyes has been discussed between RMS and the applicant. It seems reasonable to consider this phenomeone as a secondary stress response associated to the toxic exposure to sulfuryl fluoride rather than the direct irritative effect by this substance since such effect mostly occurred in those dead rats. In other acute toxicity studies has this phenomenone related to the eyes not been observed.
Conclusion	The applicant's version is adopted.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	The study is acceptable.
Remarks	No remarks.

Section A6.1.3/03 **Acute Inhalation Toxicity**

Annex Point IIA, VI.6.1.3

Acute inhalation toxicity (Mouse, IIA5.2.3/04, B05)

Report:	[redacted] (1989) Sulfuryl Fluoride: Acute LC50 Study with B6C3F1 Mice [redacted] K-9016399-028, -028A and -028B, dated 22/3/89; study began 8/9/88.
Guidelines:	US EPA 81-3, 40 CFR 158.135 Deviations from EC guideline Method B.2. Acute Toxicity (Inhalation): None
GLP:	Yes
Methodology:	Test material: Sulfuryl fluoride (Lot # 880329 752; Purity 99.6%). Groups of 5 B6C3F1 mice/sex/dose were exposed to 400, 600 or 1000 ppm (1668, 2802 or 4170 mg/m^3, respectively) for a single 4-hr period. Whole-body exposures occurred under dynamic air flow conditions. Animals were observed daily and weighed on days 1, 2, 4, 8, 11 and 15. Animals that died were necropsied; survivors were necropsied on day 15. Chamber concentrations were generated using a glass J-tube method (for mixture with compressed air) from a Saran bag. The chamber concentrations were analysed with a Miran 1A infrared spectrophotometer.
Findings:	All animals died within 90 minutes following exposure to 1000 ppm SO_2F_2 and within 5 days following exposure to 600 ppm. Body tremors were observed in several female mice shortly after exposure to 600 ppm and animals surviving after the exposure period were lethargic prior to their death. Body weights for most mice exposed to 600 ppm sulfuryl fluoride which survived one day post-exposure were decreased from pre-exposure values. Mice exposed to 400 ppm sulfuryl fluoride survived until the scheduled sacrifice. There were no body weight effects noted in male and female mice exposed to 400 ppm. There were no exposure-related gross pathologic effects noted in male and female mice exposed to 400, 600 or 1000 ppm sulfuryl fluoride. Analytical concentrations were close to target concentrations.
Conclusions:	**The 4-hour LC_{50} of sulfuryl fluoride is between 400 and 600 ppm (1.668 and 2.802 mg/L) for both male and female mice. Under the conditions of this study, sulfuryl fluoride would be classified Toxic; R23 'Toxic by inhalation'.**

Section A6.1.3/03 Evaluation by Competent Authorities

Annex point IIA, VI. 6.1.3

EVALUATION BY RAPPORTEUR MEMBER STATE

Date April 2004

Materials and methods The applicant's version is adopted.

Results and discussion The applicant's version is adopted with the following amendment.
Survival, mean body weight and clinical signs of the mice exposed to SO_2F_2 is summarized below

		SO_2F_2 (ppm)					
		400		600		1000	
		Survival (No. alive /total No)	Mean body weight (g)	Survival (No. alive /total No)	Mean body weight (g)	Survival (No. alive /total No)	Mean body weight (g)
During exposure		5/5 (♂) 5/5 (♀)		2/5 (♂) 4/5 (♀)		2/5 (♂) 2/5 (♀)	
				2 mice: body tremor			
Post-exposure (day)	1	5/5 (♂) 5/5 (♀)	25.8 (♂) 20.3 (♀)	2/5 (♂) 2/5 (♀)	25.9 (♂) 22.1 (♀)	0/5 (♂) 0/5 (♀)	21.1 (♀) Test ended
	2	All mice survived until the end of the test.	26.0 (♂) 21.0 (♀)	Weekend	22.7 (♂) 17.7 (♀)	Test ended	
	3		No record	Weekend			
	4		25.8 (♂) 21.8 (♀)	2/5 (♂) 2/5 (♀)	20.1(♂) 14.3 (♀)		
	5			4 mice: lethargy			
	6		No record	0/5 (♂) 1/5 (♀)	1 mouse: lethargy		
	7		No record	0/5 (♂) 0/5 (♀)			
	8		No record	Test ended			
	9		26.3 (♂) 21.5 (♀)				
	10		No record				
	11		27.4 (♂) 22.8 (♀)				
	12		No record				
	13		No record				
	14		No record				
	15		28.2 (♂)				

			24.0 (♀)

[a]Body weight was recorded prior to the exposure to sulfuryl fluoride on day 1 (exposure day).

Conclusion	The applicant's version is adopted.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	This study is acceptable.
Remarks	No remarks.

Section A6.1.3/04 **Acute inhalation toxicity**

Annex Point IIA, VI.6.1.3

Acute inhalation toxicity (mouse, IIA5.2.3/05, B06)

Report:	[redacted] (1990) Sulfuryl Fluoride: Acute LC50 Study with CD-1 Mice [redacted] K-016399-031, -031A, -031B, dated 21/12/90; study began 8/11/88.
Guidelines:	US EPA 81-1. Deviations from EC guideline Method B.2. Acute Toxicity (Inhalation): None
GLP:	Yes
Methodology:	Test material: sulfuryl fluoride (Lot # 880329 752 Mar/88; 99.6% pure). Groups of 5 CD-1 mice/sex/dose were exposed to 596, 692 or 806 ppm (2.485, 2.885 or 3.361 mg/L) SO_2F_2 for a single 4-hour period. Whole-body exposures occurred under dynamic airflow conditions. Animals were observed daily and weighed on days 1, 2, 4, 8, 11 and 15. Animals that died were necropsied; survivors were necropsied on day 15. Chamber concentrations were generated using a glass J-tube method (for mixture with compressed air) from a Saran bag. The chamber concentrations were analysed with a Miran 1A infrared spectrophotometer.
Findings:	Seven of ten mice exposed to 806 ppm sulfuryl fluoride and eight of ten mice exposed to 692 ppm died either during the 4-hour exposure period or within the two-week post-exposure period. Body tremors and/or lethargy were observed in several mice shortly after exposure to 692 or 806 ppm. All animals exposed to 596 ppm survived with no clinically visible effects noted. There were no exposure-related gross pathologic effects observed in male or female mice exposed to sulfuryl fluoride. However, animals which died on the day of exposure as well as those dying during the post-exposure period interval had the expected visceral congestion. The visceral congestion in animals dying during the post-exposure period was accompanied by evidence that these animals were not eating. Analytical concentrations were close to target concentrations.
Conclusions:	**The approximate LC_{50}s are 660 and 642 ppm sulfuryl fluoride for male and female CD-1 mice, respectively. This corresponds to 2.75 and 2.68 mg/L for males and females, respectively. Under the conditions of this study, sulfuryl fluoride would be classified as Harmful/Xn/R20 for CD-1 mice.**

Section A6.1.3/04 **Evaluation by Competent Authorities**

Annex point IIA, VI. 6.1.3.

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	April 2004
Materials and methods	The applicant's version is adopted with the following amendment. The LC_{50}-value was determined by non-linear interpolation.
Results and discussion	The applicant's version is adopted with the following amendment.

Survival, mean body weight and clinical signs of the mice exposed to SO_2F_2 is summarized below.

		SO_2F_2 (ppm)					
		596		692		806	
		Survival (No. alive /total No)	Mean body weight (g)	Survival (No.alive /totalNo)	Mean body weight (g)	Survival (No. alive /total No)	Mean body weight (g)
During exposure		5/5 (♂) 5/5 (♀)		5/5 (♂) 5/5 (♀)		2/5 (♂) 3/5 (♀)	
				2 mice: body tremor 5 mice: lethargy		1 mouse: body tremor	
Post-exposure (day)	1[a]	5/5 (♂) 5/5 (♀) All mice survived to the end of the test	33.4 (♂) 25.3 (♀)	4/5 (♂) 4/5 (♀)	34.4 (♂) 28.0 (♀)	2/5 (♂) 2/5 (♀)	34.0 (♂) 26.4 (♀)
						2 mice: body tremor	
	2		32.3 (♂) 25.4 (♀)	1/5 (♂) 4/5 (♀)	32.1 (♂) 24.5 (♀)	2/5 (♂) 2/5 (♀)	30.1 (♂) 25.3 (♀)
						1 mouse: body tremor	
	3		No record	1/5 (♂) 3/5 (♀)	No record	1/5 (♂) 2/5 (♀) Same number of mice survived to the end of the test	No record[4]
	4		33.2 (♂) 25.7 (♀)	1/5 (♂) 3/5 (♀)	30.5(♂) 20.9 (♀)		29.7 (♂) 26.2 (♀)
	5		No record	1/5 (♂) 1/5 (♀)	No record		No record
	6		No record	1/5 (♂) 1/5 (♀)	No record		No record
	7		No record	1/5 (♂) 1/5 (♀)	No record		No record
	8		31.9 (♂) 24.9 (♀)	0/5 (♂) 1/5 (♀) Same number of mice survived to the end of the test	27.0 (♀)		31.4 (♂) 27.0 (♀)
	9		No record		No record		No record
	10		No record		No record		No record
	11		25.6 (♂) 22.8 (♀)		27.9 (♀)		31.0 (♂) 27.6 (♀)
	12		No record		No record		No record
	13		No record		No record		No record
	14		No record		No record		No record
	15		34.1 (♂) 27.6 (♀)		28.1 (♀)		32.2 (♂) 28.1 (♀)

[a]Body weight was recorded prior to the exposure to sulfuryl fluoride on day 1 (exposure day).

Conclusion The applicant's version is adopted.

Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	The study is acceptable.
Remarks	No remarks.

Section A6.1.3/05 Acute Inhalation Toxicity

Annex Point IIA, VI.6.1.3

Study summary: refers to the publication (*Vernot EH, MacEwen JD, Haun CC, Kinkead FR [1977]. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicol Appl Pharmacol* 42:*417#*, **B08).**

Section A6.1.3/05 **Annex point IIA, VI. 6.1.3.**	**Evaluation by Competent Authorities**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2004
Materials and methods	The applicant attched the original publication (1977) by Vernot *et al.* The study failed to meet the requirements by present guideline or GLP programme. Specification of tested substance was not provided. Description of methods in the publication was general. Sprague-Dawley rats were dosed with sulfuryl fluoride in a chamber for 1 hour. Chamber concentration was measured with laboratory standard method (≤ 5% SD). Single exposure mortality was recorded.
Results and discussion	1-hour LC_{50} was 3730 ppm for male rats and 3020 ppm for female rats. This value had been pro rated to derive 4-hour exposure LC_{50} value by the applicant.
Conclusion	1-hour LC_{50} was 3730 ppm for male rats and 3020 ppm for female rats.
Reliability	Reliability indicator 3: Study with major methodological and/or reporting deficiencies.
Acceptability	The study is considered acceptable only for additional information (see Remarks).
Remarks	Due to the low reliability, the short exposure and the fact that the test substance is not specified, a reliable 4 h LC_{50}-value can not be derived from this study.

