Section A6.6.1 Annex Point IIA VI.6.VI.6.1	In-vitro Gene Mutation Study in Bacteria	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	The European Union Risk Assessment Report: Acrylaldehyde, European Chemicals Bureau, 2001 states that from the results of the bacterial mutagenicity tests it can be concluded that acrolein is a direct-acting bacterial mutagen with the Salmonella typhimurium strains TA 100, TA 104 and TA 98. However, no information has been provided on the purity of acrolein used to carry out the studies and the dose level which generated the positive result. In certain cases it is not evident whether this dose level is near the toxic dose for acrolein. Some studies have actually shown that acrolein is mutagenic at the toxic level and therefore the result is not a true positive. In the absence of information regarding the purity of the acrolein used in the studies or the dosing method employed, the positive results can be viewed with a certain degree of scepticism. This is because these results may have been caused by an impurity present in the active substance or a degradant of acrolein and not due to acrolein itself. In addition, the studies given in the ECB Risk Assessment are not supportive of each other. For example, the TA 100 and TA 98 strains of S. typhimurium have generated positive mutagenic results in some studies and negative in others. This in turn raises questions regarding the legitimacy of the results. The applicant has also provided a full set of data for both in vitro and in vivo genotoxicity studies which have all generated negative results and have been carried out using the technical grade of acrolein that is to be registered. It is therefore considered unnecessary to submit the positive in vitro mutagenicity results from the ECB Risk Assessment, based on their ambiguity and the lack of information provided and the availability of negative in vitro and in vivo genotoxicity studies conducted using the appropriate technical grade of acrolein.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	05/10/07	

Section A6.6.1 Annex Point IIA VI.6.VI.6.1	In-vitro Gene Mutation Study in Bacteria
Evaluation of applicant's justification	A review of all data is needed, especially positive results
Conclusion	Submission of all tests in the #ESR document have been requested and received
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.1/01 Annex Point IIA6.1.1		Acute Toxicity Acute oral toxicity test in the rat (LD_{50})	
		1 REFERENCE	Official use only
1.1	Reference	David, R.M. (1989) Acute Oral Toxicity Study of Acrolein, Inhibited in Rats. Microbiological Associates Inc. Study No. G-7230.220.	
1.2	Data protection	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	Yes FIFRA 81-1	
2.2	GLP	Yes	
2.3	Deviations	Yes Animal room temperature and relative humidity were out of range 63 - 67°F (67-68°F total of 4 days during main study) and 72-98% (total of 11 days during the main study), respectively. On the first day, the animals were observed twice at approximately 2 and 4 hours after dosage or approximately one hour later than the protocol dictates.	
		3 MATERIALS AND METHODS	
3.1	Test material	As given in Section 2	
3.1.1	Lot/Batch number	4035	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	
3.2	Test Animals		
3.2.1	Species	Rats	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Harlan Sprague-Dawley, Frederick, Maryland, USA.	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Age: 5 to 6 weeks Males: 254 – 307 g Females: 163 – 210 g	
3.2.6	Number of animals per group	Five animals per sex per dose level	
3.2.7	Control animals	No	

Section A6.1.1/01 Annex Point IIA6.1.1		Acute Toxicity Acute oral toxicity test in the rat (LD_{50})	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Postexposure period	15 days	
		Oral	
3.3.2	Type	Gavage	
3.3.3	Concentration	10, 15, 20, 25, 30 mg/kg bw	
3.3.4	Vehicle	Not described	
3.3.5	Concentration in vehicle	Nominal: 2, 3, 4, 5, 6 mg/ml Measured: 2.60, 3.12, 4.32, 5.18, 6.75 mg/ml	
3.3.6	Total volume applied	5 ml/kg	
3.3.7	Controls	None	
3.4	**Examinations**	Mortality, clinical observations, body weight, necropsy	
3.5	**Method of determination of LD_{50}**	Probit analysis of the mortality of each sex at each dose level	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	See Table A6_1_1-1 for mortality data. Mortality: Two males dosed with 30 mg/kg died one hour following dosing. Three males died on Test Day 2. Four males dosed with 25 mg/kg dies one hour following dosing. The fifth male dies on Test Day 2. All five males dosed with 20 mg/kg died on Test Day 2. Four males dosed with 15 mg/kg died on Test Day 2. The other males survived the 14 day observation period. Three females dosed with 30 mg/kg died one hour following dosing. Two females died on Test Day 2. Four females dosed with 25 mg/kg died one hour following dosing. The fifth female died on Test Day 2. One female dosed with 20 mg/kg died one hour following dosing. The remaining four died on Test Day 2. Four females dosed with 15 mg/kg died on Test Day 2. The other female survived the 14 day observation period. One female dosed with 10 mg/kg died on Test Day 2. The other three females survived the 14 day observation period. Clinical signs: Three males dosed with 30 mg/kg were lethargic and had hypothermia three hours following dosing. One male dosed with 25 mg/kg was lethargic and had hypothermia three hours following dosing. All five males dosed with 20 mg/kg were lethargic and had hypothermia three hours following dosing. All five males dosed with 15 mg/kg were lethargic and had hypothermia three hours following dosing. One of these males had rales from test day 9 to test day 15.	

		Two females dosed with 30 mg/kg were lethargic and had hypothermia three hours following dosing. One of these females also had respiration described as gasping one to three hours following dosing. Two females dosed with 25 mg/kg were lethargic one to three hours following dosing. One of these females had rapid and shallow respiration one hour following dosing. In addition, the other female had hypothermia three hours following dosing. Four females dosed with 20 mg/kg were lethargic and had hypothermia three hours following dosing. All five females dosed with 15 mg/kg were lethargic and had hypothermia three hours following dosing. Body weight: Body weights did not appear to be affected by test material administration.	
4.2	**Pathology**	One male dosed with 10 mg/kg had mottled kidneys. Another male had a petechial haemorrhage in the lung at the terminal necropsy. The cause of this was not known but was not considered to be the result of a dosing accident. No other gross lesions were observed at the time of necropsy.	
4.3	**Other**		
4.4	**LD_{50}**	Males : 10.3 mg/kg Females:11.8 mg/kg	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In accordance to the methodology described in FIFRA guidelines, F 81-1. Five male and five female rats per groups of approximately five to six weeks of age were used for this study. The test animals were administered a single oral dose of either 10, 15, 20, 25 or 30 mg/kg of the test material at a dosing volume of 5 ml/kg following an approximate 16 - 18 hour fast. This was accomplished by the use of a rigid oral feeding needle. All animals were observed twice (approximately 2 and 4 hours) on Day 1 following test article administration, and once daily thereafter. Bodyweights were obtained on Days 1, 8 and 15 prior to necropsy. All animals were subjected to a gross necropsy examination. The LD_{50} was calculated using a probit analysis of the mortality of each sex at each dose level.	
5.2	**Results and discussion**	100% mortality was observed for male and female rats dosed with 20, 25 and 30 mg/kg. 80% mortality was observed for rats dosed with 15 mg/kg. Mortality for the rats dosed with 10 mg/kg ranged from 40% for the males to 20% for the females. Lethargy and hypothermia was observed in the four higher dose groups for both sexes. Changes in respiration were observed in the females at the two highest doses and in the males at dose group 15 mg/kg. No gross lesions observed at necropsy were related to treatment.	
5.3	**Conclusion**	The LD_{50} was 10.3 mg/kg for the males and 11.8 mg/kg for the females. The 95% confidence limits for the males and females were 16.7 to 6.4 and 17.6 to 7.9 respectively.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes No control group used.	X

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	10/04/06	
Materials and Methods	As described by the Applicant.	
Results and discussion	As described by the Applicant.	
Conclusion	As described by the Applicant.	
Reliability	2	
Acceptability	acceptable	
Remarks	5.3.2 A control group is not necessary for acute studies, therefore lack of such a group is not a deficiency..	
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_1_1-1. Table for Acute Toxicity (modify if necessary)

Dose (mg/kg)	Number of dead / number of investigated	Time of death (after dosing)		Observations
		1hr	24hr	
10	Male: 2/5	-	2	
	Female: 1/5		1	
15	Male: 4/5	-	4	Lethargy and hypothermia
	Female: 4/5	-	4	
20	Male: 5/5	-	5	Lethargy and hypothermia
	Female: 5/5	1	4	
25	Male: 5/5	4	1	Lethargy and hypothermia
	Female: 5/5	4	1	
30	Male: 5/5	2	3	Lethargy and hypothermia
	Female: 5/5	3	2	
LD_{50} value	Males: 10.3 mg/kg Females: 11.8 mg/kg			

Section A6.1.1/02 Annex Point IIA6.1.1		Acute Toxicity Acute oral toxicity test in mice (LD_{50})	
		1 REFERENCE	Official use only
1.1	Reference	Muni, I.A. (1981b) Acute Oral LD_{50} of Acrolein in Female Mice. Bioassay Systems Corporation. BSC Project No. 10258.	
1.2	Data protection	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	No "Method used comparable to Method B1, 92/69/EEC"	
2.2	GLP	Yes	
2.3	Deviations	None	
		3 MATERIALS AND METHODS	
3.1	Test material	As given in Section 2	
3.1.1	Lot/Batch number	6072	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	
3.2	Test Animals		
3.2.1	Species	Mice	
3.2.2	Strain	CD-1	
3.2.3	Source	Charles River Breeding Laboratory, Wilmington, Massachusetts	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: 6 - 8 weeks Weight: 18 - 25 g	
3.2.6	Number of animals per group	10 animals per dose level	
3.2.7	Control animals	Yes	
3.3	Administration/ Exposure	Oral	
3.3.1	Postexposure period	15 days	

Section A6.1.1/02 **Annex Point IIA6.1.1**		**Acute Toxicity** **Acute oral toxicity test in mice (LD_{50})**	
		Oral	
3.3.2	Type	Gavage	
3.3.3	Concentration	0, 11.0, 13.2, 15.84, 19.0 mg/kg	
3.3.4	Vehicle	Deionised water	
3.3.5	Concentration in vehicle	0, 1.10, 1.32, 1.58, 1.90 mg/ml	
3.3.6	Total volume applied	10 ml/kg	
3.3.7	Controls	Yes	
3.4	**Examinations**	Clinical observations, mortality, bodyweight, necropsy	
3.5	**Method of determination of LD_{50}**	Probit analysis of the mortality at each dose level.	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Mortality: See Table A6_1_1-1 for mortality data. After acrolein administration, a total of nine mice died during the first three days of the 14 day observation period. (Six mice from the 19.0 mg/kg and three mice from the 15.8 mg/kg dose groups). Clinical signs: Immediately following dose administration, animals in the 15.84 and 19.0 mg/kg dose groups showed signs of lethargy, respiratory distress and squinted eyes until death occurred. All the surviving animals showed similar signs for varying lengths of time throughout the observation period. Blackening, followed by necrosis and breaking tip of the tails was found in several of the animals in the high dose groups. The two lowest dose groups showed similar signs (no evidence of respiratory distress) but the conditions were less severe and did not persist as long in the survivors. Control animals remained healthy throughout the observation period. Body weight: Body weights did not appear to be affected by test material administration.	
4.2	**Pathology**	Most animals which died 2-16 hours after dosing showed reddening of the lungs, haemorrhagic stomach and intestine (blood-filled), dilation of the blood vessels on the brain's surface and darkening of the medulla of the kidneys. Similar lesions were seen in the animals which died 1-3 days later. There were no specific lesions found at the terminal necropsy for the surviving animals	
4.3	**Other**	One mouse exhibited signs of gavage accident immediately following dosing. Necropsy findings indicated that the death was attributable to gavage accident. An additional animal was dosed at the same level.	