Section A6.1.4/01 Annex Point IIA, VI.6.1.4	Eye Irritation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [x] **Other justification** [ ]	
Detailed justification:	Due to the nature of the active substance, sulfuryl fluoride is a gas, it is technically difficult, if not impossible, to conduct a compliant regulatory study on this end-point. However, experience in humans over a period of 40 years of use of Vikane, indicates that sulfuryl fluoride does not present a significant hazard for eye irritation.	X1
	The above statement is correct but the human data are confounded by the presence of chloropicrin as a warning agent, which induces lachrymation at very low air concentrations. The main effect and area of concern for humans exposed to sulfuryl fluoride is inhalation toxicity. The fact that it is classified as 'Toxic' means that the 'Irritant' symbol would not appear on the label. More importantly, we would not want to suggest that eye irritation might occur when it might not, as this could conceivably mislead a person. What is clear from many controlled animal studies in which exposure was high, prolonged and 'whole-body' (and hence included eye contact), is that irritant effects on the eye were not observed. It seems unequivocal that sulfuryl fluoride is not a significant eye irritant.	
	Sulfuryl fluoride has recently been classified (ECB, 2002) without phrases for eye irritation. Please see below Summary Record taken from the ECB website on decision taking:	X2
	ECBI/15/02 Rev. 3 Ispra, 31 July 2002 SUMMARY RECORD Meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances ECB Ispra, 16 – 18 January 2002:	
	Sulphuryl difluoride (P467) (CAS No: 2699-79-8, EC No: 220-281-5, Annex I Index No: 009-015-00-7) **Classification proposal: [Xn; R20 - Xi; R37]** **Classification in Annex I, 21st ATP: T; R23/25 - Xi; R36/37/38**	
	ECBI/55/00 — UK, five substance data sheets plus classification proposals, health effects, incl. sulfuryl difluoride (P467, revision) ECBI/55/00 – Add. 3 — UK, sulfuryl difluoride (P467, revision), existing classification with Xi: R37 should be maintained. ECBI/55/00 – Add. 5 — UK, sulfuryl difluoride (P467), Acute oral toxicity ECBI/55/00 – Add. 6 — D, sulfuryl difluoride (P467),Comment on health classification - follow-up after CMR meeting - September 2001	
	In September 2001 **UK** introduced their classification proposal for the substance. As the substance was a gas industry thought the current classification with T; R25 was not justified. The **Group** agreed to classify with Xn; R20 for acute toxicity. **UK** explained that the current classification was not justified and they would prefer not to classify with R37. The **Group** provisionally agreed to this based on	

precautionary reasons but wanted to re-discuss the endpoint when more information on the impurity was available. **UK** proposed to dismiss R48/20. However some countries preferred to classify with R48 and asked for more time to look into the data. The **Group** agreed to classify sulphuryl difluoride with Xn; R20 and provisionally with Xi; R37. **F, S** and **D** put in a reservation on R48/20. During the follow-up period **D** asked for re-discussion at the next meeting with the aim to maintain the current classification as very toxic.

T; R23/25

D apologised that they had reacted so late. They presented document 55/00 Add. 6 describing fumigation incidents. There were reports on human experience leading to death of lung oedema after fumigation of house. This was of course not the same situation as at a work place, where you would not have a continuous application. Instead in the cases of fumigated houses, people who started to feel ill had often be recommended by consulted doctors to stay in the house and rest, which then instead had led to a prolonged exposure.

The **Chair** asked whether there were impurities to consider in the substance. **D** replied that it was rather sulphuryl difluoride in combination with other substances that led to irritancy of the respiratory tract.

S supported the D proposal to keep the label as toxic. There was a very steep dose response dose curve and it was needed to have very strict restrictions for the use of the substance. There were also observed brain effects in three different animal species. **UK** had proposed to re-classify the substance as harmful and that had also been agreed at the last meeting. However, listening to arguments put forward UK said that they also could go along with the D proposal for acute toxicity. However, they said that R25 would be irrelevant and that the only route of concern was inhalation. The **Group** agreed to classify the substance with T; R23.

R48/20

UK suggested not classifying with R48 on basis of similar substances that were not classified. Further the concern would now be covered by the T classification. **D** meant that it was repeated dose toxicity by inhalation and to them it seemed more logic the other way around, i.e. R48/23 rather than R23. **IRL** expressed support for T; R23 and R48/20. **DK** suggested T; R23 and R39. **D** remarked that R39 not would include death and also **IND** agreed to this. **S** said that T; R23 was the important part for them but they would also like to classify with R48/20. A majority of the Group agreed to classify with R48/20 in addition to the already agreed classification for acute toxicity.

Xi; R37

The **Group** agreed not to classify for irritancy in the respiratory system on the basis of the previous discussion.

Conclusion:
The **Group** agreed to classify **sulphuryl difluoride** as follows:
T; R23 - Xn; R48/20
Symbol: T
R-phrases: 23- 48/20

Section A6.1.4/01 Annex Point IIA, VI.6.1.4	Eye Irritation
	S-phrases: (1/2-) 45- 63 This classification and labelling will be included in the proposal for the next ATP.
Undertaking of intended data submission [ ]	No studies are planned.
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2004
Evaluation of applicant's justification	This justification is based on the following reasons according to the applicant: 1. Technical difficulty in performing the experiment for this end point due to the gas form of the substance; 2. Non-eye-hazard experiences from the early use of the product; 3. Lack of irritant effect from the acute toxicity studies in which the exposure involved "whole body", 4. ECB's classification X1: Human data: A medical surveillance of plant fumigators did not show any eye irriation related to the use of sulfuryl fluoride (III-A6.12.1). The lack of known effects from 40 years of use is only an indicator and can not be used as a sole argument to waive this end-point since it is known that not all incidents are declared. In direct observations from clinical cases and poisoning incidents, eye irritation symptom has been reported (III-A6.12.2). However, this human data was confounded by the presence of low concentration of chloropicrin during the exposure, thus, the eye irritative effect by sulfuryl fluoride from this data could not be decided. Experimental data: One acute toxicity study (III-A6.1.3/02, B03) showed that most of the dead rats during or after 0.5 hours exposure had "exudative material around external nares and/or eyes" (p21-4) at concentrations between 500 and 2000 ppm. In the same experiment 1/4 of the survived rats hade unilateral focal corneal cloudiness at 1250 ppm. But 1/10 of rats in the control group also showed a similar change. The possible mechanism for exudation around the eyes in tested rats has not been considered as direct irritation-related but a secondary response to the toxic exposure to sulfuryl fluoride (See RMS's comments in III-A6.1.3/02). Eye irriation was not observed in other acute inhalation studies, in which the eyes were exposed to sulfuryl fluoride simultanously via whole body exposure route. X2: ECB classification: The fact that a substance is not classified for the end-point eye irritation can not be used as a waiving argument if it is not shown that the lack of classification is based on data showing that it is not an eye irritant and not on lack of data. This is not clear from the study summary. In conclusion: Based on the following reasons, when weighed together, waiving of the eye irritation study on sulfuryl fluoride would be considered acceptable. 1. Sulfuryl fluoride has not shown the specific irritancy to the eyes according to the available experimental information (III-A6.1.3) and human data (III-A6.12.1 and A6.12.2). 2. When used as a biocide, eye exposure to sulfuryl fluoride during the professional application is not a common case. 3. In several acute inhalation toxicity studies in rodents the eyes were also exposed since the exposure was whole-body. There were no direct signs of eye

Section A6.1.4/01 Annex Point IIA, VI.6.1.4	Eye Irritation
	irritation observed clinically except the finding by gross pathology discussed above. 4. Practical difficulty in performing the experiment due to the fact that the substance is a gas.
Conclusion	The justification is acceptable.
Remarks	No remarks.

Section A6.1.4/02 Annex Point IIA, VI.6.1.4	Skin Irritation

Rat (IIA5.2.4/01, B07)

For the study summary, see III-A6.1.2 Acute Dermal Toxicity.

Section A6.1.4/02 Annex point IIA, VI. 6.1.4.	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2004
Materials and methods	1. The applicant uses the acute dermal toxicity (III-A6.1.2, B07) study to illustrate that sulfuryl fluoride is not a skin irritant. The study was a guideline study except that the test substance is a gas. There is no equivelant guideline for the active substance in gas form regarding the test method for skin irritation. In the study (B07), there was no skin irritation tendency observed by gross pathology or histopathology. Thus, sulfuryl fluoride is unlikely to act as a skin irritant. 2. There are a few acute inhalation studies can be considered to be references for the endpoint of skin irritation (III-A6.1.3/02, 03, 04), although these studies have not been designed particularly for that purpose. In these experiments, skin was simultanoeusly exposed to sulfuryl fluoride during the treatment. There were no skin changes reported by gross pathologic or histopathological examination.
Results and discussion	There were no indications of skin irritation by sulfuryl fluoride as examined by gross pathology or histopathology in either acute inhalation (III-A6.1.3/02, 03, 04) or acute dermal (III-A6.1.2) toxicity studies.
Conclusion	There is no skin irritation tendency for sulfuryl fluoride.
Reliability	See respective acute dermal and inhalation studies (III-A6.1.2 and A6.1.3/02, 03, 04). The conclusion derived from the comprehensive analysis from various related studies is reliable.
Acceptability	The conclusion is acceptable.
Remarks	No remarks.

Section A6.1.5 Annex Point IIA, VI.6.1.5	Skin Sensitisation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [x]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	Due to the nature of the active substance, sulfuryl fluoride is a gas, it is technically difficult, if not impossible, to conduct a compliant regulatory study on this end-point. However, experience in humans over a period of 40 years of use of Vikane, indicates that sulfuryl fluoride does not present a hazard for sensitisation.	X1
	It is practically difficult, if not impossible, to conduct a specific study on skin contact sensitisation. However, to our knowledge, there are no confirmed cases in humans to suggest that sulfuryl fluoride is a skin sensitiser.	
	Sulfuryl fluoride has recently been classified (ECB, 2002) without phrases for sensitisation. Please see below Summary Record taken from the ECB website on decision taking:	X2
	ECBI/15/02 Rev. 3 Ispra, 31 July 2002 SUMMARY RECORD Meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances ECB Ispra, 16 – 18 January 2002:	
	Sulphuryl difluoride (P467) **(CAS No: 2699-79-8, EC No: 220-281-5, Annex I Index No: 009-015-00-7)** **Classification proposal: [Xn; R20 - Xi; R37]** **Classification in Annex I, 21st ATP: T; R23/25 - Xi; R36/37/38**	
	ECBI/55/00 — UK, five substance data sheets plus classification proposals, health effects, incl. sulfuryl difluoride (P467, revision) ECBI/55/00 – Add. 3 — UK, sulfuryl difluoride (P467, revision), existing classification with Xi: R37 should be maintained. ECBI/55/00 – Add. 5 — UK, sulfuryl difluoride (P467), Acute oral toxicity ECBI/55/00 – Add. 6 — D, sulfuryl difluoride (P467),Comment on health classification - follow-up after CMR meeting - September 2001	
	In September 2001 **UK** introduced their classification proposal for the substance. As the substance was a gas industry thought the current classification with T; R25 was not justified. The **Group** agreed to classify with Xn; R20 for acute toxicity. **UK** explained that the current classification was not justified and they would prefer not to classify with R37. The **Group** provisionally agreed to this based on precautionary reasons but wanted to re-discuss the endpoint when more information on the impurity was available. **UK** proposed to dismiss R48/20. However some countries preferred to classify with	

Section A6.1.5 Annex Point IIA, VI.6.1.5	Skin Sensitisation

R48 and asked for more time to look into the data. The **Group** agreed to classify sulphuryl difluoride with Xn; R20 and provisionally with Xi; R37. **F, S** and **D** put in a reservation on R48/20. During the follow-up period **D** asked for re-discussion at the next meeting with the aim to maintain the current classification as very toxic.

T; R23/25

D apologised that they had reacted so late. They presented document 55/00 Add. 6 describing fumigation incidents. There were reports on human experience leading to death of lung oedema after fumigation of house. This was of course not the same situation as at a work place, where you would not have a continuous application. Instead in the cases of fumigated houses, people who started to feel ill had often been recommended by consulted doctors to stay in the house and rest, which then instead had led to a prolonged exposure.

The **Chair** asked whether there were impurities to consider in the substance. **D** replied that it was rather sulphuryl difluoride in combination with other substances that led to irritancy of the respiratory tract.

S supported the D proposal to keep the label as toxic. There was a very steep dose response dose curve and it was needed to have very strict restrictions for the use of the substance. There were also observed brain effects in three different animal species. **UK** had proposed to re-classify the substance as harmful and that had also been agreed at the last meeting. However, listening to arguments put forward UK said that they also could go along with the D proposal for acute toxicity. However, they said that R25 would be irrelevant and that the only route of concern was inhalation. The **Group** agreed to classify the substance with T; R23.

R48/20

UK suggested not classifying with R48 on basis of similar substances that were not classified. Further the concern would now be covered by the T classification. **D** meant that it was repeated dose toxicity by inhalation and to them it seemed more logic the other way around, i.e. R48/23 rather than R23. **IRL** expressed support for T; R23 and R48/20. **DK** suggested T; R23 and R39. **D** remarked that R39 not would include death and also **IND** agreed to this. **S** said that T; R23 was the important part for them but they would also like to classify with R48/20. A majority of the Group agreed to classify with R48/20 in addition to the already agreed classification for acute toxicity.

Xi; R37

The **Group** agreed not to classify for irritancy in the respiratory system on the basis of the previous discussion.