Section A6.1.1/02 **Annex Point IIA6.1.1**	**Acute Toxicity** **Acute oral toxicity test in mice (LD_{50})**	
4.4 LD_{50}	17.7 mg/kg (95% confidence limits: 16.3-19.2 mg/kg)	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	An acute oral LD_{50} determination of acrolein administered by gavage was conducted using female CD-1 mice (10 animals per dose level). The doses were 0.0, 11.0, 13.2, 15.84 and 19.0 mg/kg of acrolein in deionised water administered in a dose volume of 10 ml/kg.	
5.2 Results and discussion	A total of nine animals died within three days after dosing. (Six mice from the 19.0 mg/kg and three mice from the 15.8 mg/kg dose groups). Most animals which died 2 - 16 hours after dosing showed reddening of the lungs, hemorrhagic stomach and intestine, dilation of the blood vessels on the brain's surface and darkening of the medulla of the kidneys. Similar lesions were seen in the animals which died 1-3 days later. There were no specific lesions found at the terminal necropsy for the surviving animals.	
5.3 Conclusion	The LD_{50} was 17.7 mg/kg with 95% confidence limits at 16.3-19.2 mg/kg.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Yes Is not a guideline study.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	10/04/06	
Materials and Methods	As described by the Applicant.	
Results and discussion	As described by the Applicant.	
Conclusion	As described by the Applicant.	
Reliability	2	
Acceptability	acceptable	

Section A6.1.1/02 **Annex Point IIA6.1.1**	**Acute Toxicity** **Acute oral toxicity test in mice (LD_{50})**	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_1_1-1. Table for Acute Toxicity (modify if necessary)

Dose (mg/kg)	Number of dead / number of investigated	Time to death (hours after dosing)	Observations
11.0	0/10	Not applicable	-
13.20	0/10	Not applicable	-
15.84	3/10	1-6 hours	-
19.00	6/10	2 - 16 hours*	-
			2 - 3 days*

LD_{50}: 17.7 mg/kg

* Three animals died during each of the time intervals specified

Section A6.1.1/03 Annex Point IIA6.1.1		Acute Toxicity Acute oral toxicity test in mice (LD_{50})	
		1 REFERENCE	Official use only
1.1	Reference	Mansur, C.A. (1983a) Acute Oral LD_{50} of Acrolein in Male Mice. Bioassay Systems Corporation. BSC Project No. 11479.	
1.2	Data protection	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	No "Method used comparable to Method B1, 92/69/EEC"	
2.2	GLP	No GLP not compulsory when study performed.	
2.3	Deviations	The severity of the clinical signs were not noted. The lethargy seen was either mild or moderate but it was not documented.	
		3 MATERIALS AND METHODS	
3.1	Test material	As given in Section 2	
3.1.1	Lot/Batch number	6241	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	
3.2	Test Animals		
3.2.1	Species	Mice	
3.2.2	Strain	CD-1	
3.2.3	Source	Charles River Breeding Laboratory, Wilmington, Massachusetts	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Age: 5 weeks Weight: 20.3 - 24.0 g	
3.2.6	Number of animals per group	10 animals per dose level	
3.2.7	Control animals	Yes	

Section A6.1.1/03 Annex Point IIA6.1.1		Acute Toxicity Acute oral toxicity test in mice (LD_{50})	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Postexposure period	15 days	
		Oral	
3.3.2	Type	Gavage	
3.3.3	Concentration	0, 11.0, 13.2, 15.84, 19.0 mg/kg	
3.3.4	Vehicle	Deionised water	
3.3.5	Concentration in vehicle	1.10, 1.32, 1.58, 1.90 mg/ml	
3.3.6	Total volume applied	10 ml/kg	
3.3.7	Controls	Yes	
3.4	**Examinations**	Mortality, clinical signs, bodyweight, necroscopy.	
3.5	**Method of determination of LD_{50}**	Probit analysis of the mortality at each dose level	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Mortality: See Table A6_1_1-1 for mortality data. Clinical signs: After acrolein administration, a total of 22 mice died within the first two days during 14 day observation period. All other animals survived to terminal sacrifice. For the first three days, majority of the animals at all dose levels showed signs of lethargy, squinted eyes, rough coats, hunching pilo-erection. All the surviving animals showed rough coats for varying lengths of time throughout the observation period. Blackening, followed by necrosis and breaking of the tip of the tails was found in several of the survivors. Control animals remained healthy throughout the study. Bodyweight: On Day 7, all test animals showed reduced body weight gain compared to the controls. At terminal sacrifice, all the test animals had increased their rate of weight gain substantially when compared to Day 7. However, they still showed reduced weight gains of 11.6 - 28.6% compared to controls. Control animals remained healthy throughout the study.	
4.2	**Pathology**	Most animals which died in the first three days after dosing showed reddening of the lungs, and hemorrhagic stomachs and intestines. One animal at 13.2 mg/kg showed reddening of the lungs at terminal sacrifice. All other animals sacrificed at termination showed minimal, non-specific lesions.	
4.3	**Other**	No other significant effects noted	

Section A6.1.1/03 **Annex Point IIA6.1.1**	**Acute Toxicity** **Acute oral toxicity test in mice (LD_{50})**	
4.4 LD_{50}	13.9 mg/kg (95 % confidence limits: 12.8-15.1mg/kg)	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	An acute oral LD_{50} determination of acrolein administered by gavage was conducted using male CD-1 mice (10 animals per dose level). The doses were 0.0, 11.0, 13.2, 15.84, and 19.0 mg/kg of acrolein in deionised water administered in a dose volume of 10 ml/kg.	
5.2 Results and discussion	A total of 22 animals died within two days after dosing. For the first three days, a majority of the animals at all dose levels showed signs of lethargy, squinted eyes, rough coats, hunching, and pilo-erection. All of the surviving animals showed rough coats for varying lengths of time throughout the observation period. Blackening, followed by necrosis and breaking of the tip of the tails was found in several of the test animals. Control animals remained healthy throughout the study. On Day 7, all dose levels showed reduced body weight gain compared to the controls. At terminal sacrifice, all the animals had increased their rate of weight gain substantially when compared to Day 7, but still showed reduced weight gain compared to controls.	
5.3 Conclusion	The LD_{50} was 13.9mg/kg with 95% confidence limits of 12.8 - 15.1 mg/kg.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Yes It is not a guideline study.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	10/04/06	
Materials and Methods	As described by the Applicant.	
Results and discussion	As described by the Applicant.	
Conclusion	As described by the Applicant.	
Reliability	2	
Acceptability	acceptable	
Remarks		

Section A6.1.1/03 **Annex Point IIA6.1.1**	**Acute Toxicity** **Acute oral toxicity test in mice (LD_{50})**	
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_1_1-1. Table for Acute Toxicity

Dose (mg/kg)	Number of dead / number of investigated	Time to death (after dosing)	Observations
11.0	4/10	18-24h (2)* 24-30h (2)	Hunched posture, lethargy, rough coat, piloerection, squinted eyes
13.20	4/10	18-24h (2) 30-48h (2)	Piloerection, hunched posture, lethargy, squinted eyes, rough coat
15.84	8/10	1-6h (2) 18-24h (4) 24-30h (1) 30-48h (1)	Hunched posture, lethargy, squinted eyes, piloerection, rough coat
19.00	6/10	1-6h (3) 18-24h (2) 30-48h (1)	Hunched posture, lethargy, piloerection, squinted eyes, rough coat
LD_{50} value	13.9 mg/kg 95% confidence limit: 12.8 - 15.1 mg/kg		

- number of animals ( ) died during the time interval specified

Section A6.1.2 Annex Point IIA6.1.2		Acute Toxicity Acute dermal toxicity test in the rabbit (LD_{50})	
		1 REFERENCE	Official use only
1.1	Reference	Muni, I.A. (1981a) Acute Dermal Toxicity (LD_{50}) of Acrolein (Lot no. SFSL-5993) in Rabbits. Bioassay Systems Corporation. BSC Project No 10258.	
1.2	Data protection	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	Yes FIFRA 43, Part II (1978)	
2.2	GLP	Yes	
2.3	Deviations	No	
		3 MATERIALS AND METHODS	
3.1	Test material	As given in Section 2	
3.1.1	Lot/Batch number	SFSL-5993	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	
3.2	Test Animals		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	H.A.R.E. Rabbits for Research, Hewitt, New Jersey	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Age: 10-12 weeks Weight: 2.3 – 3.2 kg	
3.2.6	Number of animals per group	Ten animals per sex per dose level Control animals: Untreated controls, four animals per sex Vehicle treated controls, six animals per sex	
3.2.7	Control animals	Yes	

Section A6.1.2 Annex Point IIA6.1.2		Acute Toxicity **Acute dermal toxicity test in the rabbit (LD_{50})**	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Postexposure period	14 days	
3.3.2	Area covered	Not stated	
3.3.3	Occlusion	Not stated	
3.3.4	Vehicle	Absolute ethanol - water, 50:50 v/v	
3.3.5	Concentration in vehicle	100, 120, 144 mg acrolein/ml	
3.3.6	Total volume applied	2 ml/kg i.e. 200, 240, 288 mg acrolein/kg	
3.3.7	Duration of exposure	Once on Day one	
3.3.8	Removal of test substance	Not stated	
3.3.9	Controls	None	X
3.4	**Examinations**	Mortality, clinical observations, necropsy, histopathology	
3.5	**Method of determination of LD_{50}**	Modified method of Karber. Reference: Cornfield and Montel, (1950) JASA. 45:193-210.	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Mortality: See Table A6_1_2-1 for mortality data. After acrolein administration, a total of 34 rabbits died during the 14-day study period. (Three males and four females at 200 mg/kg, seven males and seven females at 240 mg/kg, and five males and eight females at 280 mg/kg). One female from the 200 mg/kg dose group was sacrificed moribund on the fourth day. Clinical signs: Approximately one to two minutes after dosing, all animals exhibited signs of severe pain including screaming, and severely hyperactive behaviour. These conditions were exhibited for 10-20 minutes following dosing. After this initial reaction, animals became lethargic, had squinted eyes, and evidence of respiratory distress. Cyanosis became evident in varying intensity on the day of dosing in acrolein-treated rabbits. Evidence of lethargy, mild respiratory problems and mild nasal discharge continued for varying lengths of time throughout the study period. Skin lesions of varying severity were noted throughout the observation period for acrolein-treated rabbits. Control animals remained healthy throughout the study period.	

Section A6.1.2 Annex Point IIA6.1.2		Acute Toxicity **Acute dermal toxicity test in the rabbit (LD_{50})**	
4.2	Pathology (necropsy)	All acrolein-treated animals showed signs of dermatopathy. In this condition, the shaved skin of the flank is discoloured yellow-brown. When ulcerated, the skin is characterized by an intimately adherent red-brown scab, under which rarely there is a pus filled cavity. When not ulcerated the overlying skin is firm, wrinkled, nonpliable, brittle and puckered. The subjacent hypodermis is markedly edematous and often hemorrhagic; frequently the glistening and gelatinous appearance of the subcutis extends to the axillary and inguinal regions. The thickened and immovable skin and subcutis are not, however, adherent to the musculature of the body wall. Other then dermotapathy, the only gross lesions occurring consistently at all treatment levels were pulmonary petechiae, atelectasis, and discoloration.	
4.3	Histopathology		
4.4	Other		
4.5	LD_{50}	Males: 240.0 mg/kg (95% confidence limits: 217.3-265.1 mg/kg) Females: 233.1 mg/kg (95% confidence limits: 200.1-248.7 mg/kg) All animals: 231.4 mg/kg (95% confidence limits: 216.5-247.4 mg/kg)	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	This study was conducted in accordance to the methodology described in FIFRA guidelines, 43, part II (1978). Ten rabbits of each sex were dosed via dermal application at three dose levels, 200, 240, 288 mg/kg of acrolein in a mixture of absolute ethanol and deionised water (50:50v/v). In each dose group, equal numbers of abraded and unabraded skin conditions were represented. A total volume of 2 ml/kg of test material was administered once on Day 1 and all the animals were observed for overt signs until Day 14. All animals were subjected to a gross necropsy examination.	
5.2	Results and discussion	Of 60 acrolein-treated animals a total of 11 males and 18 females died within 24 hours of dosing. All animals were observed for 14 days, during which time four more males died for a total of 15 over the course of the study. Terminally sacrificed animals showed minimal signs of toxicity except at the dosing site on the skin. All acrolein-treated animals showed signs of dermatopathy, pulmonary petechiae, atelectasis, and discoloration.	
5.3	Conclusion	Males: 240.0 mg/kg (95% confidence limits: 217.3-265.1 mg/kg) Females: 233.1 mg/kg (95% confidence limits: 200.1-248.7 mg/kg) All animals: 231.4 mg/kg (95% confidence limits: 216.5-247.4 mg/kg)	

Section A6.1.2 **Annex Point IIA6.1.2**	**Acute Toxicity** **Acute dermal toxicity test in the rabbit (LD_{50})**	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Yes Incomplete reporting/minor methodological deficiencies: Study does not state the area covered by administration of the test material, and does not state how or if the test substance was removed. It is felt that these points do not affect the quality of the results.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	10/04/06	
Materials and Methods	3.3.9 There were in fact 4/sex untreated controls and 6/sex vehicle treated controls.	
Results and discussion	As described by the Applicant.	
Conclusion	As described by the Applicant.	
Reliability	2	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	

Section A6.1.2 **Annex Point IIA6.1.2**	**Acute Toxicity** **Acute dermal toxicity test in the rabbit (LD_{50})**	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_1_2-1. Table for Acute Toxicity

Dose mg/kg	Number of dead / number of investigated	Time of death (range)	Observations
200	Males: 3/10 Females: 4/10	2 hours to Day 9 after dosing	Acrolein-associated dermatopathy, pulmonary petechiae, atelectasis and discoloration
240	Males: 7/10 Females: 7/10	2 hours to Day 6 after dosing	Acrolein-associated dermatopathy, pulmonary petechiae, atelectasis and discoloration
288	Males: 5/10 Females: 8/10	2 hours to Day 3 after dosing	Acrolein-associated dermatopathy, pulmonary petechiae, atelectasis and discoloration
LD_{50} value	Males: 240.0 mg/kg (95% confidence limits: 217.3-265.1 mg/kg) Females: 233.1 mg/kg (95% confidence limits: 200.1-248.7 mg/kg) All animals: 231.4 mg/kg (95% confidence limits: 216.5-247.4 mg/kg)		

Reference – [1]Cornfield and Montel, JASA, 45: 193-210 (1950)

Section A6.1.3 Annex Point IIA6.1.3		Acute Toxicity **Acute inhalation toxicity study in the rat (LC_{50})**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Nachreiner, D.J et al. (1987) Acute Inhalation toxicity of Acrolein Vapour by One and Four Hour Exposures. Bushy Run Research Centre. BSC Project No. 49-170.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not specifically stated Study followed laboratories own specific protocol.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Several female animals (4 hour exposure) were below the weight range specified in the protocol (200 - 350g) This deviation does not affect the integrity of the study	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2.	
3.1.1	Lot/Batch number	70526 JM	
3.1.2	Specification	Test material was 99+ % Gold label reagent and was inhibited with approximately 3% water and 200 ppm hydroquinone	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	

Section A6.1.3 Annex Point IIA6.1.3		Acute Toxicity Acute inhalation toxicity study in the rat (LC_{50})		
3.2	**Test Animals**			
3.2.1	Species	Rat		
3.2.2	Strain	Sprague-Dawley		
3.2.3	Source	Harlan Sprague-Dawley, Inc., Indianapolis, Indiana, USA		
3.2.4	Sex	Males and females		
3.2.5	Age/weight at study initiation	Age Males: 56 - 73 days Females: 56 - 93 days Weight Males: 250 - 323 g Females: 168 - 247 g		
3.2.6	Number of animals per group	Five animals per sex per dose level		
3.2.7	Control animals	No		
3.3	**Administration/ Exposure**	Inhalation		
3.3.1	Postexposure period	14 days		
		Inhalation		
3.3.2	Concentrations	Nominal concentration:	1hour – 50, 69, 72, 104, 240 mg/m³ 4 hours - 13.6, 18.5, 27.8, 32.5 mg/m³	
		Analytical concentration:	1 hour - 180.2, 69.0, 53.4, 49.0, 31.2 mg/m³ 4 hours - 26.9, 20.2, 15.6, 10.7 mg/m³	
3.3.3	Particle size	Not applicable - Vapour exposure		
3.3.4	Type or preparation of particles	Not applicable		
3.3.5	Type of exposure	Whole body		
3.3.6	Vehicle	Not used		
3.3.7	Concentration in vehicle	Not applicable		
3.3.8	Duration of exposure	1 and 4 hours		
3.3.9	Controls	None		
3.4	**Examinations**	Mortality, clinical observations, body weight, necropsy,		

Section A6.1.3 Annex Point IIA6.1.3		Acute Toxicity **Acute inhalation toxicity study in the rat (LC_{50})**	
3.5	**Method of determination of LD_{50}**	Probit Analysis, Finney (1964) Moving average method of Thompson (1947) – female 4hr only	
3.6	**Further remarks**	mg/m^3 concentration calculated from ppm Data at 1 ppm = 2.225 mg/m^3 (at 24°C)	

Section A6.1.3 Annex Point IIA6.1.3	Acute Toxicity Acute inhalation toxicity study in the rat (LC_{50})	
	4 RESULTS AND DISCUSSION	
4.1 Clinical signs	See Table A6_1_3-1 for mortality data. Single 1 hour vapour exposures to acrolein produced mortalities in the 180.2, 69, 53.4 and 49 mg/m^3 exposure groups. Single four hour vapour exposures to acrolein produced mortalities in the 26.9, 20.2, 15.6 mg/m^3 exposure groups. Observations during exposure included lacrimation, perinasal and perioral wetness, and mouth breathing. Clinical signs observed following exposure or during the first week post-exposure included perinasal and perioral wetness and encrustation, unkempt fur, respiratory difficulties (mouth breathing, audible respiration, decreased respiration rate) and hypoactivity. An additional sign observed following exposure for the 10.68 mg/m^3 (four hours) exposure group was gas-filled distended stomachs. The only signs observed during the second week post-exposure included perinasal and periocular encrustation and unkempt fur. A loss of bodyweight was observed for both sexes in the first week of the post exposure period for all exposure groups. During the second week post-exposure bodyweight gains were observed for all males with the exception of those in the 53.4 mg/m^3 (one hour) and 20.2 mg/m^3 (four hours) exposure groups. Mean bodyweight gains were observed during the second week for females in the 53.4, 49, 31.2 mg/m^3 (one hour) exposure groups and in the 26.9, 15.6, 10.7.8 (four hours) exposure groups. However further loss of bodyweight was observed for one female in the 49 mg/m^3 (one hour) and 26.9 mg/m^3 (four hours) exposure groups. In addition, a depression in bodyweight gain was observed for females in the 15.575mg/m^3 exposure groups relative to females of comparable age in the 10.7 mg/m^3 exposure groups.	
4.2 Pathology	Gross lesions were observed in animals which died and included, mottled discoloration of the lungs and liver, clear fluid in the trachea and the thoracic cavity, red discoloration of the submandibular lymph nodes, gas-filled stomach and intestines, opaque or cloudy eyes, and subdural haemorrhage. Perinasal and perioral encrustation was also noted. No macroscopic lesions were observed in rats sacrificed after the 14-day recovery period.	
4.3 Other		
4.4 LC_{50}	(95% confidence limits) Males: 1 hour – 57.9 (51-62) mg/m^3 4 hours – 16.5 (13-21) mg/m^3 Females: 1 hour – 53.4 (45-67) mg/m^3 4 hours – 19.6 (16-24) mg/m^3 Combined: 1 hour - 57.9 (53-60) mg/m^3 4 hours - 18.5 (16-22) mg/m^3	

Section A6.1.3 **Annex Point IIA6.1.3**		**Acute Toxicity** **Acute inhalation toxicity study in the rat (LC_{50})**	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study followed specific protocol BRRC Project 86-15-40187 prepared by Bushy Run Research Center. The test material was 99+ % gold Label reagent and was inhibited with approximately 3% water and 445 mg/m^3 hydroquinone. Nine groups, each containing five male and five female rats, were exposed once for either one hour or four hours to vapour dynamically-generated from acrolein test material. The whole body was exposed. No control exposures were performed. All animals were observed for signs of toxic effects on the day of exposure and daily for 14 days following exposure. All animals were subjected to a gross necropsy examination.	
5.2	**Results and discussion**	Single one hour vapour exposures to acrolein produced mortalities in the 180.2, 69, 53.4, 49 mg/m^3 exposure groups. Single four hour vapour exposures to acrolein produced mortalities in the 26.9, 20.2, 15.6 mg/m^3 exposure groups. Clinical signs were observed in all exposure groups and included signs of ocular and respiratory irritation and hypoactivity. The only signs observed during second week post-exposure were perinasal and pericocular encrustation and unkempt fur. A loss of bodyweight was observed for all exposure groups during the first week post-exposure. Body weight gains were observed for most animals (both sexes) during the second week post-exposure. Macroscopic lesions were found only in animals which died and included mottled discoloration of the lungs and liver, clear fluid in the trachea and thoracic cavity, red discoloration of the submandibular lymph nodes, gas-filled stomach and intestines, opaque eyes and subdural haemorrhage. Due to low incidence of subdural haemorrhage (3/90), this finding was not considered biologically significant.	
5.3	**Conclusion**	**LC_{50}** Males: 1 hour –57.9 (51-62) mg/m^3 4 hours – 16.5 (13-21) mg/m^3 Females: 1 hour – 53.4 (45-67) mg/m^3 4 hours – 19.6 (16-24) mg/m^3 Combined: 1 hour - 57.9 (53-60) mg/m^3 4 hours - 18.5 (16-22) mg/m^3	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A6.1.3 **Annex Point IIA6.1.3**	**Acute Toxicity** **Acute inhalation toxicity study in the rat (LC_{50})**	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	19/04/06	
Materials and Methods	As described by the Applicant	
Results and discussion	Mouth breathing is described in the study as one of the clinical signs. Rats are obligate nasal breathers so the accuracy and reliability of reporting for this clinical sign is questionable.	
Conclusion	As described by the Applicant	
Reliability	2	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_1_3-1. Table for Acute Toxicity

Exposure (ppm)	Esposure duration (h)	Sex	Time of death								Total incidence
			During exposure	Post exposure day							
				0	1	2	3	4	5	6	13	
81	1	M	0	4*	0	0	1	0	0	0	0	5/5
81	1	F	0	2*	0	2	1	0	0	0	0	5/5
31	1	M	0	2*	0	3	0	0	0	0	0	5/5
31	1	F	0	0	1	2	0	1	0	1	0	5/5
24	1	M	0	0	0	0	2	0	0	0	0	2/5
24	1	F	0	0	1	0	0	0	0	0	0	1/5
22	1	M	0	0	0	0	0	0	0	0	0	0/5
22	1	F	0	0	0	1	0	0	0	0	0	1/5
14	1	M	0	0	0	0	0	0	0	0	0	0/5
14	1	F	0	0	0	0	0	0	0	0	0	0/5
12.1	4	M	0	0	2	2	0	0	0	0	0	5/5
12.1	4	F	0	0	0	3	0	0	0	0	0	3/5
9.1	4	M	0	0	1	2	0	0	0	0	0	3/5
9.1	4	F	0	0	0	3	0	0	0	0	1	4/5
7.0	4	M	0	0	2	0	0	0	1	0	0	3/5
7.0	4	F	0	0	0	0	0	0	0	0	0	0/5
4.8	4	M	0	0	0	0	0	0	0	0	0	0/5
4.8	4	F	0	0	0	0	0	0	0	0	0	0/5

* Mortalities occurred within four hours following exposure.