Conclusion:

Section A6.1.5 Annex Point IIA, VI.6.1.5	Skin Sensitisation
	The **Group** agreed to classify **sulphuryl difluoride** as follows: **T; R23 - Xn; R48/20** **Symbol: T** **R-phrases: 23- 48/20** **S-phrases: (1/2-) 45- 63** This classification and labelling will be included in the proposal for the next ATP.
Undertaking of intended data submission [ ]	No studies are planned.
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2004
Evaluation of applicant's justification	There are two reasons for waiving the skin sensitization study according to the applicant: 1) technical difficulty in performing the experiment as the active substance is in gas form; 2) There is no evidence whatsoever indicating that sulfuryl fluoride acts as an allergen. X1: The lack of known effects from 40 years of use is only an indicator and can not be used as a sole argument to waive this end-point since it is known that not all incidents are declared. X2: The fact that a substance is not classified for the end-point skin sensitisation can not be used as a waiving argument if it is not shown that the lack of classification is based on data showing that it is not an sensitiser and not on lack of data. This is not clear from the study summary When the arguments are weighed together, and since the use of the formulated product does not normally involve direct skin contact, and because of the relative difficulty in performing the experiment due to the gaseous form of the substance, waiving is acceptable.
Conclusion	Justification is acceptable
Remarks	No remarks.

Section A6.2
Annex Point IIA, VI.6.2

Metabolism Studies in Mammals: Studies on Absorption, Distribution, Excretion and Metabolism in Mammals

Single low and high dose, inhalation route (Rat, IIA 5.1a/b/01, H01)

Report: [redacted] (2002)
Sulfuryl Fluoride: Pharmacokinetics and Metabolism in Fischer 344 Rats
[redacted]
DECO HET K-016399-059, dated 22 May 2002; study began 26 March 2001

Guidelines: USEPA, OPPTS 870.7485, 1998
OECD, Guideline 417, 1984
EEC, Part B.36, 87/302/EEC, 1986
Deviations from EC guideline: None

GLP: Yes

Methodology: Test material: sulfuryl fluoride (Non-radiolabelled TSN 102173, Lot Number OC16160101, purity 99.8%; Radiolabelled Lot Number: 901-059-010, 100% radiochemically pure).

This study was conducted to determine the pharmacokinetics and metabolism of inhaled sulfuryl fluoride (SO_2F_2) in the rat. Groups of rats were given single, four-hour exposures to target concentrations of 30 or 300 ppm sulfuryl fluoride by inhalation via a nose-only inhalation chamber according to the following design:

30 ppm ^{35}S-sulfuryl fluoride group:
4 jugular vein cannulated rats and 4 non-cannulated rats
300 ppm ^{35}S-sulfuryl fluoride group:
4 jugular vein cannulated rats and 4 non-cannulated rats
30 ppm non-radiolabelled sulfuryl fluoride group: 18 non-cannulated rats
300 ppm non-radiolabelled sulfuryl fluoride group: 18 non-cannulated rats
0 ppm sham exposed control group: 8 non-cannulated rats.

The jugular vein cannulated animals were used only during the radiolabelled portion of the study for blood collection during and post-exposure. All other specimens were collected from non-cannulated animals only.

Rats exposed to ^{35}S-labelled sulfuryl fluoride had blood (~0.15 ml), urine, and faeces collected during and following the exposures and analysed for radioactivity, ^{35}S-labelled fluorosulfate and sulfate and fluoride (urine and faeces only). Selected tissues were collected 7-days post exposure and analysed for radioactivity. For the radiolabelled portion of the study, venous blood samples were collected via the indwelling jugular cannulae at 0.25, 0.5, 1, 2, 3 and 4-hour during the inhalation exposure and at 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, 120, 144 and 168-hour post-exposure. Blood was obtained at sacrifice via cardiac puncture. Plasma and RBC were analysed for radioactivity from which plasma and RBC ^{35}S concentration-time courses were constructed. Aliquots of plasma were counted directly by LSC while RBC were solubilised and then analysed by LSC. In addition, approximately equal-volume aliquots of blood was pooled (per sample time) and stored at -80 °C for chemical analysis.

Groups of rats exposed to unlabelled sulfuryl fluoride had blood, brain and kidney tissues collected during and following the exposures and analysed for fluoride ion. During the non-radiolabelled portion of the study, in order to obtain a volume of plasma large enough for fluoride analysis, groups of three rats were sacrificed at -2 hour (during exposure), at 0 hour (end of exposure), and at 2, 4, 8 and 20 hour post-exposure. Sham-exposed control animals in groups of two were sacrificed at -4, 0, 4 and 8 hour. Blood was obtained at sacrifice via cardiac puncture. The blood was centrifuged to separate plasma and plasma analysed by fluoride-ion specific probe for fluoride ion content and a fluoride ion concentration-time course was constructed. Plasma was not pooled for fluoride analysis.

Urine voided during the study was collected in dry-ice cooled traps. The urine traps were changed at the end of exposure (0 hour) and at 6, 12, 24, 48, 72, 96, 120, 144 and 168-hour post-exposure. The cages were rinsed with water at the time the traps were changed and the rinse collected. Each urine specimen and urine/cage rinse were weighed, and a weighed aliquot of each sample was analysed for radioactivity by LSC. Equal volume aliquots of urine samples from the 0–6 hour and 6–12 hour post-exposure collection intervals were pooled and stored at -80 °C and selected urine samples underwent radiochemical profile analysis.

Faeces were collected in dry-ice chilled containers through the exposure period (0 hour) and at 24, 48, 72, 96, 120, 144 and 168-hr post-exposure. Aqueous homogenates (~25% w/w) were prepared and weighed aliquots of these homogenates were placed in scintillation vials, solubilised and quantitated for radioactivity by LSC. In addition, equal volume aliquots of faecal homogenates from each animal were taken from the 0-24 hr post-exposure collection interval and pooled. These pooled samples were stored at -80 °C prior to radiochemical profile analysis.

Air was drawn through the metabolism cage at approximately 500 ml/min. Upon exiting the cage, volatiles in the expired air were trapped on charcoal by passage of expired air through a charcoal trap. The charcoal was then desorbed with toluene and aliquots analysed for radioactivity by LSC. No radioactivity was detected in the 0-24 hr post-dosing charcoal traps following the 300 ppm exposure. Therefore, expired air was not scrubbed through charcoal for the remainder of the 300 ppm sample collection intervals nor for any of the sample collection intervals for the 30 ppm exposure.

At 168-hr post-exposure, the animals were anaesthetised with CO_2 and sacrificed by exsanguination via cardiac puncture. Following sacrifice, the Roth cages were washed, and the cage wash analysed for radioactivity. The brain, GI tract plus contents, kidneys, liver, lungs, carcass and spleen were collected, homogenised individually (~33% homogenate), and a weighed aliquot solubilised and analysed for radioactivity by LSC. Blood was obtained at sacrifice via cardiac puncture and an aliquot also assessed for radioactivity by LSC. The skin was removed from the carcass and a representative skin sample was solubilised and analysed for radioactivity by LSC. Perirenal fat and the nasal turbinates, both olfactory and respiratory, were directly solubilised without homogenisation.

For the non-radiolabelled fluoride ion portion of the study, homogenates of brain and kidney from groups of three rats sacrificed at -2 hr (during exposure), 0 hr (end of exposure), 2 hr and 4 hr post-exposure were analysed for fluoride ion by fluoride-ion specific probe.

Following the terminal sacrifice of the animals, a final cage wash was performed. The final cage wash and contents were collected and the weight of the sample was determined. A weighed aliquot of the final cage wash was analysed for radioactivity.

Control urine and faeces were collected in dry-ice cooled traps from male rats not

exposed to sulfuryl fluoride. During the non-radiolabelled fluoride ion portion of the study, a control group of rats was sham exposed to dry air using a nose-only inhalation chamber. Two rats were sacrificed at each sacrifice time. Animals were sacrificed prior to the sham exposure (-4 hr), at 0 hr (end of exposure), and at 4 and 8 hr post-exposure. Blood was obtained at sacrifice via cardiac puncture. Plasma was analysed by fluoride-ion specific probe for fluoride ion content and a fluoride ion concentration-time course was constructed. Plasma was not pooled for fluoride analysis. Brain and kidney tissue were also analysed for fluoride ion as stated above.

The ^{35}S-sulfuryl fluoride was analysed for radiochemical purity and structural confirmation. The radiochemical purity of ^{35}S-sulfuryl fluoride, with retention time confirmation using an authentic standard of sulfuryl fluoride, was determined using gas chromatography with flow radiogas monitoring (GC/RAM) and thermal conductivity detection (GC/TCD). The structural confirmation was accomplished using gas chromatography/electron impact ionisation/mass spectrometry (GC/EI/MS).

^{35}S Analysis: Radioactivity was quantified in a liquid scintillation spectrometer. Counts per minute (cpm) were corrected for quench and background, and converted to disintegrations per minute (dpm). At least one sealed ^{14}C-standard was counted with each group of samples to monitor the performance of the liquid scintillation counter (LSC). Samples with dpm less than twice the concurrently run background (blanks) were considered to contain insufficient radioactivity to reliably quantify. Samples were counted as soon as possible after collection, therefore no correction for radioactive decay was performed (half-life ^{35}S = 88 days).

The LSC data were used to generate reconstructed radiochromatograms and these radiochromatograms were integrated to determine peak area response of the radiolabelled peaks. The concentration of sulfate and fluorosulfate in these samples were estimated from the calculated specific activity of sulfate and fluorosulfate and the total radioactivity in the radiolabelled peaks.

The concentration of fluoride ion in the urine of rats exposed to both concentrations of ^{35}S-sulfuryl fluoride was determined by ion selective electrode (ISE). Also, ISE determination of the fluoride ion concentration in the plasma, and the brain and kidney tissue homogenates, of rats exposed to both concentrations of non-radiolabelled sulfuryl fluoride was conducted.

Findings:

The target exposure concentration of 300 ppm ^{35}S-sulfuryl fluoride had an actual time-weighted average (TWA) of 274 ppm through the 4-hr exposure period and a ^{35}S concentration of approximately 2.8 µCi/L of atmosphere (specific activity 0.25 mCi/mmole). The non-radiolabelled 300 ppm sulfuryl fluoride exposure had a TWA of 312 ppm. The target exposure concentration of 30 ppm ^{35}S-sulfuryl fluoride had an actual TWA of 28.4 ppm through the 4-hr exposure period and a ^{35}S concentration of approximately 0.26 µCi/L of atmosphere (specific activity 0.22 mCi/mmole). The non-radiolabelled 30 ppm sulfuryl fluoride exposure had a TWA of 31.2 ppm. Sulfuryl fluoride was not detected in any of the analyses of the control chamber atmosphere at a level exceeding the lowest level quantified (~1 ppm).

All animals survived the 4-hr sulfuryl fluoride exposure. Those animals exposed to 30 and 300 ppm radiolabelled sulfuryl fluoride were maintained for 1-week post-exposure prior to sacrifice. Animals exposed to non-radiolabelled sulfuryl fluoride were sacrificed at the specified times pre-, during and post-exposure.

Radiolabelled sulfuryl fluoride was rapidly absorbed via inhalation exposure, achieving maximum concentrations of ^{35}S-sulfuryl fluoride-derived radioactivity in both plasma and red blood cells near the end of the 4-hr exposure periods. Once absorbed, the ^{35}S was rapidly excreted, primarily via the urine. Absorbed ^{35}S was excreted in the urine even during the 4-hr nose-only exposure.

Recovery: Plasma Radioactivity

During the 30 ppm exposure period, radioactivity derived from ^{35}S-sulfuryl fluoride achieved quantifiable plasma levels by 15 to 30 minutes after initiation of the exposure (-3.75 to -3.5 hr). Plasma levels of radioactivity continuously increased throughout the exposure period, ultimately achieving a peak mean plasma concentration of 5.2 µg-equivalents/g (µg-eq./g) at the end of exposure. Plasma levels of radioactivity during the 4 hr exposure never reached steady-state conditions most likely because of rapid elimination of radioactivity from the plasma. Following termination of ^{35}S-sulfuryl fluoride exposure the plasma radioactivity decreased rapidly during an initial (α) phase with a half-life of 2.6 hr. A second β phase beginning about 24 hr post-exposure was observed with a half-life calculated as 82.7 hr. The $AUC_{(-4\ hr-\infty)}$ for the 30 ppm exposure of ^{35}S-sulfuryl fluoride-derived radioactivity was calculated as 96.7 µg-hr ml^{-1}.