Section A6.1.4/01 Annex Point IIA6.1.4		Acute Dermal Irritation Primary dermal irritation test in the rabbit	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Goodband, J. (1981) Primary Skin Irritation Study of Acrolein in Rabbits. Bioassay Systems Corporation. BSC Project No. 10258.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes 16 CFR	
2.2	**GLP**	No GLP not compulsory when study performed.	
2.3	**Deviations**	Yes	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	Lot No. SFSL-5893	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	MHF	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	2.0 - 2.5 kg	
3.2.6	Number of animals per group	Six	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Application		

Section A6.1.4/01 Annex Point IIA6.1.4		Acute Dermal Irritation Primary dermal irritation test in the rabbit	
3.3.1.1	Preparation of test substance	Test substance was used as delivered.	
3.3.1.2	Test site and Preparation of Test Site	One intact and one abraded skin site on each animal. No cleaning of skin took place prior to testing.	
3.3.2	Occlusion	Semiocclusive	
3.3.3	Vehicle	None	
3.3.4	Concentration in vehicle	Not applicable	
3.3.5	Total volume applied	0.5 ml	
3.3.6	Removal of test substance	Not stated	
3.3.7	Duration of exposure	24 hours	
3.3.8	Postexposure period	Three days	
3.3.9	Controls		
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Dermal examination	Yes	
3.4.2.1	Scoring system	Grading scale Primary irritation was scored using the following scale: Draize, J.H. (1959) The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the United States, Austin, Texas.	

Erythema and Eschar Formation	**Value**
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema Formation	**Value**
No oedema	0
Very slight oedema (barely perceptible)	1

Section A6.1.4/01 **Annex Point IIA6.1.4**		**Acute Dermal Irritation** Primary dermal irritation test in the rabbit	
		Very slight oedema (edges of area well-defined by definite raising) 2 Moderate oedema (raised approximately 1 mm) 3 Severe oedema (raised more than 1 mm and extending beyond the area of exposure) 4 The scores erythema and oedema at the 24 and 72 hour readings were totalled for the intact and abraded sites, and this total was divided by 24 to give the primary irritation index of the test material. The test material was classified according to the following scheme: Primary Irritation Index — Classification of Irritancy 0 — Non-irritant >0 - 2 — Mild irritant >2 - 5 — Moderate irritant <5 - 8 — Severe irritant	
3.4.2.2	Examination time points	24 and 72 hours	
3.4.3	Other examinations	None	
3.5	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Average score**		
4.1.1	Erythema	24 hours - 1 unabraded, 1 abraded 72 hours - 1 unabraded, 1 abraded	
4.1.2	Edema	24 hours - 3 unabraded, 3 abraded 72 hours - 3 unabraded, 3 abraded	
4.2	**Reversibility**	No	
4.3	**Other examinations**	None	
4.4	**Overall result**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Six male rabbits were used for the primary dermal irritation study. A dose of 0.5 ml of acrolein was introduced under 1-inch square gauze patches. The patches were applied to one intact and one abraded skin site on each animal. Control patches were similarly placed on the contralateral abraded and unabraded sites. The test substance was kept in contact kept with the skin for 24 hours. Signs of erythema and oedema were scored 24 and 72 hours after application of the acrolein.	

Section A6.1.4/01 **Annex Point IIA6.1.4**	**Acute Dermal Irritation** Primary dermal irritation test in the rabbit	
5.2 Results and discussion	Two animals died on the day after dosing. Using the Draize method, the average score for erythema is 1 and for oedema it is 3. Acrolein is a skin irritant.	
5.3 Conclusion	Acrolein is a skin irritant.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Yes It is not a guideline study.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	19/04/06	
Materials and Methods	As described by the Applicant.	
Results and discussion	As described by the Applicant.	
Conclusion	As described by the Applicant.	
Reliability	2	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_1-S1 Table for skin irritation study (modify if necessary)

Score (average animals investigated)	Time	Erythema		Oedema	
		UA	A	UA	A
average score Draize scores (0 to maximum 4)	24 h	1	1	3	3
	48 h**	-		-	
	72 h	1	1	3	3
average score	24h, 72h	1		3	
reversibility: *					
average time for reversibility					

UA: Unabraded

A: Abraded

* c : completely reversible
n c : not completely reversible
n : not reversible

** : Not determined

Section 6.1.4/02 **Annex Point IIA6.1.4**		**Acute Eye Irritation** Primary eye irritation test in the rabbit	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Goodband, J. & Dunn, G.R. (1980) Primary Eye Irritation Study of Acrolein in Rabbits. Bioassay Systems Corporation. BSC Project No. 10258.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No "Method used comparable to EU Method B.5"	
2.2	**GLP**	No GLP not compulsory when study performed.	
2.3	**Deviations**	Yes	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot number	SFSL 5893	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	MHF	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	2.1 - 2.6 kg	
3.2.6	Number of animals per group	Nine	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**		
3.3.1	Preparation of test substance	Test substance was used as delivered	

Section 6.1.4/02 **Annex Point IIA6.1.4**		**Acute Eye Irritation** Primary eye irritation test in the rabbit	
3.3.2	Amount of active substance instilled	0.1 ml	
3.3.3	Exposure period	Three rabbits were flushed with lukewarm water after 20-30 seconds of instillation, whereas the remaining six were left unwashed.	
3.3.4	Ophthalmoscopic examination	No	
3.4	**Examinations**		
3.4.1	Ophthalmoscopic examination	No	
3.4.1.1	Scoring system	**Cornea** No ulceration or opacity **0** Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible **1** Easily discernible translucent areas, details of iris slightly obscured **2** Necreous areas, no details of iris visible, size of pupil barely discernible- **3** Complete corneal opacity, iris not discernible **4** **Iris** Normal **1** Markedly deepened folds, congestion, swelling, moderate circum-corneal injection (any of these or combination thereof) **2** No reaction to light, haemorrhage, gross destruction (any or all of these)- **3** **Conjunctival** Redness (refers to palpebral and bulbar conjunctive excluding cornea and iris) Vessels normal **0** Some vessels definitely injected above normal **1** Diffuse, crimson red, individual vessels not easily discernible **2** Diffuse beet red **3** **Chemosis** No swelling **0** Any swelling above normal (includes nictitating membrane) **1** Obvious swelling with partial eversion at lids **2** Swelling with lids about half closed **3** Swelling with lids more than half closed **4**	

Section 6.1.4/02 **Annex Point IIA6.1.4**		**Acute Eye Irritation** Primary eye irritation test in the rabbit	
		Reference: Draize, J.H., Woodward, G. & Calvery, H.O. (1944). Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. J. Pharm. and Exper. Ther. 82: 77-390.	
3.4.1.2	Examination time points	24h, 48h, 72h, Days 4 and 7.	
3.4.2	Other investigations		
3.5	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Yes One rabbit died on Day 4	
4.2	**Average score**		
4.2.1	Cornea	See Table A6_1_4E-1.	
4.2.2	Iris	See Table A6_1_4E-1.	
4.2.3	Conjunctiva		
4.2.3.1	Redness	See Table A6_1_4E-1.	
4.2.3.2	Chemosis	See Table A6_1_4E-1.	
4.3	**Reversibility**	No	
4.4	**Other**	One rabbit died on Day 4.	
4.5	**Overall result**	Acrolein is an eye irritant	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Nine male rabbits were used for the study. A dose of 0.1 ml of acrolein (density 0.86) was applied to each test eye. The test substance was placed in the everted lower lid of the eye; the upper and lower lids were then gently held together for a few seconds before releasing to prevent the loss of test substance. The other eye remained untreated, and served as a control. The treated eyes of three rabbits were flushed with lukewarm water for 1 - 2 minutes, 20 - 30 seconds after instillation. The treated eyes of the remaining six rabbits were left unwashed. Readings of ocular lesions were made at 24, 48, 72 hours and 4 and 7 days after treatment.	
5.2	**Results and discussion**	All animals displayed positive responses concluding that acrolein is an eye irritant.	X
5.3	**Conclusion**	Acrolein is an eye irritant.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes Not a guideline study.	

Section 6.1.4/02 **Annex Point IIA6.1.4**	**Acute Eye Irritation** Primary eye irritation test in the rabbit	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	19/04/06	
Materials and Methods	As described by the Applicant.	
Results and discussion	As described by the Applicant.	
Conclusion	5.2 Average scores were: Cornea opacity 4, iris 2, conjunctival redness 2, chemosis 4.	
Reliability	2	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_1_4E-1. Results of eye irritation study

	Cornea	Iris	Conjunctiva	
			redness	chemosis
score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to4
24 h	4	2	2	4
48 h	4	2	2	4
72 h	4	2	2	4
Average 24h, 48h, 72h	4	2	2	4
Area effected				
Maximum average score (including area affected, max 110)				
Reversibility*				
average time for reversion				
Give method of calculation maximum average score. * *c :* *completely reversible* *n c :* *not completely reversible* *n :* *not reversible*				

Section A6.1.5 **Annex Point IIA6.1.5**		**Skin sensitisation** Guinea pig maximisation test (GPMT)	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Susten, AS and Breitenstein, MJ (1990). Failure of acrolein to produce sensitisation in the guinea pig maximisation test. Contact Dermatitis; 22(5): 299-300	
1.2	**Data protection**	n/a	
1.2.1	Data owner	Unknown	
1.2.2			
1.2.3	Criteria for data protection	Data on new a.s for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No Methods used comparable to OECD guideline for the Testing of Chemicals (1981), No. 406s.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Yes Skin reaction scoring differs from OECD guidelines (see 5.2)	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Not specified	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	
3.1.2.4	Preparation of test substance for application	a) <u>For induction:</u> intradermal: 0.01% acrolein in water topical: 2.5% acrolein in water b) <u>For challenge</u>: 0.5% acrolein in water	
3.1.2.5	Pretest performed on irritant effects	Not specified	
3.2	**Test Animals**		
3.2.1	Species	Guinea pigs	
3.2.2	Strain	Not specified	

Section A6.1.5 Annex Point IIA6.1.5		Skin sensitisation Guinea pig maximisation test (GPMT)	
3.2.3	Source	Not specified	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Not specified	
3.2.6	Number of animals per group	7	X
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	State study type: Not specified	
3.3.1	Induction schedule	Not specified	
3.3.2	Way of Induction	Intradermal and topical	
		Occlusive or semi-occlusive, Not specified	
3.3.3	Concentrations used for induction	Intradermal: 0.01% acrolein in water Topical: 2.5% acrolein in water	
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	Not specified	
3.3.5	Challenge schedule	Not specified	
3.3.6	Concentrations used for challenge	0.5% Acrolein in water	
3.3.7	Rechallenge	Not specified	
3.3.8	Scoring schedule	Not specified	
3.3.9	Removal of the test substance	Not specified	
3.3.10	Positive control substance	DNCB in ethanol 70%	
3.4	**Examinations**	Non-entry field	
3.4.1	Pilot study	Not specified	
3.5	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Results of pilot studies**	Not specified	
4.2	**Results of test**	See table 6_1_5-2	
4.2.1	24h after challenge	Not specified	
4.2.2	48h after challenge	A maximum of seven animals showed signs of allergic reactions.	
4.2.3	Other findings	The animals scored 0.5 (only one control animal showed the same score)	

Section A6.1.5 **Annex Point IIA6.1.5**	**Skin sensitisation** Guinea pig maximisation test (GPMT)	
4.3 Overall result	The incidence of skin reactions was much higher in test animals than in control animals.	X
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	In this study female guinea pigs were treated with acrolein in water. The concentrations used for the intradermal and topical induction phases and for the topical challenge phase were 0.01%, 2.5% and 0.5%, respectively. DNCB in ethanol 70% was used as positive control. Skin reactions were scored on a scale 0.5, 1, 2 and 3.	
5.2 Results and discussion	Challenge treatment induced skin reactions in a maximum of seven test animals (score 0.5), whereas only one control animal showed the same score. The difference between score 0.5 and 1 is not distinguished in the OECD-guidelines. According to the authors, score 0.5 is defined as patches of redness, not confluent, and score 1 as mild redness, confluent. Given this description and the description in the OECD-guidelines (score1: discrete or patchy erythema), score 0.5 should be interpreted as score 1 according to OECD.	
5.3 Conclusion	Since the incidence of skin reactions was much higher in test animals than in control animals it seems dubious to conclude that the substance is not a skin sensitiser, however no definite conclusion with respect to the sensitisation potential can be made on the basis of this study.	
5.3.1 Reliability	2	X
5.3.2 Deficiencies	Yes Although the test method deviates from OECD guidelines and the study was poorly reported, the raw data of this study is acceptable for use.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	21/04/06	
Materials and Methods	3.2.6 There were 15 animals in each group.	
Results and discussion	4.3 The authors of this study state in their report that ' challenge treatment with acrolein failed to produce positive skin reactions in any of the fifteen acrolein exposed animals or in a similar number of vehicle treated controls. Sensitisation was observed in each of the fifteen guinea pigs treated with DNCB.'	
Conclusion	Other conclusions:	
Reliability	3	

Section A6.1.5 Annex Point IIA6.1.5	Skin sensitisation Guinea pig maximisation test (GPMT)	
Acceptability	Not acceptable This study states that acrolein failed to produce effects of sensitisation in any of the fifteen exposed animals. However, on examination of the raw data provided seven of the animals had skin reactions which were scored as 0.5. This score is given for 'patches of redness, non-confluent' and so equates to an OECD score of 1. The incidence of skin reactions was hence higher in treated animals than controls. There is no explanation as to why the study Authors concluded that this study was negative. The discrepancies in the proposed finding lead to uncertainty as to the reliability of this study. A further sensitisation study has not been conducted due to animal welfare concerns because of acrolein's corrosive nature; an approach with which the UK CA agrees.	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_1_5-1. Detailed information including induction/challenge/scoring schedule for skin sensitisation test

Inductions	**GPMT**		**Buehler test**	**Observations/Remarks** *give information on irritation effects*
	day of treatment	application	day of treatment	
Induction 1	0	intradermal	day 0	
pretreatment for non-irritating substances	4-6	0.5 ml 10 % SDS in vaseline	--	
Induction 2	5-7	topical	6-8	
Induction 3	6-8	topical	13-15	
challenge	20-22	topical	27-29	
(rechallenge)	27-29		34-36	
scoring 1	21-23		35-37	
scoring 2	22-24		36-38	

Table A6_1_5-2. Result of skin sensitisation test (modify if necessary)

	Number of animals with signs of allergic reactions / number of animals in group		
	Negative control	**Test group**	**Positive control**
scored after 24h	**Not stated**	**Not stated**	**Not stated**
scored after 48h	**1 / 7**	**7 /7**	**Not stated**

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 [X] **Limited exposure [ ]**	**Technically not feasible [X]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	In the guinea pig maximisation test (Susten et al., Contact Dermatitis, 1990: 22: 299-300 Section IIIA 6.1.5), acrolein is reported to be negative. However, the study was poorly reported but the raw data was submitted by industry on request by the European Commission. Skin reactions were scored on scale 0.5, 1, 2 and 3. Challenge treatment induced skin reactions in a maximum of 7 test animals (score 0.5) whereas only one control animal showed the same score. According to the OECD score 0.5 should actually be interpreted as score 1. Since the incidence of skin reactions was much higher in test animals than in control animals it seems dubious to conclude that the test substance is not a skin sensitiser (European Union Risk Assessment Report: Acrylaldehyde, European Chemicals Bureau 2001). Aldehydes are a well known class of skin sensitisers. The chemical class is very diverse in terms of structure and therefore there are varying degrees of sensitisation potential within the group. The reactivity is because the carbonyl group (C=O) is thought to act as an electrophilic site for haptenation via Schiff base formation, or by activating another site. Therefore, lypophilicity (Log Pow), size of molecule and the reactivity of the carbonyl group all play a part in determining the sensitisation potential of the chemical. Several studies have been performed using groups of aldehydes to test the sensitivity of the Local Lymph Node Assay (LLNA) and the validity of QSARs (i.e. DEREK prediction system) for skin sensitisation potential. (Patlewicz, G et al (2001) Contact Dermatitis 44 (6) page 331-; Patlewicz, G et al (2004) Contact Dermatitis 50 (2) page 91-; Basketter, DA et al (2001) Contact Dermatitis 45 (2) page 89-; Roberts, DW and Patlewicz, G (2002) SAR QSAR Environ. Res. 13(1): 145 – 152). Chemicals are assigned to one of 5 discrete classes of sensitising potency, Class 1 being strong allergens to Class 5: non-sensitisers. During development of the LLNA as a stand-alone test for skin sensitisation potential, studies of allergenic potential in humans were compared with responses in the LLNA to determine if the test was sufficiently sensitive. (Basketter et al (2001) Contact Dermatitis 45: 89-94 Referenced in Dearman, RJ and Kimber I (2001) Development of the Local Lymph Node Assay for risk assessment of chemicals and formulations. HSE Contract Research Report 399/2001).	

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

Ten aldehydes of varying degrees (Classes 2 to 5) were assessed in man and compared to responses in the LLNA. The results of the study indicate that aldehydes either similar in structure or that are potential metabolites of acrolein (e.g. formaldehyde) are moderate to weak sensitisers. Patlewicz, G et al (2004) Contact Dermatitis 50 (2) page 91-, identifies structural groups which indicate increasing sensitising potency of the aldehydes. With a small aliphatic chain length and a $CH=CH_2$ group, it is expected that acrolein would, by QSAR prediction, be identified as a moderate sensitiser. The potential analogue 2, 4-hexadienal was shown by both QSAR and LLNA to be moderately sensitising. It should also be noted that hexyl cinnamic aldehyde is the preferred positive control in the LLNA for Regulatory testing performed in the UK (Dearman, RJ et al (2001) Contact Dermatitis 44: 357 – 361).

Acrolein is currently classified as R24/25: Toxic in contact with skin and if swallowed and R34: Causes burns. The local lymph node assay (LLNA) study has to use live animals with the test material applied to the back of the ear of the animal. The test requires a repeated application of the test material on the back of the ear (3 applications over 3 days). Existing toxicity studies where acrolein has been applied to the skin – dermal irritation, acute dermal toxicity and 21-day dermal toxicity studies have shown that application of the test material causes immediate irritation responses. In the 21-day dermal study (Section IIIA6.3.2) application of the lowest dose to skin, 7 mg/kg (equivalent to a 3.5 mg/litre solution) caused irritation after the initial 6 hour application. For a LLNA study the lowest dose normally considered for application is a 0.1% dilution, equivalent to a 1 g/litre solution which is one order of magnitude greater than the dosage causing irritation in the dermal toxicity study. The existing studies also indicate that when the test material is left on the skin, irritation increases over time. In the dermal irritation study (Section IIIA 6.1.4) it can be seen that by 7 days after initial application there are signs of necrosis, with erythema and oedema greater at 14 days than up to 72 hours. The OECD Guideline for an LLNA study indicates that the highest dose tested should avoid systemic toxicity and an excessive level of skin irritation. The existing toxicity studies have indicated that systemic toxicity occurs after one dose and in the 21-day dermal study, there are indications both clinical signs (nasal mucus discharge) and evidence from the histopathology (lesions in lungs) that systemic toxicity via inhalation of the test material is occurring even though the application sites are covered. In addition, in both the dermal irritation study and the acute toxicity study, mortalities occurred within one day of dosing. In the 21-day dermal toxicity study where lower doses were applied, mortalities occurred within 5 days of initial dosing and two animals had to be sacrificed in the same time period due to injuries considered to be triggered by the severe pain response to application of the test material, as initially observed in the acute dermal toxicity study. It is therefore considered that application of the test material at the required doses for the correct performance of the LLNA may cause undue pain and discomfort to the animals involved. According to the Home Office License, studies should not be conducted using a test material which has the potential to cause the animal pain.

Section A6.1.5 Annex Point IIA VI.6.1.5	Skin Sensitisation
	It should also be noted that the test substance is a highly volatile liquid (vapour pressure = 31920 Pa at RTP) and will produce formaldehyde and other aldehydes such as glyoxal and glycoaldehyde upon degradation in air. The speed of volatilisation (from 23 hours) and rapid degradation (photo-oxidation with OH radicals in 29 hours) of the test material even in solution is such that it would be difficult to guarantee the correct dosage had been applied to the ear for the correct period of time. As already stated, evidence from previous toxicity studies where the test material had been applied dermally and the application sites covered indicate that some test material was being inhaled and causing systemic toxicity. It would therefore be difficult assure dosing was to the correct level, plus the presence of other degradation products such as 3-hydroxypropanal to which acrolein rapidly hydrolyses in contact with moisture could affect the results of the study. In addition to potential sensitising degradants, hydroquinone is present in the technical grade active substance and the biocidal product at a typical concentration of 0.3 % and is classified as a Category 3 carcinogen and skin sensitiser. According to the Dangerous Preparations Directive hydroquinone is present at a concentration below that which would attract R43 classification of acrolein. However, it is possible that the combined concentration of hydroquione plus sensitising degradant species would be above the level requiring classification of acrolein as R43. To summarise, based on the ambiguous data from the ECB Risk Assessment, the presence of hydroquinone as a stabiliser, structural alerts for aldehydes as potential skin sensitisers, and the rapid degradation of the active substance to other potentially sensitising aldehyde species, acrolein is to be provisionally classified by the applicant as R43: May cause sensitisation by skin contact. It is also considered that a LLNA study would be unethical based on animal welfare grounds as existing evidence indicates that the application of acrolein to the animal would cause pain and that any results obtained from a LLNA study may be unreliable due to the reactivity of the test material. As already indicated acrolein is already classified as Corrosive (R34) and Toxic in contact with the skin (R24). As such, any risk management measures put in place to deal with these hazards would also be appropriate for controlling exposure to a substance that is potentially a skin sensitiser.
Undertaking of intended data submission [ ]	
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