During the 300 ppm exposure period, radioactivity derived from ^{35}S-sulfuryl fluoride achieved detectable plasma levels in all rats by 15 minutes after initiation of the exposure. Plasma levels of radioactivity rapidly increased throughout the exposure period and, similar to the 30 ppm exposure, never reached steady-state conditions. A peak mean level of plasma radioactivity of 37.7 µg-eq./g, approximately 7-fold higher than obtained during the 30 ppm exposure, was obtained by 15 minutes after termination of ^{35}S-sulfuryl fluoride exposure. As observed with the 30 ppm exposure, plasma radioactivity decreased rapidly following termination of exposure. During an initial α-phase, a plasma radioactivity half-life of 2.4 hr was calculated. Similar to the lower concentration, a longer β-phase beginning about 24 hr post-exposure was observed with a half-life calculated as 56.2 hr. The 300 ppm exposure $AUC_{(-4\ hr-\infty)}$ of ^{35}S-sulfuryl fluoride-derived radioactivity was calculated as 756.3 µg-hr ml^{-1}, nearly 8-fold higher then that obtained for the 30 ppm exposure.

RBC Radioactivity:

RBC kinetics were similar to that of plasma in uptake and initial distribution phases but a longer terminal half-life for the elimination of RBC radioactivity was observed. Peak RBC radioactivity was measured at the end of the 4-hr exposure period for both 30 and 300 ppm ^{35}S-sulfuryl fluoride exposures. Immediately following the ^{35}S-sulfuryl fluoride exposures, mean peak RBC radioactivity reached 4.7 and 40.3 µg-eq./g RBC for the 30 and 300 ppm ^{35}S-sulfuryl fluoride exposure concentrations, respectively. The similar peak concentration of radioactivity measured with plasma and RBC suggests the ^{35}S initially was evenly distributed between these two compartments. Following the peak RBC-radioactivity levels at termination of exposure, an initial α elimination phase half-life of 2.5 and 1.1 hr was calculated for the 30 and 300 ppm exposure levels. This α-phase was similar to that observed with plasma. But, the RBC terminal β-phase beginning about 12-hr post-exposure was approximately 2.5 times longer than that calculated with plasma for both sulfuryl fluoride exposure concentrations. Following the 30 ppm exposure the β-phase was estimated to be 222 hr and following the 300 ppm exposure the β-phase was estimated at 139 hr. These longer terminal half-lives may be a result of either non-specific incorporation of the ^{35}S radiolabel as the radiolabel becomes available through the sulfate pool, transulfuryl fluorideormed into amino acids and incorporated into tissues, or a result of binding of some unknown ^{35}S-containing component to RBC. Radioactivity remained at measurable, albeit small, concentrations until the time of terminal sacrifice, 168-hr post-exposure. The $AUC_{(-4\ hr-\infty)}$ values for RBC, 863 and 5492 µg-hr ml^{-1} for 30 and 300 ppm exposure concentrations, respectively, are 9- to 7-fold larger than the AUC obtained for plasma.

Absorption: Summation of the recovered radioactivity from urine, faeces and

tissues gives an estimate of the total absorbed radioactivity (Table 5.1a/b/01-1), giving an estimate of the amount of sulfuryl fluoride that became systemically available during the 4-hr exposures. Following the 30 ppm exposure, 581, 73, and 35 µg-eq. sulfuryl fluoride (5.7, 0.7, and 0.34 µmole-eq.) were recovered in the urine, faeces and tissues, respectively. Therefore, about 688 µg-eq. sulfuryl fluoride (~6.7 µmole-eq.) was absorbed during the 4-hr 30 ppm exposure and ~85% was eliminated in the urine. Following the 300 ppm exposure, 4618, 777, and 298 µg-eq. sulfuryl fluoride (45, 7.6, and 2.9 µmole-eq.) were recovered in the urine, faeces and tissues, respectively. Therefore, about 5690 µg-eq. sulfuryl fluoride (~55 µmole-eq.) was absorbed during the 4-hr 300 ppm exposure and 81% was eliminated in the urine. The 5% of the ^{35}S radiolabel recovered from tissues 7 days post-exposure is likely due to non-specific incorporation of the ^{35}S radiolabel as absorbed radiolabelled sulfur compounds are transulfuryl fluorideormed into amino acids and become incorporated into tissues.

Excretion: Urinary Excretion (Table 5.1a/b/01-1)

Once absorbed, the ^{35}S-sulfuryl fluoride-derived radioactivity rapidly appeared in the urine and faeces. Urine contained 88.9 and 85.6% of the total excreted radioactivity through 7 days post-exposure. The ^{35}S absorbed from inhaled sulfuryl fluoride was excreted even during the exposure, with the majority of the radioactivity recovered in the urine during the nose-only exposure period (-4 to 0-hr). These urine samples contained 273 and 2766 µg-eq., or 47 and 60% of the total urinary radioactivity, for the 30 and 300 ppm sulfuryl fluoride exposures, respectively. The 0 to 6-hr interval urine samples collected immediately following the end of the exposures contained 167 and 936 µg-eq., or an additional 29 and 20% of the total urinary radioactivity, for the 30 and 300 ppm sulfuryl fluoride exposures, respectively. Conversion of the amounts excreted to elimination rates to correct for unequal collection intervals enabled estimation of half-life of elimination. Initial urinary elimination rates of 68 and 691 µg-eq./hr for 30 and 300 ppm exposures, respectively, rapidly decreased through subsequent collection intervals. An initial urinary half-life for ^{35}S-sulfuryl fluoride-derived radioactivity was estimated as approximately 4 hr at both exposure concentrations. This was followed by a second urinary elimination phase with a half-life of approximately 40 hr. Radioactivity remained detectable in the urine through 7-days post-dosing.

Faecal Excretion (Table 5.1a/b/01-1)

Faecal excretion data is presented as µg- and µmole-eq. sulfuryl fluoride for each collection interval and as cumulative µg- and µmole-eq. sulfuryl fluoride. Radioactivity was detected in faeces collected during the exposure period, probably from contamination of fecal pellets with radioactive urine which may have occurred during the exposure period. Separation and collection of urine and faeces during the nose-only exposure period was difficult due to the animal restraint device used with the nose-only exposure chamber. Following the exposures, when cleaner separation of urine and faeces was possible, less than 10% of the total radioactivity recovered in the urine and faeces was collected with the faeces. Through 48 hr post-exposure, 70 and 704 µg-eq. of sulfuryl fluoride was collected in the faeces from the 30 and 300 ppm exposures, respectively, representing 91-93% of the total amount of radioactivity recovered in the faeces. In total, through 7-days post-dosing, 73 and 777 µg-eq. sulfuryl fluoride were recovered in the faeces from the 30 and 300 ppm exposure groups, respectively.

Distribution: The highest concentration of radioactivity was detected in tissues at the site of first exposure to the gas. Following both the 30 and 300 ppm exposures, the lungs had the highest concentration of radioactivity, 0.77 and 6.30 µg-eq. ^{35}S-sulfuryl fluoride/g tissue. With the 30 ppm exposure, the lung radioactivity concentration was followed by spleen, kidneys, nasal turbinates (respiratory and

olfactory), brain, skin, carcass, liver, GI tract and fat. The rank-order following the 300 ppm exposure was similar to that obtained with the 30 ppm exposure except that the respiratory and olfactory turbinates followed the lungs in the concentration of radioactivity in the tissues. In general, the concentration of radioactivity in the tissues was 7- to 11-fold higher following the 300 ppm exposure than the 30 ppm exposure.

Table 5.1a/b/01-1: Excretion data

Excretion after inhalation exposure (µg.eq sulfuryl fluoride)		
Group	**Low dose 30 ppm**	**High dose 300 ppm**
Urine (+rinse)	272.9	2766.0
0-6h	167.4	935.6
6-12h	54.2	429.4
12-24h	43.2	198.9
24-48h	20.1	123.5
48-72h	8.6	55.7
72-96h	5.3	39.2
96-120h	3.9	34.7
120-144h	2.9	21.4
144-168h	2.0	13.6
subtotal	580.6	4618.1
Faeces -4-0h	9.6	324.7
0-24h	32.4	221.6
24-48h	21.8	158.0
48-72h	5.8	38.3
72-96h	2.1	19.4
96-120h	1.0	9.4
120-144h	0	0.6
144-168h	0	4.4
subtotal	72.7	776.5
Expired air	-	-
Cage Wash	-	-
Tissues (Day 7)	35.0	297.6
Total Recovery	**688.3**	**5692.2**

Metabolism: Chemical Analysis – Blood

Blood extract samples showed only two radiolabelled components, tentatively identified as sulfate and fluorosulfate. No analysis for parent sulfuryl fluoride in blood was performed because methods development work previously done in this laboratory showed, *in vitro*, rapid removal of sulfuryl fluoride from rat blood fortified with high levels of sulfuryl fluoride. The identification of fluorosulfate was confirmed by ^{19}F NMR spectroscopy. The amount of fluorosulfate is approximately 2-fold higher than sulfate at all sample times post-exposure,

except 15 minutes after the beginning of the 300 ppm exposure where the concentration of fluorosulfate is 6.5-fold higher, and 4 hr after the end of the 300 ppm sulfuryl fluoride exposure where only a small amount of fluorosulfate was detected. At all sample times, sulfate and fluorosulfate are approximately 3- to 5-fold higher following the 300 ppm exposure than following the 30 ppm exposure. Based on the limited amount of data available, a half-life for fluorosulfate elimination from whole blood was calculated to be 48 to 73 minutes while the half-life for sulfate elimination from whole blood was calculated to be 50 to 64 minutes.

Chemical Analysis – Urine

Two radioactive peaks tentatively identified as sulfate and fluorosulfate were detected in urine. Analysis for parent sulfuryl fluoride in urine was not conducted as sulfuryl fluoride has been shown to be rapidly hydrolysed in aqueous solutions. During the exposure, the amount of fluorosulfate eliminated in the urine was 3- to 3.5-fold higher than the amount of sulfate (Table 5.1a/b/01-2). Following the exposures, the amount of sulfate recovered in the urine was greater than fluorosulfate recovered in urine. By 12 hr post-exposure, 5- to 7-fold more sulfate was being recovered in the urine than fluorosulfate. The total µmoles of sulfate plus fluorosulfate recovered in the urine as determined by HPLC/RAM compares well with the µmole-eq. sulfuryl fluoride in the urine + rinse as determined from the radiolabelled portion of this study.

Conversion of sulfate and fluorosulfate urine concentrations to rate estimates to correct for unequal collection intervals allowed calculation of half-lives for the elimination of sulfate and fluorosulfate in urine. Sulfate was eliminated in the urine with a half-life of 2.2 and 3.8 hr for the 30 and 300 ppm exposure groups, respectively. Fluorosulfate was eliminated slightly faster with a half-life estimate of 1.2 and 2.4 hr for the 30 and 300 ppm exposure groups, respectively.

Fluoride Analysis

Elevated levels of fluoride ion were detected in urine during and after the sulfuryl fluoride exposures (Table 5.1a/b/01-2). Non-exposed control rats had concentrations of fluoride ion of approximately 2.5 µg/g urine. By the end of the 4-hr 30 ppm exposure the urine levels of fluoride ion reached a maximum concentration of 9.3 µg/g urine. This concentration was maintained through 6 hr post-exposure. By 12 hr post-exposure the concentration had diminished to near background levels, 2.7 µg/g urine. In a similar fashion, by the end of the 4 hr 300 ppm exposure the urine levels of fluoride ion reached a maximum concentration of 76 µg/g urine, about 8-fold higher than that obtained following the 30 ppm exposure. But this concentration diminished through 6 hr post-exposure to 32 µg/g urine and by 24 hr post-exposure to 5.0 µg/g urine. The overall shape of the three curves for urine HPLC and ISE data during and following the sulfuryl fluoride exposures are quite similar indicating that the kinetics of formation and elimination of the three metabolites (urine sulfate, fluorosulfate and fluoride) are similar or are interrelated.