Section A6.1.5 Annex Point IIA VI.6.1.5	Skin Sensitisation
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*05/10/07*
Evaluation of applicant's justification	
Conclusion	The UK CA accepts the Applicants justification for non-submission of further skin sensitisation studies.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2/02 **Annex Point IIA6.2**		**Toxicokinetic study** Metabolic fate of ^{14}C-acrolein orally administered to laying hens	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Berge, M.A. & Hennes, M. (1996) Metabolic Fate of ^{14}C-Acrolein Orally Administered to Laying Hens. Hazleton Wisconsin, Inc. Laboratory Project ID HWI No. 6318-103	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US EPA 171-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	Non- radiolabelled: 060889-89446A Radiolabelled: 119F9242	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	
3.1.2.4	Radiolabelling	^{14}C $CH_2 = CH - CHO$	
3.2	**Test Animals**		
3.2.1	Species	Hens	
3.2.2	Strain	White Leghorn	
3.2.3	Source	Milton Waldbaum Company, Hudson, Colorado	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: 9 months Weight: 1512 g	
3.2.6	Number of animals	14 laying females	
3.2.7	Control animals	Yes	X

Section A6.2/02 **Annex Point IIA6.2**		**Toxicokinetic study** Metabolic fate of ^{14}C-acrolein orally administered to laying hens	
3.3	**Administration/ Exposure**	Oral	X
3.3.1	Preparation of test site		
3.3.2	Test material administration	Oral gavage Five days a week	X
3.3.3	Bodyweight	Start of acclimation period, prior to first dose, and at termination	
3.3.4	Clinical signs	Yes, twice daily for 15 days	
3.3.5	Sample collection	Eggs and excreta: Predose eggs and excreta collected over a 24 hour period during acclimation. After first dose, eggs were collected twice daily and counted. The eggs whites were separated from egg yolks. Volatile sampling: Predose samples collected over a 10 hour period during acclimation. Six hens were monitored for expired ^{14}C-volatiles by using modified indirect calorimetry chamber system. After the third dose, six hens were placed in a respiration chamber for 10 hours. The air from the chamber was pumped through a flow meter that recorded a total air flow. After the flow meter, the scrub fraction (0.864%) was pumped through a second flow meter and then through two carbon dioxide scrubbers, each containing 200ml of R.J Harvey Carbon 14 Cocktail. The collection solutions were removed from the scrubbers and were transferred to glass bottles and stored frozen. Tissues collected at termination: Blood, liver, kidney, breast muscle, thigh muscle fat, and gastrointestinal tract with its contents.	X
3.3.6	Sample Preparation for Radioanalysis	Blood, tissue and eggs were homogenised Excreta was homogenised with water	
3.3.7	Sampling time		
3.3.8	Samples	Egg yolk, egg white, excreta, fat, kidney, liver muscle, breast and thigh muscle	
3.3.9	Tissues retained for radioanalysis		
		4 RESULTS AND DISCUSSION	
4.1	**Excretion studies**		
4.2	**Distribution**	Acrolein was almost entirely metabolised to small endogenous molecules that enter standard metabolic pathways of the hen to produce incorporation into natural products. The small molecules would enter into energy metabolism to product Carbon dioxide which also can be incorporated into natural products.	

Section A6.2/02 **Annex Point IIA6.2**		**Toxicokinetic study** Metabolic fate of ^{14}C-acrolein orally administered to laying hens	
4.3	**Recovery of labelled compound**	^{14}C-Acrolein was not found in any matrix. In egg yolk (1.25ppm), the fatty acids represented 58.8% of the total radioactive residue (TRR) with lesser amounts of radioactivity present as glycerol (3.84%TRR), and cholesterol (3.83% TRR). A minor residue was identified as allantoin (0.14%). Hydrolysis of the egg yolk residue showed incorporation of radioactivity into amino acids. The amino acids from residue hydrolysis and free amino acids represented 9.50% TRR. The major metabolites identified in egg white (0.127ppm) were amino acids (26.55%). Also identified were minor amounts (2%) of lactic acid, glyceric acid, and 1,3-propanediol. The fat (0.137 ppm) was extracted and saponified to give a fatty acid fraction (54.87% TRR) and an aqueous fraction that yielded glycerol (9.72% TRR). In kidney (0.839 ppm), the major metabolise were amino acids (14.31% TRR), with lesser amounts of fatty acids (4.31% TRR) and cholesterol (2.05% TRR). In the liver (0.731 ppm), amino acids and fatty acids represented 15.16% TRR and 12.39% TRR, respectively with minor amounts of cholesterol (3.21% TRR)) and creatine (2.62% TRR). In breast muscle (0.091 ppm), lactic acid and amino acids were the major residues with 16% TRR and 11.12% respectively, as well as 11.61% TRR and 10.75% TRR, respectively in the thigh muscle (0.135ppm).	X
4.4	**Percutaneous absorption**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was conducted in accordance to US EPA, Section 171-4. . The hens were acclimatised for 31 days. Hens were divided into two dosing groups: Control = four hens, dose of 1.09 mg/kg bw/day (equivalent to 4.8 ppm in the drinking water) = 10 hens. The hens were weighed at the start of acclimation period, just before the first dose, and at termination. Each dose was administered by oral gavage. Each hen was dosed once daily for 5 days, after the morning feed. Control dosing solution consisted of 0.5% DMF/4% ACD/0.01% hydroquinone in distilled water. The control hens were given 3.0-3.9 ml of this solution per day. Total ^{14}C-residue levels were determined in the tissue, egg, excreta, gastrointestinal tract, and volatile samples. The extracts from eggs, tissues, and excreta were analysed by thin layer chromatography. Ion-exchange chromatography was used to classify the ^{14}C-residues present in the aqueous fractions of the tissues and eggs.	X

Section A6.2/02 **Annex Point IIA6.2**	**Toxicokinetic study** Metabolic fate of ^{14}C-acrolein orally administered to laying hens	
5.2 Results and discussion	Acrolein did not affect body weight of the hens. With the exception of one hen, acrolein dosing had no adverse effects on the feed and water consumption. All hens maintained normal egg production during the treatment period.	X
5.3 Conclusion		X
5.3.1 Reliability	2	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*06/04/06*	
Materials and Methods	*3.1 Acrolein* *3.2.7 Four control hens were used.* *3.3.2, 5.1 Dosing was once daily for 5 days in drinking water.* *3.3 10 Females were exposed to 4.8ppm (equivalent to 1.09 mg/kg/d) for 5 days. Preliminary studies showed that 15 and 75ppm dose levels caused toxic effects.* *3.3.5 Hens were killed 12-14 hours after the 5th daily dose of acrolein.*	
Results and discussion	*4.3 The following should also be included in this section:* *Accountability of the administered dose was as follows: eggs, 0.5%; tissues, 1.1%; GI tract 2.8%; excreta 66.3%; and volatiles 4.6%. A total of 75% of the administered dose was recovered and is considered to be acceptable for this type of study.* *Most of the radioactivity appeared in the excreta, with very little radioactivity in the eggs.* *5.2 The following should also be included in this section:* *The levels of radioactivity incorporated into glycerol, fatty acids, cholesterol, amino acids, glyceric acid, and lactic acid suggest that the 3-carbon unit of acrolein is metabolised to a form which can be readily converted to form endogenous molecules that enter the standard metabolic pathway of the hen.*	
Conclusion	*5.3 Acrolein is metabolised into materials associated with intermediary metabolism: no major metabolites were found in tissues and eggs of laying hens.*	
Reliability	*1*	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ...	

Section A6.2/02 **Annex Point IIA6.2**	**Toxicokinetic study** Metabolic fate of ^{14}C-acrolein orally administered to laying hens	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.2/03 **Annex Point IIA6.2**		**Toxicokinetic study** **Metabolic fate of acrolein orally administered to lactating goats**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Berge, M.A. & Paust, D (1996) Metabolic Fate of ^{14}C-Acrolein Orally Administered to Lactating Goats. Hazleton Wisconsin, Inc. Laboratory Project ID HWI No. 6318-104	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US EPA 171-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	Non- radiolabelled: 060889-89446A Radiolabelled: 070H9208	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2.	
3.1.2.3	Stability	See 3.1.2.	
3.1.2.4	Radiolabelling	^{14}C or other * * $CH_2 = CH - CHO$	
3.2	**Test Animals**		
3.2.1	Species	Goats	
3.2.2	Strain		
3.2.3	Source		
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Goat 1 = 51.75 kg Goat 2 = 65.0 kg	
3.2.6	Number of animals per group	2 females	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	

Section A6.2/03 Annex Point IIA6.2		Toxicokinetic study Metabolic fate of acrolein orally administered to lactating goats	
3.3.1	Preparation of test site		
3.3.2	Test material administration	Oral gavage Goat 1: Dosed for 5 days Goat 2: Dosed for 4 days	
3.3.3	Bodyweight	Day 0 and termination	
3.3.4	Clinical signs	Once daily	
3.3.5	Sample collection	Milk, faeces and urine Predose samples of milk, urine and faeces were collected over 24 hours during acclimation. Each goat was milked each morning and evening. Total Faeces and urine were collected from each treated goat once daily. Each bulk sample was weighed and stored frozen. The total weight of urine collected was weighed. Volatile sampling The goats were monitored for expired ^{14}C-volatiles by using a modified indirect calorimetry chamber system. Predose samples were collected over a 10 hour period during acclimation. After the first dose of the second day, the treated goats were placed in individual respiration chamber for 10.9 hours. The collection solutions were removed from the scrubbers and were transferred to glass bottles. These were stored frozen.	
3.3.6	Sample Preparation for Radioanalysis	Tissues: Samples were homogenised. Blood, gut content, milk, urine and volatile samples: samples were homogeneous and did not require further processing Faeces: Homogenised with water.	
3.3.7	Sampling time	Milk : Predose sample collected 24 hrs during acclimation. Milk collected morning and evening. Faeces and urine: Once daily Volatile sample: Predose 10 hour period, After first dose of the second day.	
3.3.8	Samples	Milk, urine, faeces, exhaled air Tissues: Blood, liver, kidneys, skeletal muscle, composite fat, contents of stomach, small intestine, large intestine.	
3.3.9	Tissues retained for radioanalysis	Blood, Liver, kidneys, skeletal muscle, composite fat, contents of stomach, small intestine, large intestine.	
		4 RESULTS AND DISCUSSION	
4.1	**Excretion studies**	The percentage recovery of the total dose in urine was 9.8% for goat 1 and 8.5% for goat 2. In faeces the percentage recovery of total dose was 14.9% in goat 1 and 5.5% in goat 2. Studies indicated that the residues in urine were polar and water- soluble. There were no glucuronide or sulfate conjugates present. Acid hydrolysis of urine resulted in the disappearence of a major metabolite indicating that at least one metabolite is acid-labile, possibly a conjugate. Treatment with glutathione and Raney nickel produced no significant effect	X

Section A6.2/03 Annex Point IIA6.2	Toxicokinetic study Metabolic fate of acrolein orally administered to lactating goats	
	indicating the absence of reducible disulfide linkages. The metabolites did not correspond to the cysteine conjugates already identified. The study indicated that the major metabolites found in the liver, kidney and muscles may also be present in urine. Extraction and identification of metabolites in faeces was poor and definitive quantitation results were not obtained.	
4.2 Distribution	Tissue residue levels were as follows: Goat 1: Liver (9.1 ppm), kidney (1.7 ppm), muscle (0.4 ppm) and fat (0.2 ppm). For both goats the residue levels were high in the milk (upto 10 ppm). A high percentage of the radioactive dose was released as volatiles (14.2% for goat 1 and 12.5% for goat 2).	
4.3 Recovery of labelled compound	Sample: Milk Urine Faeces Tissues Gut Volatiles % Recovery of Total Dose Goat 1: 22.9 9.8 14.9 8.0 6.6 14.2 Goat 2: 13.0 8.5 5.5 6.4 15.4 61.3 Sample: Total % Recovery of Total Dose: Goat 1: 76.4 Goat 2: 61.3	
4.4 Percutaneous absorption	Not applicable	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The study was conducted in accordance to US EPA, Section 171-, subdivision F. Two goats were dosed orally with ^{14}C-acrolein at a nominal treatment level of 7.5 ppm, based on water consumption during the acclimation period. A control goat was used in the study. Dosing was once daily by oral intubation at 7.5 ppm. Goat 1 was dosed for 5 days. Goat 2 was only dosed for 4 days due to toxic effects due to acrolein. Goat 1 was killed 12 hours after its final dose, whereas goat 2 was killed 34 hours after its final dose. Total ^{14}C- residues levels were determined in the tissue, milk, urine, faeces, gut content, and volatiles samples for both goats. Due to the toxic effects exhibited by goat 2, the results were not considered representative. All metabolism work was carried out using samples from goat 1. Milk and tissues were extracted and analysed by thin layer chromatography (TLC). For lyophilized milk, approximately 86% of the total ^{14}C-residue was extracted with methanol; for whole milk, approximately 77 - 84% was extracted.	