ISE analysis of plasma and tissues was conducted with rats exposed to non-radiolabelled sulfuryl fluoride and with control rats exposed to clean air (Table 5.1a/b/01-3). Levels of plasma fluoride ion in control animals ranged from 0.430 to 1.338 µg/g plasma throughout the 24 hr cycle. An apparent slight elevation of plasma fluoride from control levels was observed during the 30 ppm and 300 ppm exposures that rapidly returned to control levels by about 2 hr after exposure was terminated. Plasma fluoride was 1.6- and 5.4-fold higher than control levels at the end of exposure for the 30 and 300 ppm sulfuryl fluoride exposures, respectively. The maximum concentration of plasma fluoride measured at the termination of the 4-hr 30 ppm sulfuryl fluoride exposure is

similar to that reported by Eisenbrandt and Nitschke (1989; study ref. **D04**) following six hr/day, five days/week for 13 weeks exposure to 30 ppm sulfuryl fluoride, although considerable variation is reported. The plasma concentration reported here following the 300 ppm exposure is nearly twice as large as that reported by Eisenbrandt and Nitschke (1989; study ref. **D04**). But, our data indicate a rapid clearance of fluoride from the plasma and a return to background levels by roughly 2 hr after termination of exposure. Because fluoride is rapidly cleared, a delay in sacrificing the animals after exposure by Eisenbrandt and Nitschke (1989; IIA 5.3.3.2a/01, **D04**) may have resulted in measurements of fluoride that were below peak levels. As seen with urine, the overall shape of the three curves during and following the sulfuryl fluoride exposures are quite similar indicating that the kinetics of formation and elimination from plasma of the three metabolites (urine sulfate, fluorosulfate and fluoride) are similar or are interrelated.

Fluoride levels in kidney tissue during and after exposure to 30 and 300 ppm sulfuryl fluoride were roughly 2- to 2.5-fold higher at all collection times than control rats. Control rats had mean fluoride levels of 2.2 µg/g (0.12 µmole/g) kidney tissue while both 30 and 300 ppm exposure levels achieved concentrations of about 5 µg/g (0.26 µmole/g) kidney tissue. These levels were measured by the second hour of exposure and were maintained through 4 hr post-exposure.

A slight 1.5-fold elevation in fluoride levels in brain tissue relative to control rats was observed during and after exposure to 30 ppm sulfuryl fluoride. Control brain tissue fluoride ion concentrations were roughly 0.6 µg/g (0.03 µmole/g) tissue at all sacrifice times, increasing to 0.8 µg/g (0.04 µmole/g) brain following the 30 ppm sulfuryl fluoride exposure. At the termination of the 300 ppm exposure, a range of 1.3 to 3.7 µg/g (0.05 to 0.12 µmole/g) tissue (mean of 2.3 µg/g) was measured, 2- to 5-fold higher than controls. These concentrations of fluoride approached the control levels by 4 hours post-exposure.

Seven days post-exposure, the ^{35}S was not localised to any specific target or non-target tissue but small amounts of radiolabel were evenly distributed among the tissues, possibly a result of incorporation of the ^{35}S into amino acids. The highest concentration of radioactivity was detected in portal of entry tissues. The lungs had the highest levels of radioactivity 7 days post-exposure and the nasal turbinates also had detectable radioactivity. Radioactivity associated with the red blood cells (RBC) remained elevated 7 days post-exposure and highly perfused tissues such as the spleen and kidneys had higher levels of radioactivity than other non-respiratory tissues, probably due to the radioactivity in the blood. Radioactivity was cleared from plasma and RBC with initial half-lives of ~2.5 hr following the 30 ppm exposure and 1-2.5 hr following the 300 ppm exposure. But the terminal half-life of radioactivity was ~2.5-fold longer in RBC than plasma. Although not directly assayed, there was no evidence of parent ^{35}S-sulfuryl fluoride in the blood based on the radiochemical profiles. The identification of fluorosulfate and sulfate in blood and urine suggests that sulfuryl fluoride is first hydrolysed to fluorosulfate, with release of fluoride, followed by further hydrolysis to sulfate and release of the remaining fluoride. This is supported by the increases in fluoride detected in the blood following exposure of rats to sulfuryl fluoride.

Table 5.1a/b/01-2: Metabolism in the rat - urinary metabolite profile

	µmole Metabolite recovered in urine				µg Fluoride ion/g urine	
	30 ppm		300 ppm		30 ppm	300 ppm
Metabolite Fraction	S	FS	S	FS	F^-	F^-
Non-exposed	-	-	-	-	2.23	2.55
During exposure: -4-0h	0.58	1.68	6.35	22.36	9.33	76.25
Post-exposure: 0-6h	0.64	0.46	5.38	3.96	9.21	31.90
6-12h	0.37	0.06	2.59	0.59	2.71	6.34
12-24h	-		1.43	0.22	-	5.04
Total	**1.59**	**2.2**	**15.76**	**27.14**	**1.119**	**6.29**

S: sulfate; FS: fluorosulfate; F^-: fluoride ion

Table 5.1a/b/01-3: Metabolism in the rat – fluoride ion levels

Sacrifice time	µg Fluoride ion/g tissue								
	Plasma			Brain			Kidney		
	0 ppm	30 ppm	300 ppm	0 ppm	30 ppm	300 ppm	0 ppm	30 ppm	300 ppm
Pre-exposure: -4h	0.631	-	-	0.460	-	-	2.254	-	-
During exposure: -2h	-	0.867	2.514	-	1.106	1.475	-	4.815	5.354
End of exposure: 0h	0.461	0.757*	2.506*	0.597	0.791	2.268	2.251	5.378*	5.538*
Post-exposure: 2h	-	0.562	0.864	-	0.776	1.323	-	5.375	4.884
4h	0.443	0.534*	0.708*	0.457	0.798	0.983	2.464	5.694*	5.038*
8h	1.239	0.375*	0.553*	0.755	-	-	1.909	-	-
20h	0.564	0.471	0.518	-	-	-	-	-	-

* Significant difference from control at the indicated sacrifice time; alpha = 0.05

Conclusions: Radiolabelled sulfuryl fluoride was rapidly absorbed via inhalation exposure, achieving maximum concentrations of ^{35}S-sulfuryl fluoride-derived radioactivity in both plasma and red blood cells near the end of the 4-hr exposure period. Once absorbed, the ^{35}S was rapidly excreted, primarily via the urine. A large portion of the absorbed ^{35}S was excreted in the urine even during the 4-hour exposure period.

Radioactivity was rapidly cleared from plasma and RBC with initial half-lives of ~2.5 hr following a 30 ppm exposure and 1-2.5 hr following a 300 ppm exposure, but, the terminal half-life of radioactivity was ~2.5-fold longer in RBC than plasma. The identification of fluorosulfate and sulfate in blood and urine suggests that sulfuryl fluoride is first hydrolysed to fluorosulfate, with release of fluoride, followed by further hydrolysis to sulfate and release of the remaining fluoride. This is supported by increases in fluoride in blood and urine following exposure of rats to sulfuryl

fluoride.

Seven days post-exposure, the ^{35}S was not localised to any specific target or non-target tissue but small amounts of radiolabel were evenly distributed among the tissues, suggesting incorporation into the tissues of ^{35}S that had been transulfuryl fluorideormed into amino acids, but the exact chemical composition was not determined. Radioactivity was recovered mainly in tissues at the site of first exposure to the gas.

The data suggest that the systemic toxicity elicited by sulfuryl fluoride may be due to the release of fluoride ions, rather than a direct toxic action of sulfuryl fluoride.

During the 98/8/EC completeness check for PT8, the Rapporteur Member State Sweden (KemI) asked:

The total uptake of sulfuryl fluoride was not presented. There was no exhalation measurement data mentioned in this study. Explain the total excreted radioactivity. Is it possible that sulfuryl fluoride excreted via expiration? What is the total recovery of radioactivity concerning the exposure concentrations?

Time period expression in table 5.1a/b-1 and -2 should be more accurate, e.g. should be 0-4h, 5-12h instead of 0-4h, 4-12h, etc.?

Waiving of repeated dose exposure via inhalation routes results in missing data of F bioaccumulation in connection to the exposure to sulfuryl fluoride. Waiving can be considered unless the applicant can abstract the relevant information by using published data.

Dow AgroScience answer:

1) The total uptake of sulfuryl fluoride was not presented.

   The animals were exposed to measured concentrations of sulfuryl fluoride in a 2-liter nose-only chamber for 4-hr. The majority of the test material passed through the flow-through chamber, the actual amount of sulfuryl fluoride removed from the airstream by the animals is not known. Several assumptions would have to be made to estimate total uptake from the inspired air, including the minute volume and the percent of sulfuryl fluoride in the atmosphere that would be utilized during respiration. After removal of the animals from the nose-only chamber, expired air was passed through charcoal traps to capture expired volatiles. Post-exposure, no radioactivity was detected in these traps. Total absorption was estimated by summation of radioactivity from urine, feces and tissues. Therefore, the total uptake is likely comparable to the total amount absorbed, which was 11- 14%.

2) There was no exhalation measurement data mentioned in this study.

   As indicated in the Specimen Collection section of the report, after removal of the animals from the nose-only chamber, expired air was passed through charcoal traps to capture expired volatiles. No radioactivity was detected in these traps – also refer to the answer number 4, below.

3) Explain the total excreted radioactivity.

   Unlike oral gavage or intravenous administration, the actual "dose administered" in this and all inhalation studies is not known. Therefore, urine and fecal data are presented as µg and µmole equivalents. Total absorption was estimated by summation of radioactivity from urine, feces and tissues and, from this estimate of total absorption, 81-85% of the radioactivity recovered from the animals was eliminated in the urine.

4) Is it possible that sulfuryl fluoride excreted via expiration?

As indicated in the Specimen Collection section of the report, expired air was passed through charcoal traps to capture expired volatiles. Preliminary experiments indicated that charcoal traps were sufficient (100% efficiency) for trapping sulfuryl fluoride. In fact, charcoal canisters were used to remove ^{35}S-sulfuryl fluoride from the airstream after exiting the chamber and before being released to the outdoors. That there was no radioactivity recovered in the charcoal traps used to trap expired volatiles post-exposure indicates that sulfuryl fluoride is not excreted via expiration.

5) What is the total recovery of radioactivity concerning the exposure concentrations.

As indicated in the report (Total Absorption), 688 and 5690 µg-equivalents were recovered in the urine, feces, and tissues following the 4-hr 30 and 300 ppm exposures. Since we have no way of determining the total radioactive "dose administered", the data are presented as µg and µmole equivalents. We assume that we recovered 100% of the radioactivity absorbed.

6) Time period expression in table 5.1a/b-1 and -2 should be more accurate, e.g. should be 0-4h, 5-12h instead of 0-4h, 4-12h, etc.?

The first time interval indicated as -4 to 0 hr is the exposure interval, the interval where the animals were being exposed to sulfuryl in the chamber. Time "0" is when the exposure ended and, therefore, 0 to 6 hr is the first interval following exposure, 6 to 12 hr would be the second. Time periods expressed as 0-4 hr and 5-12 hr would not include the 4-5 hr interval.

7) Waiving of repeated dose exposure via inhalation routes results in missing data of F bioaccumulation in connection to the exposure to sulfuryl fluoride. Waiving can be considered unless the applicant can abstract the relevant information by using published data.

Regarding the bioaccumulation of fluoride, we would not expect it to be any different than that from fluoride supplements and the ADME data for sulfuryl fluoride indicate a rapid clearance of plasma fluoride and return to background levels by ~2 hr post-exposure. Since sulfuryl fluoride had a 35S label and only soft tissues were examined, it would not have been possible to monitor F accumulation.

Section A6.2 **Annex Point IIA, VI.6.2**	**Evaluation by Competent Authorities**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2004
Materials and methods	The applicant's version is adopted with some amendments. Only single dose exposure is performed. There is no data about dermal absorption. Male F344 rats were used.
Results and discussion	The applicant's version is adopted.
Conclusion	The applicant's version is adopted.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	This study is acceptable.
Remarks	Dermal absorption study is considered irrelevant as sulfuryl fluoride is a gas and the toxic effect is caused via inhalation route. This single dose metabolism study has provided sufficient information on the sulfuryl fluoride metabolism, therefore, waiving of repeated dose studies can be considered acceptable. There is no information in the study about the distribution of fluoride in bones or

	teeth. However, this is documented and some of the relevant open literature is summarised in the report (Doc III-A6.6.7)

Section A6.3 Repeated Dose Toxicity
Annex Point IIA,VI.6.3

Section A6.3.1 Repeated Dose Toxicity (Oral, 28-days)
Annex Point IIA,VI.6.3

Oral 28-day toxicity – Rat

There are no oral 28-day toxicity studies on sulfuryl fluoride. Sulfuryl fluoride is a gas and as such, all regulatory studies have been conducted by the inhalation route. A study was done on feed fumigated with sulfuryl fluoride and this is summarised below.

Rat (IIA 5.3.1a/01, D01)

Report: Anon. (1959)
Short Term Dietary Feeding Study of Commercial Laboratory Diet Fumigated with Vikane
[redacted]
Report K-16399-005, dated 22/10/59

Guidelines: This study is pre-guideline
Deviations from EC guideline Method B.7. Repeated Dose (28 Days) Toxicity (Oral): This study was not conducted in the usual manner. The duration was 66 days. It was conducted to test whether fumigation of feed with Vikane would cause the feed to become toxic. Food consumption data not reported; body weight data not tabulated; no clinical blood chemistry; tissues and urine were tested for fluoride content; routine urinalysis was not conducted; tissues histologically examined did not necessarily reflect those with effects (bone/teeth not done); teeth were examined for fluorosis; report is sketchy.
However, given the lack of other related information, this report still provides useful information.

GLP: No – this study pre-dates the GLP Compliance Programme

Methodology: Test material: Vikane not specified. Separate samples of Purina Laboratory Chow fumigated with Vikane for 24 hours at 80°C with 200, 100, 10 and 2 lbs, respectively, of Vikane/1000 cu ft. Samples of feed from each of the diets were then analysed for fluoride. Results are shown in Table 5.3.1a/01-1 below.
Groups of 10 rats/sex/dose (diet) were maintained for 66 days on the diets fumigated with 0, 200, 100, 10 and 2 pounds/1000 cu ft. of Vikane. Two extra rats of each sex were set up at each dietary level for necropsy after 30 days. The rats were at least 50 days of age when the study began.
The rats were weighed twice weekly for 28 days and then weekly. They were observed 'frequently' for gross changes in appearance and behaviour. The teeth of all rats were checked for any visual evidence of fluorosis. Food consumption was recorded for the first month. Samples of urine were obtained prior to termination from all males for fluoride analysis; terminal haematology values were obtained from 5 female rats on the 0, 2 and 10 lbs diets and from 2 males from each dietary level. At necropsy, the lungs, heart, liver, kidneys, spleen, and testes were weighed. Portions of these organs as well as pancreas and adrenals were examined

histologically. Samples of blood, urine (male), kidney, lung, liver and bones were collected and analysed for fluorides from all rats killed at 30 days.

Table 5.3.1a/01-1: Fluoride (ppm) in Diets Fumigated with Vikane for Use in Rat Dietary Feeding Study

Rate of Fumigation with Vikane (lbs/1000 cu. ft)	Average ppm Fluorides	
	Total	Net
Control Diet (no fumigation)	36	36
2	55	19
10	89	53
100	386	350
200	740	704

Findings: See Table 5.3.1a/01-2 for overall results. There is no statement about what the fluoride added to the diet really is, in terms of molecular identification.

Table 5.3.1a/01-2: Overall Results

Diet (net ppm fluoride)	Growth	Fluorosis (teeth)	Organ Wt.	Microscopic Examination
19	None noted	None noted	None noted	None noted
53	Slight ↓ in final mean body wts.	Early indications (whiter)	Slight ↑ in relative liver wt (males)	None noted
350	Retardation (males)	Darkening/ Banding	↑ in relative testes wt.	None noted
704	Severe retardation	Darkening/ Banding	↑ in relative liver and testes wt. (males)	Glomerular 'involvement' of kidneys

Mortality: There was no mortality reported.

Clinical signs: No findings were given in the report.

Body weight: Growth was retarded in a dose-related manner in all but the lowest dose (19 ppm fluoride) group. There is no tablular body weight data, only a graph which is not included here for technical (computer memory) reasons.

Food Consumption: There is no food consumption data or statement about it in the results.

Ophthalmology: Not conducted.

Haematology: No treatment-related effect.

Clinical chemistry: Not conducted.

Urine & organ fluoride content: Urinalysis was not conducted. Fluoride content in urine and bone increased in dose-related manner, as shown in Table 5.3.1a/01-3. Tissue fluoride content could not be correlated with dose.

Table 5.3.1a/01-3: Urinary Fluoride Content (ave. ppm) after 30 Days

Sex	Dose (net ppm fluoride)	Blood	Urine	Kidney	Lung	Liver	Bone
Males	0	0.0	9.9	0.9	13.5	0.0	260
	19	2.7	11.9	2.4	5.0	0.0	408
	53	0.4	13.3	10.3	1.0	0.3	413
	350	0.4	85.6	2.3	17.6	0.0	1615
	704	6.2	174.4	2.3	6.0	8.4	1920
Females	0	4.4		1.3	20.3	1.6	276
	19	3.0		1.3	13.0	2.0	339
	53	0.0		9.6	20.4	2.0	339
	350	0.6		0.0	17.2	0.0	1875
	704	2.9		5.7	25.3	0.3	3445

Organ weights: Relative liver and testes weights were increased in all but the lowest dose group, as shown in table 5.3.1a/01-4.

Table 5.3.1a/01-4: Final Body Weights, Liver and Testes Weights

Sex	Diet (net ppm fluoride)	No. of Rats	Body Weight (g)	Liver		Testes	
				g	g/100g	g	g/100g
Males	0	8	306	8.72	2.86	3.02	0.99
	19	9	285	8.43	2.96	2.86	1.01
	53	10	275*	8.36	3.03*	2.78	1.01
	350	9	247**	7.31	2.96	2.80	1.09*
	704	10	207**	6.23	3.00*	2.72	1.33*
females	0	9	173	5.40	3.12		
	19	10	185	5.66	3.06		
	53	10	171	540	3.15		
	350	9	169	5.28	3.12		
	704	10	145**	4.52	3.12		

*P = 0.01 to 0.05 (statistical test not specified); **P = <0.01 (statistical test not specified)

Gross pathology: Teeth noted to have fluorosis banding and staining.

Histopathology: Findings quoted as "glomerular involvement of the kidneys."

Conclusions: **Diets fumigated with Vikane were found to be 'rather high' in repeated oral toxicity when fed to groups of male and female rats for 66 days. The identity of the 'fluoride' in the diets was not given. In this study, the NOEL/NOAEL was a net 19 ppm 'fluoride' in the diet.**

Section A6.3.1 Annex Point IIA, VI.6.3	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2004
Materials and methods	The study was performed in 1959. It failed to meet guidelines or GLP requirements. There are a number of deviations according to the TNsG for Data Requirement (see the description of the applicant).
Results and discussion	The applicant's version is adopted.
Conclusion	Diets fumigated with Vikane were found to be 'rather high' in repeated oral toxicity when fed to groups of male and female rats for 66 days. The identity of the 'fluoride' in the diets was not given. In this study, the NOEL/NOAEL was a net 19 ppm 'fluoride' in the diet based on significantly decreased body weight and increased relative liver weight at 53 ppm diet fluoride.
Reliability	Reliability indicator 3: Study with major methodological and/or reporting deficiencies.
Acceptability	The study is considered acceptable as additional information (see Remarks).
Remarks	Food contamination by Vikane is not a common case. This study provides general toxicity information on repeated dose of sulfuryl fluoride exposure via oral route. Specifically, the fluoride concentration was correlated to sulfuryl fluoride concentration in the diet and the level of fluoride in various tissues was analysed in this study.

Section A6.3.2 Annex Point IIA, VI.6.3	Repeated Dose Toxicity (Dermal)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Not relevant – sulfuryl fluoride is a gas. There is no significant dermal exposure. Since the active ingredient is a gas, therefore main route of exposure is inhalation. Exposure through skin will be negligible if not zero. To conduct a dermal repeated dose toxicity study would constitute unjustified use of animals. Sulfuryl fluoride is a gas therefore the primary route of exposure to humans is via the lungs. All regulatory toxicity studies have therefore been conducted by the inhalation route. However, all inhalation studies were conducted by whole-body exposure so dermal (percutaneous) contact was an integral part of the study design.	
Undertaking of intended data submission [ ]	No studies are planned.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	May 2004	
Evaluation of applicant's justification	Sulfuryl fluoride is a gas. Exposure to sulfuryl fluoride is via inhalation rather than via dermal route. In all regulatory inhalation toxicity studies, dermal exposure was an integral part of the whole body treatment of sulfuryl fluoride. There was no dermal toxicity indicated. The potential toxicity after dermal exposure to sulfuryl fluoride is, thus not considered to be significant.	
Conclusion	The justification is acceptable.	
Remarks	No remarks.	

Section A6.3.3/01 **Repeated Dose Toxicity (Inhalation, 28-days)**
Annex Point IIA, VI.6.3

28-day inhalation toxicity

There are no 28-day inhalation toxicity studies on sulfuryl fluoride. The following non-regulatory, 14-day inhalation studies in rats, dogs, mice and rabbits are included for completeness as they provide useful information.

Rat (IIA 5.3.3.1a /01, D02)

Report:	Eisenbrandt, D.L., Nitschke, K.D., Streeter, C.M., 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, The Dow Chemical Company, USA, Midland, Michigan, USA Report K-16399-022, dated 2/4/85; study started 27/11/83. Also published in: Fundam. Appl. Toxicol., 12: 540 (1989).
Guidelines:	None cited. This study was conducted as a 'probe' for a 13-week study. Deviations from EC guideline Method B.8. Repeated Dose (28 Days) Toxicity (Inhalation): This was a 2-week probe study. It meets the guidelines for a 28-day study.
GLP:	Yes
Methodology:	Test material: Vikane gas fumigant (Lot #TWP 830919-408; 99.8% sulfuryl fluoride). Rats were exposed to 0, 100, 300 or 600 ppm sulfuryl fluoride (VIKANE gas fumigant) for six hours/day, five days/week for nine exposures. Exposure groups consisted of five Fischer 344 rats of each sex. Animals were observed daily and body weights were recorded several times throughout the study (prior to 1st, 5th, 6th and 9th day of exposure. Prior to the 9th exposure, blood samples were collected from rats for haematology (PCV, Hgb, RBC, WBC & differential counts, MCV, MCH, MCHC and platelets) and urine samples were collected for urinalysis (bilirubin, glucose, ketones, blood, pH, protein, urobilinogen and specific gravity). Serum samples were collected for clinical chemistry (UN, SGPT, SGOT, AP, glucose, total protein, albumin and globulins) and organs (brain, heart, liver, kidneys, thymus (rats), and testes) were weighed at the terminal sacrifice. A complete necropsy was performed on all animals and extensive histopathology was completed on controls and high dose groups. Also, a similar set of tissues was examined for rats in the 300 ppm group because of the early deaths in the 600 ppm group. Otherwise, examination of tissues in intermediate dose groups was confined to target organs and several other tissues. Special stains were used on brain, lung, kidney, heart and stomach from top dose rats in order to accurately evaluate effects.
Findings:	
Mortality:	All males and 4 of 5 females at 600 ppm died or were moribund between the 2nd and 6th exposures. No other rats died in this study.
Clinical signs:	Not specified in the report.
Body weight:	Body weights were statistically reduced at the top dose, as shown in Table 5.3.3.1a/01-1.

Table 5.3.3.1a/01-1: Mean Body Weights (g)

Experimental Day		Concentration (ppm)			
		0	100	300	600
Males	1	170.5	165.5	170.5	167.9
	5	179.3	174.2	182.4	120.7*
	8	190.1	184.1	193.0	--
	11	194.5	188.9	194.3	--
Females	1	115.0	113.5	112.6	112.0
	5	121.2	118.0	117.3	85.9*
	8	127.8	125.4	123.8	89.3*
	11	129.9	126.8	123.6	80.3*

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

Food consumption: Not conducted.

Ophthalmology: Not conducted. However, eyes were examined at necropsy and histopathology.

Haematology: WBC was elevated in both sexes and red blood cell parameters were elevated in high dose females, as shown in Table 5.3.3.1a/01-2.

Table 5.3.3.1a/01-2: Haematology

Sex	Dose (ppm)	No.	RBC x 10^6/mm^3	HGB g/dl	PCV %	WBC x 10^3/mm^3
Males	0	5				6.7
	100	5				7.4
	300	5				8.8*
	600	0				--
Females	0	5	7.78	17.0	48.0	5.3
	100	5	7.87	17.0	48.4	6.6*
	300	5	7.80	17.0	48.0	7.9*
	600	1	8.76*	19.2*	53.0*	11.6*

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

Clinical chemistry: There were some statistically identified alterations in the surviving female rat at the top dose, as shown in Table 5.3.3.1a/01-3.

Table 5.3.3.1a/01-2: Haematology

Dose (ppm)	UN (mg/dl)	SGPT (mU/ml)	Glucose (mg/dl)	Albumin (g/dl)	Globulin (g/dl)
0	19	37	123	3.5	1.6
100	21	39	115	3.4	1.7
300	17	41	126	3.3*	2.0
600	213*	132*	1483*	2.9*	2.3*

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

Urinalysis: No changes specifically identified in survivors.