Section A6.2/03 **Annex Point IIA6.2**	**Toxicokinetic study** **Metabolic fate of acrolein orally administered to lactating goats**	
5.2 Results and discussion	There was no effect on body weight during the study. During the treatment period, both feed and water consumption declined for goat 2 and control. The milks production also decreased for both of the goats. Neither radiolabelled acrolein nor any of the potential conjugated metabolites were found in any matrix. All of the identified ^{14}C-residues were the result of the incorporation of radioactivity into natural products of goat metabolism. In milk, lactose represented 79.28% of the TRR with lesser amounts of radioactivity present in the lipid fractions and the casein fraction. Hydrolysis of the casein fraction showed incorporation of radioactivity into amino acids. The major metabolite in liver was glucose. Lesser amounts of lactic acid, and a residue that could be hydrolysed to glucose that appeared to contain glycogen and glucose-1-phophates and glucose-6-phosphates, as well as incorporation into triglycerides and protein. The majority of the radioactivity in the triglyceride fraction was found as glycerol after saponification although incorporation into fatty acids was also seen. An extract of fat was saponified to give a aqueous fraction which yielded glycerol and a fatty acid fraction. Because of the low specific activity of the fatty acid fraction, it was not possible to demonstrate incorporation into specific fatty acids. In muscle, lactic aid was the major residue. Minor amounts of glyceric acid and oxalic acid were also found. In kidney, the major metabolites were amino acids, either free or in proteins, as well as creatinine, hydantoin/allantoin and uric acid. The levels of radioactivity incorporated into glycerol, glucose and lactic acid suggest that the very reactive acrolein is metabolised to a 3-carbon unit, which can be readily converted to similar endogenous molecules that enter the standard metabolic pathways of the goat. The residues enter the energy metabolism pathways that produce carbon dioxide, which would be incorporated into additional natural products. The goat has rich and diverse gut flora that also could contribute to the metabolism of acrolein and the production of the 3- carbon precursors needed for biosynthesis of natural products.	
5.3 Conclusion	The levels of radioactivity incorporated into glycerol, glucose and lactic acid suggest that acrolein is metabolised into a 3-carbon unit, which can be readily converted to similar endogenous molecules that enter the standard metabolic pathways of the goat. These pathways represent the TCA cycle, gluconeogenesis, glycolysis, lipid synthesis and the urea cycle. Residues also enter the energy metabolism pathways that produce CO_2. As a ruminent, the goat has a rich and diverse gut flora that would have contributed to the metabolism of acrolein.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

Section A6.2/03 **Annex Point IIA6.2**	**Toxicokinetic study** **Metabolic fate of acrolein orally administered to lactating goats**	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	06/04/06	
Materials and Methods	As described by the Applicant.	
Results and discussion	4.1 The percentage recovery of the total dose in urine was 14.2% for goat 1 and 61.3% for goat 2.	
Conclusion	In addition: Acrolein was widely distributed, with high levels found in the milk, indicating that acrolein or metabolites may be transferred to milk.	
Reliability	1	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_2-1. Table for Metabolic Fate Study

	Labelled compound			
	Absolute amount (ppm)		% of dose	
Sample collection	Goat 1	Goat 2	Goat 1	Goat 2
1. Gut contents			6.6	15.4
2. Urine			9.8	8.5
3. Faeces			14.9	5.5
4. Milk			22.9	13.0
5. Removed organs				
Liver	9.1			
Kidney	1.7			
Muscle	0.4			
Fat	0.2			
			8.0	6.4
6. Remaining carcass			Not measured	Not measured
7. Exhaled air			14.2	12.5
Sum of #2 – 6: blood, excreta, removed organs, remaining carcass (= absorption)			69.8	45.9
Sum of all detected labelled compound (#1 – 7) (=recovery)			76.4	61.3

The substance was dosed via the oral route.

Section A6.2/04 **Annex Point IIA6.2**		**Toxicokinetics study** Metabolism of ^{14}C-acrolein in Rats (Preliminary and Definitive Phases)	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Sharp, D.E. (1991a,b) Metabolism of Acrolein in Rats (Preliminary and Definitive Phases). Hazleton Laboratories America, Inc. Laboratory Project ID HLA No. 6318-101.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EPA Metabolism Guidelines, Subdivision F, Section 85-1	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	Non- radiolabelled: 119F9242 Radiolabelled: 060889-89446	
3.1.2	Specification	Deviating from specification given in section 2 as follows: Radiolabelled	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	Purity: 96.1% Radiochemical purity: 93%	
3.1.2.3	Stability	See 3.1.2	
3.1.2.4	Radiolabelling	^{14}C or other $CH_2 = CH - CHO$	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague Dawley Crl:CD BR	
3.2.3	Source	Charles River Laboratories, Portage, Michigan	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Age: 5 to 9 weeks Weight: 125 – 200g	
3.2.6	Number of animals per group	Preliminary study: 2 male, 2 female Definitive study: 5 animals/sex/group	X

Section A6.2/04 **Annex Point IIA6.2**	**Toxicokinetics study** Metabolism of ^{14}C-acrolein in Rats (Preliminary and Definitive Phases)	
	Group 1 Phase: Preliminary Dose level: 2.5 mg/kg Dosing route: Oral[a] Number of males: 2 Number of females: 2 Group 2 Phase: Definitive Dose level: 2.5 mg/kg Dosing route: IV[a] Number of males: 5 Number of females: 5 Group 3 Phase: Definitive Dose level: 2.5 mg/kg Dosing route: Oral[a] Number of males: 5 Number of females: 5 Group 4 Phase: Definitive Dose level: 2.5 mg/kg Dosing route: Oral[b] Number of males: 5[c] Number of females: 5[c] Group 5 Phase: Definitive Dose level: 15 mg/kg Dosing route: Oral[a] Number of males: 5 Number of females: 5 IV = Intravenous	

Section A6.2/04 **Annex Point IIA6.2**		**Toxicokinetics study** Metabolism of ^{14}C-acrolein in Rats (Preliminary and Definitive Phases)	
		a Single dose b Fourteen daily nonradiolabelled doses, followed by a single radiolabelled dose on the 15th day c Seven animals per sex were dosed with non-radiolabelled material for 14 days	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Oral and intravenous	
3.3.1	Preparation of test site		
3.3.2	Test material administration	Oral gavage and intravenous injection into the tail vein The dose amount was based on individual body weight. After being dosed with the radiolabelled test material, the treated animals were housed in individual glass metabolism chambers for the collection of expired air and the separation and collection of urine and faeces.	
3.3.3	Bodyweight	Day 0, 8, 15	X
3.3.4	Clinical signs	Twice daily for 15 days	
3.3.5	Sample collection	Expired carbon dioxide was trapped in a solution of ethanolamine:ethoxyethanol. Activated charcoal, was used to trap organic volatiles. These were collected at 4, 8, 12 and 24 hours. Urine and faeces samples were collected at 4,8,12 and 24 hours. Blood (2 to 5ml) was collected and weighed in heparinised tubes and saved for radioanalysis.	X
3.3.6	Sample Preparation for Radioanalysis	Tissues: Samples were homogenised and duplicate aliquots were weighed for combustion. Blood: Whole blood samples were homogenised by inverting several times and weighed for combustion. Faeces: Homogenised with water and weighed for combustion. Samples of carbon and urine were analysed by direct LSC.	
3.3.7	Sampling time	6 hours and 24 hours after initiation of skin contact, other time points possible.	X
3.3.8	Samples	Urine, faeces, exhaled air, organs, carcass, skin with substance not removable, liquid used for washing the skin, protective appliances	X
3.3.9	Tissues retained for radioanalysis	Adrenals, bone (femur), brain, fat, ovaries, testes, heart, large intestines and contents, liver, kidneys, lungs, muscle (thigh), pancreas, pituitary, spleen, stomach and contents, small intestine and contents, uterus, urinary bladder, residual carcass.	
		4 RESULTS AND DISCUSSION	

Section A6.2/04 Annex Point IIA6.2	Toxicokinetics study Metabolism of ^{14}C-acrolein in Rats (Preliminary and Definitive Phases)	
4.1 Excretion studies	Preliminary studies, Group 1 - 2.5 mg/kg The major route of excretion was via the kidneys. Urine contained 33.5% of the total dose for males and 41.4% for females. Male rats excreted 31.7% of the dose in faeces, compared with 23.9% for females when a single oral dose of 2.5mg/kg was given. Total radioactivity in expired air was 36.6% for males and 38.5% for females. The majority of radioactivity was eliminated within the 24 hours after dosing. Single intravenous, Group 2 – 2.5 mg/kg The overall recovery was 101% for both sexes. Urine contained an average of 66.6% of the total dose for males and 69.3% for females. Total radioactivity in expired air was 26.7% for males and 26.9% for females. Male rats excreted 1.92% of the dose in faeces, compared with 1.42% for females. The majority was eliminated within the first 48 hours after dosing. Single oral low dose, Group 3 – 2.5 mg/kg The overall recovery was 111% for males and 98.8% for females. Urine contained 63.4% of the dose for males and 52.2% for females. Total radioactivity in expired air was 30.3% for males and 31.5% for females. Males rats excreted 14.8% of the dose in faeces compared with 12.8% for females. Multiple oral low dose, Group 4 – 2.5 mg/kg The overall recovery was 101% for both males and females. Urine contained 55.9% if the dose for males and 55.7% for females. Total radioactivity in expired air was 29.7% for males and 31.5% for females. Male rats excreted 12.6% of the dose in faeces compared with 11.9% for females. Tissues and carcass contained a total of (2.60%) male and 1.96% (female). Single oral high dose, Group5 – 15 mg/kg The total mean recovery was 98.8% for males and 96.6% for females. Urine contained 40.6% of the dose for males and 36.5% for females. Total radioactivity in expired air was 27.45% for males and 27.66% for females. Male rats excreted 28.4% of the dose in faeces compared with 30.6% for females. Tissues and carcass contained a total of (2.35%) male and 1.86% (female).	X