Organ weights: Data for heart and kidney weights are shown in Table 5.3.3.1a/01-4. There were some very slight increases in male rat kidney weight at the 300 ppm dose level and a statistically significant increase in relative kidney weight in female rats at the top 2 doses. The heart weights of rats were variable but no treatment-related explanation was possible for males. The relative heart weight was elevated for females at 300 and 600 ppm though the absolute weight was significantly reduced for the surviving female at 600 ppm.

Table 5.3.3.1a/01-4: Organ Weights

Sex	Dose (ppm)	No.	Terminal Body Weight (g)	Heart		Kidney	
				(g)	(g/100g)	(g)	(g/100g)
Males	**0**	5	173.7	0.617	0.355	1.452	0.834
	100	5	167.7	0.637	0.380*	1.443	0.861
	300	5	172.3	0.614	0.356	1.545	0.896
	600	0	--	--	--	--	--
Females	**0**	5	112.2	0.459	0.409	1.071	0.955
	100	5	109.4	0.467	0.427	1.123	1.028
	300	5	107.6	0.471	0.438*	1.155	1.075*
	600	1	67.6*	0.313*	0.463*	0.961	1.422*

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

Histopathology: A summary of the findings in the rats is given in Table 5.3.3.1a/01-5. Decedents had severe microscopic kidney lesions that included papillary necrosis as well as degeneration and regeneration of collecting tubules and proximal tubules. Numerous microscopic pathologic observations in these rats were considered secondary to the renal effects or terminal changes. The single female rat that survived until termination had moderate degenerative and regenerative changes in renal collecting tubules, hyperplasia of renal papillary epithelium and basophilic epithelial cells in the proximal tubules. This rat also had severe inflammation of the nasal mucosa and slight bronchioalveolar inflammation. In addition, this female was in poor condition and had a variety of secondary changes. Five of 10 rats exposed to 300 ppm sulfuryl fluoride had minimal renal changes.

Table 5.3.3.1a/01-5: Histopathology

Sex	Males				Females			
Dose (ppm)	0	100	300	600	0	100	300	600
Number of Rats Examined	5	5	5	(5)	5	5	5	5 (4)
Kidney								
Number of Tissues Examined	5	5	5	(5)	5	5	5	5 (4)
Within Normal Limits	2	3	2	(0)	1	4	0	0 (0)
Altered tinctorial properties - increased basophilia, proximal tubule(s): - very slight	0	0	2	(0)	0	0	1	0 (0)
- slight	0	0	0	(0)	0	0	0	1 (0)
Hyperplasia, collecting ducts: - very slight	0	0	1	(0)	0	0	2	0 (0)
- slight	0	0	1	(0)	0	0	1	0 (0)
Hyperplasia – epithelial, papilla(e): - slight	0	0	1	(0)	0	0	1	0 (0)
- moderate	0	0	0	(5)	0	0	0	5 (4)
Necrosis, papilla(e):	0	0	0	(5)	0	0	0	4 (4)
Degeneration/regeneration, collecting ducts: - slight	0	0	0	(0)	0	0	0	1 (1)
- moderate	0	0	0	(0)	0	0	0	1 (0)
- severe	0	0	0	(5)	0	0	0	3 (3)
Degeneration/regeneration, proximal tubule(s): - moderate	0	0	0	(5)	0	0	0	4 (4)
Atrophy, individual nephron(s):	0	0	0	(0)	1	0	0	0 (0)
Cyst, unilateral, multifocal:	0	1	0	(0)	0	0	0	0 (0)
Mineralisation: - very slight	2	1	0	(0)	2	0	0	0 (0)
- slight	1	0	1	(1)	1	1	3	3 (2)
Lung								
Number of Tissues Examined	5	5	5	(5)	5	5	5	5 (4)
Within Normal Limits	2	3	2	(1)	4	4	3	0 (0)
Alveolar histiocytosis, focal:	1	1	0	(0)	0	0	1	0 (0)
Oedema: - slight	0	0	0	(1)	0	0	0	2 (2)
Fibrin, alveoli, multifocal: - slight	0	0	0	(0)	0	0	0	1 (1)
Fibrin, alveoli, diffuse: - moderate	0	0	0	(1)	0	0	0	0 (0)
Haemorrhage: - slight	0	0	0	(0)	0	0	0	1 (1)
Inflammation – subacute, pleura: - slight	0	0	0	(3)	0	0	0	2 (2)
Inflammation – subacute, bronchioalveolar, multifocal: - slight	0	0	0	(0)	0	0	0	1 (0)
Mineralisation, alveoli: - slight	0	0	0	(0)	0	0	0	1 (0)
- moderate	0	0	0	(1)	0	0	0	0 (0)
Thrombus acute or recent, capillaries: - very slight	0	0	0	(1)	0	0	0	1 (1)
- moderate	0	0	0	(1)	0	0	0	0 (0)
Inflammation – subacute to chronic, interstitium: - very slight or slight	2	1	3	(1)	1	1	1	1 (0)

Sex	Males				Females			
Dose (ppm)	0	100	300	600	0	100	300	600
Trachea								
Number of Tissues Examined	5	5	5	(5)	5	5	5	5 (4)
Within normal limits:	5	5	5	(4)	5	5	5	4 (4)
Mineralisation, mucosa: - slight	0	0	0	(1)	0	0	0	0 (0)
Necrosis – individual cell(s), respiratory epithelium:	0	0	0	(0)	0	0	0	1 (0)
Nasal Tissues								
Number of Tissues Examined	5	5	5	(5)	5	5	5	5 (4)
Within normal limits:	5	5	5	(0)	4	4	4	4 (4)
Degeneration, olfactory epithelium, focal:	0	0	0	(0)	0	0	1	0 (0)
Degeneration, respiratory epithelium, focal:	0	0	0	(0)	0	1	0	0 (0)
Inflammation – subacute, mucosa, diffuse, with multifocal ulceration and mucopurulent exudate: - severe	0	0	0	(0)	0	0	0	1 (0)
Inflammation – subacute, olfactory epithelium, focal: - very slight	0	0	0	(0)	2	0	0	0 (0)
Necrosis – individual cell(s), olfactory epithelium:	0	0	0	(5)	0	0	0	0 (0)
Larynx								
Number of Tissues Examined	5	5	5	(5)	5	5	5	5 (4)
Within normal limits:	5	4	4	(4)	4	5	5	3 (3)
Inflammation – subacute, mucosa: - slight	0	1	0	(0)	0	0	0	0 (0)
Mineralisation, mucosa: - slight	0	0	1	(1)	1	0	0	2 (1)
Necrosis – individual cell(s), respiratory epithelium: - slight	0	0	0	(0)	0	0	0	1 (0)

( ) denotes decedent animals

Conclusions: **In this 14-day inhalation study in F344 rats, the NOAEL was 100 ppm based on very slight or slight renal hyperplasia and mineralisation at 300 ppm.**

Section A6.3.3/01 **Annex Point IIA, VI.6.3**	**Evaluation by Competent Authorities**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2004
Materials and methods	The applicant's version is adopted with the amendment that the exposure was whole-body in chambers.
Results and discussion	The applicant's version is adopted.
Conclusion	The applicant's version is adopted.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	This study is acceptable.
Remarks	The rabbit part of this study is presented in section A.6.3.3/04

Section A6.3.3/02 **Repeated Dose Toxicity (Inhalation, 28-days)**
Annex Point IIA, VI.6.3

Dog (IIA 5.3.3.1b/01, D03)

Report:	[redacted] (1991) Sulfuryl Fluoride: Two-Week Inhalation Toxicity Study in Beagle Dogs [redacted] Report K-016399-038, dated 30/4/91; study began 26/3/90.
Guidelines:	US EPA 82-4, Supplemental. Deviations from EC guideline Method B.8. Repeated Dose (28 Days) Toxicity (Inhalation): The study was conducted in dogs and it was a probe study using only one dog/sex/dose level. Histologic examination of likely target tissues was conducted on all dogs rather than a wider range of tissues on controls and high dose.
GLP:	Yes
Methodology:	Test material: Sulfuryl fluoride (Lot # 880329 752 MAR/88; 99.6% pure). One male and one female Beagle dog were each exposed via inhalation to 0, 30, 100 or 300 ppm (0, 0.13, 0.42 or 1.25 mg/L, respectively) SO_2F_2 for 6 hours/day, 5 days/week for 9 exposures. Whole-body exposures were conducted under dynamic airflow conditions. Animals were observed daily and weighed on test days -7, -5, -3, 1, 3, 5, 8 and 11. Animals were necropsied on the day after the last exposure. Serum samples were obtained from each animal at least twice prior to the initial exposure to SO_2F_2 and again at necropsy for haematology (HCT, Hgb, RBC, WBC/differential count, and platelets) and clinical chemistry (AP, AST, ALT, CK, UN, Creatinine, total portein, albumin, globulin, glucose, cholesterol, triglycerides, total bilirubin, Na, K, P, Cl, Ca and F) determinations. Urinalysis (pH, bilirubin, glucose, proteins, ketones, occult blood and urobilinogen, specific gravity) was conducted on urine obtained from the urinary bladders at necropsy. An ophthalmologic examination was conducted prior to the study and after the last exposure. Major organs were weighed and selected tissues examined histopathologically. Vapour was generated using a glass 'J-tube' method and the concentrations were checked using a portable Miran 1A IR.
Findings:	Chamber concentrations were determined to be 30 = 28.9; 100 = 96.3; and 300 = 298.2 ppm
Mortality:	All dogs survived the nine exposures.
Clinical signs:	At 300 ppm, infrequent intermittent episodes of tremors and tetany were observed in both dogs beginning with the 5th exposure. On day 9, during the 7th exposure, the tremors and tetany were severe enough that the exposure was terminated after approximately 5.5 hours. Within thirty minutes after terminating the exposure, both dogs appeared to be normal. Similar clinical effects were noted during subsequent exposure periods and were rapidly reversible even during the exposure period. There were no exposure-related effects noted in dogs exposed to 30 or 100 ppm sulfuryl fluoride.
Body weight:	There was a larger than normal body weight range between dogs in this study since these dogs were the extras from an animal shipment for another study. Due to the large body weight variation and so that each dog could basically serve as its own

control, body weights were obtained several times prior to the first exposure to sulfuryl fluoride. The body weight on day 11 of the female dog exposed to 300 ppm was decreased by approximately 500 g from pre-exposure values (mean of pre-exposure values - 9337 g - minus day 11 value - 8844 g). Body weights for the male dog exposed to 300 ppm or male or female dogs exposed to 30 or 100 ppm were comparable to pre-exposure values, as shown in Table 5.3.3.1b/01-1.

Table 5.3.3.1b/01-1: Body Weights (g)

Conc. (ppm)	Days on Test							
	-7	-5	-3	1	3	5	8	11
Males								
0	7800	7693	7921	7801	7818	7742	8090	8044
30	10400	10675	10558	10661	10300	10676	10897	10708
100	10600	10469	10639	10786	01718	10484	01494	10761
300	10200	10319	10550	10672	10558	10757	10776	10510
Females								
0	8400	8403	8516	8744	8553	8575	8568	8907
30	8600	8624	8612	8865	8635	8619	9053	8410
100	5850	5887	5962	6132	6145	6124	6421	6437
300	9300	9090	9331	9626	9209	8877	8870	8844

Food consumption: Not conducted.

Ophthalmology: There were no effects noted in the report.

Haematology: Haematology values for dogs exposed to concentrations as high as 300 *ppm* sulfuryl fluoride were comparable to pre-exposure values.

Clinical chemistry: Serum fluoride levels of dogs exposed to 100 or 300 ppm were elevated to 2-4 fold higher than control values. Serum fluoride levels of dogs exposed to 30 ppm and all other clinical chemistry values of dogs exposed to concentrations as high as 300 ppm were comparable to control and/or pre-exposure values.

Urinalysis: Urinalysis values for male and female dogs exposed to 300 ppm sulfuryl fluoride were comparable to control values. The specific gravity for the male dog exposed to 100 ppm was lower than for the control or 300 ppm exposed dog. This was not considered to be exposure-related, since there was no apparent dose-response relationship.

Serum samples were collected shortly after exposure on days 5 and 9 to measure calcium levels in an attempt to determine if the tremors and tetany observed during exposure at 300 ppm were due to an electrolyte imbalance. At the time the blood samples were drawn, the animals appeared to be clinically normal. While the tremors and tetany noted may be due to a localised electrolyte imbalance, total serum calcium levels were comparable to control values. No attempt was made to measure ionic versus non-ionic calcium levels in the serum.

Organ weights: There were no apparent exposure-related effects noted in absolute or relative organ weights of the single dog of each sex used per exposure level.

Gross pathology: There were no exposure-related effects noted upon gross examination of dogs exposed to concentrations as high as 300 ppm.

Histopathology: The target organ based on histopathologic examination was the upper respiratory tract, as shown in Table 5.3.3.1b/01-2. Minimal microscopic inflammatory changes were observed in the nasal turbinates of the male and female dog and trachea of the female dog exposed to 300 ppm. Although numerous microscopic sections were examined from the cerebral cortex, brain stem, cerebellum and medulla oblongata, there were no changes detected in dogs exposed to 300 ppm sulfuryl fluoride. There were no exposure-related effects noted in dogs exposed to 30 or 100 ppm sulfuryl fluoride.

Table 5.3.3.1b/01-2: Histopathology (Nasal Turbinates, Trachea)

Sex	**Males**				**Females**			
Dose	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Number of Dogs Examined	1	1	1	1	1	1	1	1
Nasal turbinates (# of tissues examined)	1	1	1	1	1	1	1	1
Within normal limits:	0	0	0	0	0	0	0	0
Exudate, lumen: - slight	0	0	0	0	0	0	0	1
Inflammation - subacute to chronic, respiratory epithelium, multifocal: - very slight	1	1	1	0	1	1	1	0
- slight	0	0	0	1	0	0	0	1
Trachea (# of tissues examined)	1	1	1	1	1	1	1	1
Within normal limits:	1	1	1	1	1	1	1	0
Inflammation - subacute, mucosa: - slight	0	0	0	0	0	0	0	1

Conclusions: **In this 14-day inhalation study in Beagle dogs the NOAEL was 100 ppm based on infrequent, intermittent episodes of tremors and tetany beginning with the 5th exposure at 300 ppm. There were no histologic CNS changes found to explain this effect. Also, at 300 ppm, the upper respiratory tract showed histologic signs of inflammatory changes.**

Section A6.3.3/02 **Annex Point IIA, VI.6.3**	**Evaluation by Competent Authorities**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2004
Materials and methods	The applicant's version is adopted with the amendment that the exposure was only two weeks (5 days/week for 9 exposures).
Results and discussion	The applicant's version is adopted.
Conclusion	In this 14-day inhalation study, the NOAEL was 100 ppm based on the clinical observations of tremor and tetany as well as slight inflammation in the up respiratory tract at 300 ppm. NOEL was 30 ppm based on increased serum fluoride concentrations at 100 ppm.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	This study is acceptable.
Remarks	No remarks.

Section A6.3.3/03 **Repeated Dose Toxicity (Inhalation, 28-days)**
Annex Point IIA, VI.6.3

Mouse (IIA 5.3.3.1c/01, D08)

Report: [redacted] (2002)
Sulfuryl Fluoride: Two-Week Inhalation Toxicity Study in CD-1 Mice
[redacted]
Report DECE HET K-016399-029, dated 11/2/02, study start date 10/10/88.

Guidelines: OECD 412 (19)
84/449/EEC, Method B8 (1984)
Deviations from EC guideline Method B.8. Repeated Dose (28 Days) Toxicity (Inhalation): None, except that this was a 2-week study.

GLP: Yes

Methodology: Test material: Sulfuryl fluoride (Lot WP 880329 752; 99.6% sulfuryl fluoride).
Groups of 5 male and 5 female mice were exposed to 0, 30, 100, or 300 ppm (0, 0.13, 0.42, or 1.3 mg/L, respectively) sulfuryl fluoride for 6 hours/day, 5 days/week for 9 exposures during a two-week period.
Each animal was examined ophthalmologically with a penlight prior to the initial exposure to SO_2F_2; all animals appeared normal. All animals were observed daily for overt signs of toxicity or changes in demeanor. These observations included an evaluation of the fur, eyes, mucous membranes, and respiration. Behavior pattern and nervous system activity were assessed by specific observations for lethargy, tremors, convulsions, salivation, lacrimation, diarrhea, or other signs of altered central nervous system functions. An additional daily observation and routine monitoring on weekends was limited to the removal of dead animals and animal husbandry procedures required to ensure the availability of food and water. All animals were weighed on test days 1, 3, 5, 8, and 11.
Blood samples were collected by orbital sinus puncture from mice anaesthetised with methoxyflurane immediately prior to necropsy. The following haematologic parameters were evaluated for each animal: haematocrit (HCT), haemoglobin concentration (HGB), and erythrocyte (RBC), total leukocyte (WBC) and platelet (PLAT) counts. Blood samples for serum analyses were collected at the terminal sacrifice from the orbital sinuses of mice. Serum samples were chilled with crushed ice or refrigerated until analysed. The following parameters were measured: alanine aminotransulfuryl fluorideerase (ALT), aspartate aminotransulfuryl fluorideerase (AST) and alkaline phosphatase (AP) activities, and urea nitrogen (UN).
All surviving animals were necropsied the day following the last exposure to the test material. Each animal was weighed, anaesthetised with methoxyflurane, and humanely euthanatised. Weights of the brain, heart, liver, kidneys and testes (males) were recorded from all animals at the scheduled sacrifice. All animals were examined for gross pathological alterations. A complete set of tissues was collected from each animal and preserved in neutral phosphate-buffered 10% formalin. The lungs were infused with buffered formalin to their approximate normal inspiratory volume and the nasal cavity was flushed with formalin via the pharyngeal duct to ensure rapid fixation. The necropsy included *in situ* examination of the eyes by a glass-slide technique with fluorescent illumination. Histopathologic examination of

brain, liver, kidney and respiratory tract (nasal tissue, trachea, larynx, and lungs) was conducted on all animals. Tissues examined histopathologically were processed by conventional techniques, sectioned at approximately 6 mm, stained with haematoxylin and eosin and evaluated by light microscopy.

Findings: Groups of 5 male and 5 female mice were exposed to analytically measured time weighted average (TWA) concentrations of 0, 30.3, 99.7 and 301.5 ppm SO_2F_2, which corresponded to target concentrations of 0, 30, 100 and 300 ppm, respectively.

Chamber airflow, temperature and relative humidity values were comparable.

Mortality: At 300 ppm, all male and 4/5 female mice died during the second week of the study.

Clinical signs: Decedents were thin and many had roughened haircoat and body tremors (males only). All animals exposed to lower concentrations of SO_2F_2 survived to the end of the study with no overt signs of toxicity or changes in demeanor.

Body weight: Body weights of mice exposed to 300 ppm were decreased from control values and statistically identified (Table 5.3.3.1c/01-1). Mean body weights of male and female mice exposed to 30 or 100 ppm SO_2F_2 were comparable to control values.

Table 5.3.3.1c/01-1: Mean Body Weights (g)

Sex	Test Day	Concentration (ppm)			
		0	30	100	300*
Males	1	33.2	32.1	31.2	32.8
	3	30.3	31.8	30.5	30.2
	5	32.8	32.9	31.5	27.9
	8	34.2	33.4	31.9	24.4
	11	33.7	32.8	31.1	20.5
Females	1	25.3	25.2	25.4	24.5
	3	25.2	23.4	26.1	22.2
	5	25.9	25.9	26.8	20.2
	8	26.5	26.2	27.2	20.7
	11	26.7	26.3	26.6	20.2

*Time-dose interaction was highly statistically significant for males and females at the high dose, alpha = 0.05.

Food intake: Not conducted.

Ophthalmology: Not conducted post-exposure.

Haematology: Red blood cell counts in male mice and haemoglobin concentrations in male and female mice exposed to 100 ppm SO_2F_2 were elevated from control values and statistically identified. However, these values were within the range of the historical control data from this laboratory. Consequently, the red blood cell counts in male mice and haemoglobin concentrations in male and female mice were considered to be within the range of normal. All remaining haematological parameters in male and female mice exposed to 30 or 100 ppm SO_2F_2 were comparable to control values.

Clinical chemistry: All clinical chemistry parameters measured in mice exposed to SO_2F_2 were comparable to control values.

Urinalysis: Not conducted.

Organ weights: The terminal body weights and absolute and relative organ weights of male and female mice exposed to 30 or 100 ppm SO_2F_2 were comparable to control values. Due to the decreased body weight in the sole surviving female mouse exposed to 300 ppm, several absolute and/or relative organ weight values, while not statistically different, were different from control values. These differences included an increase in relative brain, heart and kidney weights and a decrease in absolute liver weight.

Gross pathology: Several stress-related gross pathologic changes were observed in male and female mice exposed to 300 ppm SO_2F_2 that died prior to scheduled necropsy. These stress-related changes included decreased amount of ingesta in the digestive tract, decreased fat and erosions of the stomach. All other gross lesions were incidental findings unrelated to exposure to the test material. There were no gross lesions noted in the sole female surviving to the scheduled necropsy.

Histopathology: Exposure-related histopathologic effects were observed in the cerebrum and medulla of the brain of mice exposed to 100 or 300 ppm SO_2F_2 (Table 5.3.3.1c/01-2). Multifocal vacuoles, which were very slight in degree, were observed in the cerebrum of 4 male and 2 female mice exposed to 100 ppm. More severe vacuoles graded as moderate were observed in the cerebrum of the majority of mice exposed to 300 ppm. In addition, 4 male and 1 female mice exposed to 300 ppm had vacuoles in the medulla, which were very slight or moderate in severity. There were no histopathologic changes in the medulla of mice exposed to 100 ppm SO_2F_2 and no lesions noted in the cerebrum or cerebellum of mice exposed to 30 ppm.

Nine of ten mice exposed to 300 ppm SO_2F_2 had hepatocellular and lacrimal gland atrophy, which were secondary to inanition/moribund condition of these animals.

All other microscopic changes in mice were considered incidental findings unrelated to exposure to sulfuryl fluoride.

Table 5.3.3.1c/01-2: Summary of Histopathology Findings

Sex	Males				Females			
Dose (ppm)	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Number of Mice Examined	5	5	5	(5)	5	5	5	1 (4)
Brain								
Number of Tissues Examined	5	5	5	(5)	5	5	5	1 (4)
Within Normal Limits	5	5	1	0	5	5	3	1 (1)
Vacuolation, cerebrum, bilateral, multifocal								
- very slight	0	0	4	(1)	0	0	2	0
- moderate	0	0	0	(4)	0	0	0	(3)
Vacuolation, medulla, bilateral, multifocal:								
- very slight	0	0	0	(2)	0	0	0	(1)
- moderate	0	0	0	(2)	0	0	0	0
Liver								
Number of Tissues Examined	5	5	5	(5)	5	5	5	1 (4)
Within Normal Limits	3	2	3	0	2	4	2	1
Aggregate(s) of reticuloendothelial cells:	2	3	2	0	3	1	3	0
Atrophy, secondary to inanition:	0	0	0	(5)	0	0	0	(4)
Kidneys								
Number of Tissues Examined	5	5	5	(5)	5	5	5	1 (4)
Within Normal Limits:	4	5	5	(5)	3	3	3	1 (4)
Atrophy, tubule, unilateral, focal: - very slight	1	0	0	0	1	2	0	0

Sex	Males				Females			
Dose (ppm)	0	30	100	300	0	30	100	300
multifocal: - very slight	0	0	0	0	0	0	1	0
Atrophy, tubule, bilateral, focal: - very slight	0	0	0	0	1	0	1	0
Lacrimal/Harderian Gland								
Number of Tissues Examined	0	0	0	(5)	0	0	0	(4)
Atrophy:				(5)				(4)

Conclusions: **In this 14-day inhalation study in CD-1 mice, the NOEL for males and females was 30 ppm based on very slight vacuolation in the cerebrum at 100 ppm.**

Section A6.3.3/03 **Annex Point IIA, VI.6.3**	**Evaluation by Competent Authorities**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2004
Materials and methods	The applicant's version is adopted with some amendments. The exposure was whole-body in an exposure chamber under dynamic airflow conditions. The mice were of the strain CD-1. The highest dose resulted in many fatalities. According to the EC method the highest dose should result in no or few fatalities.
Results and discussion	The applicant's version is adopted with the following amendment: The males died at day 10 (4) and 12 (1). For females death occurred at day 8 (2) and 10 (2).
Conclusion	The applicant's version is adopted with the revision that NOEL/NOAEL was 30 ppm.
Reliability	Reliability indicator 1: Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Acceptability	This study is acceptable.
Remarks	No remarks.