Section A6.2/04 Annex Point IIA6.2	Toxicokinetics study Metabolism of ^{14}C-acrolein in Rats (Preliminary and Definitive Phases)	
4.2 Distribution	Single Intravenous, Group 2 – 2.5 mg/kg The highest concentration was found in kidneys, 0.410 ppm in males and 0.231 ppm in females. Other organs where acrolein was found was spleen, approximately 0.4 ppm in males and females, lungs, approximately 0.25 ppm for both sexes. The mean radioactivity concentration in the blood at sacrifice was 0.636ppm for males and 0.656ppm for females. The mean residue concentration in the ovaries was 0.073ppm. The highest dose percentage of dose recovered in tissues was in the residual carcass for males (4.47%) and females (2.46%). The mean percent of dose in the remaining tissues was 0.67% for males and 0.58% for females. Single oral low dose, Group 3 – 2.5 mg/kg The highest concentration was found in the liver, 0.165 ppm in males and 0.214 ppm in females. Other organs included fat, 0.104 ppm in males and 0.078 ppm in females, and stomach contained 0.064 ppm in males and 0.074 ppm in females. The mean radioactivity concentration in the blood at sacrifice was 0.071 ppm for males and females. The mean radioactivity concentration in the ovaries was 0.04 ppm. The highest mean percent of dose was recovered in the residual carcass for male (2.06%) and females (1.78%). The percentage of dose remaining tissues was 0.22% for both sexes. Multiple Oral low Dose, Group 4 – 2.5 mg/kg The highest concentration, 0.211 ppm, was found in liver. Other organs included adrenals (0.094 ppm in males and 0.097ppm in females) and fat, (0.096 ppm in males and 0.089 ppm in females). The mean radioactivity concentration in the ovaries of females rats was 0.19 ppm. The mean radioactivity concentration in blood at sacrifice was 0.049 ppm for males and 0.058 ppm for females. The highest percent dose recovered in the residual carcass for both males (2%) and females (1.47%). The mean percent of dose in the remaining tissues was 0.18% for males and 0.22% for females. Single oral high dose, Group 5 – 15 mg/kg Majority of the residue was found in liver, (0.936 ppm) for males, (1.19 ppm) for females. Kidneys contained 0.904 ppm in males and 0.552 ppm for females. Adrenals contain 0.799ppm in males and 0.860ppm in females. The mean residue concentration in the ovaries of female rats was 0.249 ppm. The mean concentration in blood at sacrifice was approximately 0.5 ppm for both sexes. The highest dose recovered in residual carcass for males (1.72%) and females (1.24%). The liver contained the next highest percent of dose, with 0.35% for males and 0.37% for females. The mean percent of dose in the remaining tissues was 0.28% for males and 0.25% for females.	X
4.3 Recovery of labelled compound		
4.4 Percutaneous absorption		

Section A6.2/04 **Annex Point IIA6.2**		**Toxicokinetics study** Metabolism of ^{14}C-acrolein in Rats (Preliminary and Definitive Phases)	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was conducted in accordance to EPA Metabolism Guidelines, Section 85-1, subdivision F. The metabolism and disposition of acrolein were studied in rats. The 48 treated animals in this study were divided into five groups. A preliminary group (Group 1) of four animals (2/sex) were dose orally at 2.5 mg/kg to determine whether expired carbon dioxide and organic volatiles needed to be collected in the definitive study. The remaining 44 animals were divided into three groups of 10 animals (5/sex) and one group of 14 animals (7/sex). A single intravenous low dose group (Group 2) at 2.5 mg/kg, a single oral low dose group (Group 3) at 2.5 mg/kg, a multiple oral low dose group (Group 4) at 2.5 mg/kg (14 daily nonradiolabelled doses followed by a single oral high dose given to 10 animals (5/sex) on the 15th day), and a single oral high dose group (Group 5) at 15 mg/kg. All groups had urine, faeces, expired carbon dioxide and organic volatiles collected. The animals were sacrificed 7 days after administration of the radiolabelled dose, and various tissues were collected and analysed for total radioactivity.	X
5.2	**Results and discussion**	Acrolein was well absorbed after oral administration. The percentage of absorption was estimated by comparing the percentages of the radioactive dose found in various matrices after oral dosing, with those found in the same matrices after intravenous dosing. Using the urine as a basis for comparison, absorption values of 95.2% for males and 75.3% for females are obtained for a single oral dose of 2.5 mg/kg. The corresponding values for Groups 4 and 5 are 83.9% for males and 80.45 for females in Group 4, and 61.0% for males and 52.7% for females in Group 5. This indicates that absorption is reduced at the 15 mg/kg dose relative to the 2.5 mg/kg dose, as is also indicated by the increased percentage of the dose found in the faeces. The use of expired radiolabelled carbon dioxide to estimate absorption provides estimates of the percentage absorbed of 114% for males and 115% for females in Group 3, 112% for males and 116% females in Group 4, and 103% for males and 102% for females in Group 5. These values are higher than the values obtained from urine and are in excess of 100%. A possible explanation for this is that some organic volatiles are being trapped in the carbon dioxide traps after oral dosing but not after intravenous dosing. Tissues residues were very low after a single oral dose of 2.5 mg/kg. Nearly all the tissues showed low levels of radioactivity, as would be expected from a compound largely metabolised to carbon dioxide. There was no apparent increase in tissue concentrations upon repeated dosing. Tissue residues were increased by greater than 6-fold compared to the 2.5 mg/kg dose when a single 15 mg/kg oral dose was given. This indicates that a dose-dependent phenomenon may be occurring.	X
5.3	**Conclusion**		X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A6.2/04 **Annex Point IIA6.2**	**Toxicokinetics study** Metabolism of ^{14}C-acrolein in Rats (Preliminary and Definitive Phases)	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*05/04/06*	
Materials and Methods	*2.1 Conducted to OECD test guideline 417* *3.1 Test material should be Acrolein* *3.2.6 The preliminary study was to determine whether expired CO_2 and organic volatiles needed to be collected in the definitive study.* *Only 5 animals have been reported for group c, not 7 as stated here.* *3.3.3 Bodyweight was measured on day 0 for Groups 1,2,3 and 5 and also on days 8 and 15 for Group 4.* *3.3.5 Expired carbon dioxide and urine and faeces samples were also collected daily for 7 days post dosing.* *3.3.7 Samples were collected for 4, 8, 12 and 24 hours (12 and 24 hours for Group 1) after radiolabelled dose, and then daily for 7 days, not 6 and 12 hours after skin contact.* *3.3.8 Blood was also collected.* *In addition the urine from Groups 2, 3, 4 and 5 and the faeces from Groups 3, 4 and 5 were pooled by sex and collection time and analysed by HPLC for the presence of metabolites.*	

Section A6.2/04 Annex Point IIA6.2	**Toxicokinetics study** Metabolism of ^{14}C-acrolein in Rats (Preliminary and Definitive Phases)	
Results and discussion	*4. Data regarding absorption and metabolism should also be included in this section.* *Absorption data to be taken from section 5.2.* *Metabolism: Six urinary metabolites were found; five were identified as oxalic acid (non-detectable in the i.v. groups), malonic acid, glyceric acid, N-acetyl-S-3-hydroxypropylcysteine, and N-acetyl-S-2-carboxyethylcysteine. One metabolite was unknown. There was no apparent difference in metabolism between single and multiple or low or high dosed animals. No discrete metabolites were present in the faeces. The major metabolic pathways are likely to involve oxidation/hydrolysis and glutathione conjugation. The concordance of metabolites observed after oral and i.v. administration indicates that first-pass metabolism does not occur.* *4.1 The majority of radioactivity was eliminated within the first 48 hours of dosing for Groups 3, 4 and 5.* *4.2 The following concentrations of acrolein reported in ppm are given as percentages for males and females, respectively:* *Group 2:Liver (0.26, 0.26%), kidney (0.13, 0.07%), spleen (0.04, 0.04%), lung (0.04,0.06%), blood (0.143, 0.54%), ovary (<0.01%), testis (0.02%).* *Group 3: Liver (0.33, 0.36%), fat (0.03, 0.03%), stomach (0.02, 0.02%), blood (0.06, 0.07%), ovaries (>0.01%), testis (0.02%).* *Group 4: Liver (0.41, 0.2%), adrenals (<0.01, <0.01%), fat (0.03, 0.03%), blood (0.03, 0.05%), ovaries (<0.01%), testis (0.02%).* *Group 5: Liver (0.35, 0.37%), kidneys (0.05, 0.03%), adrenals (<0.01, <0.01%), blood (0.07, 0.08%), ovaries (<0.01%), testis (0.02%).* *5.1 The absorption and excretion of acrolein were also studied in rats. The group sizes were one group of 2/sex and 4 groups of 5/sex.* *5.2 The first and second paragraphs should be included in section 4.* *5.2 The text in this section should be replaced by the following:* *Acrolein is well absorbed after oral administration (75 to 95%). A slight decrease in absorption occurs with increasing dose (52 to 61%).* *Acrolein was biotransformed into six urinary metabolites. There was no apparent difference in metabolism between single and multiple or low or high dosed animals. Radioactive carbon dioxide was also present in exhaled air. No discrete metabolites were present in the faeces, but there is evidence that acrolein can polymerise during passage through the GI tract although it is likely that this was derived from unabsorbed material. The major metabolic pathways are likely to involve oxidation/hydrolysis and glutathione conjugation. Less than 2.3% of an oral dose was present, widely distributed, in the tissues at 7 days post administration, indicating that acrolein has limited potential for bioaccumulation.* *The main concentration of acrolein after dosing was found in the liver in all dose groups, apart from the i.v. dosed group where the highest concentration was found in the kidney. Acrolein was also distributed to fat, spleen, lung, liver, stomach, the kidneys, and adrenal glands. A small amount of acrolein was deposited in the ovaries (slightly higher concentration for high and repeated dose groups) and in blood (slightly higher in the high dose group and, as to be expected in the blood).* *The major route of excretion is via the urine (33 to 63%), with a large proportion being exhaled (27 to 38%). Excretion via the faeces is minimal after i.v. administration (2%), however, a greater amount is excreted via this route after oral administration (12 to 32%). This suggests that biliary excretion is minor, and the amount determined in faeces after oral dosing is due to non –absorption. Excretion via the faeces was increased in the 15mg/kg dose group and relates to the decreased absorption at this dose. The majority of acrolein and/or its metabolites was excreted within 48 hours of dosing via the oral or i.v. route.*	

Section A6.2/04 **Annex Point IIA6.2**	**Toxicokinetics study** Metabolism of ^{14}C-acrolein in Rats (Preliminary and Definitive Phases)	
Conclusion	Other conclusions: *5.3 Acrolein is well absorbed and rapidly excreted and after oral administration distribution is widespread. The major metabolic pathways are likely to involve oxidation/hydrolysis and glutathione conjugation. The major route of excretion is via the urine, with large amounts also being exhaled as CO_2.* *No differences occurred between sexes.*	
Reliability	*1*	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_2-1. Table for absorption (in vivo test)

		labelled compound				
		% of dose				
Compound applied		100				
Compartments with compound detected		1	2	3	4	5
1. Protective appliances						
2. Liquid used for washing the skin						
3. Skin (with substance not removable)						
4. Blood (mean)	M	0.05				
	F	0.07	-	-	-	
5. Carcass (mean)	M	3.02	4.47	2.06	2.00	1.72
	F	2.56	2.46	1.78	1.47	1.24
6. Urine	M	33.6	66.6	63.4	55.9	40.6
	F	41.4	69.3	52.2	55.7	36.5
7. Faeces	M	31.7	1.92	14.8	12.6	28.4
	F	23.9	1.42	12.8	11.9	30.6
8. Carbon dioxide	M	36.5	26.4	30.1	29.5	27.3
	F	38.5	26.6	31.0	31.3	27.4
9. Exhaled air	M	0.13	0.33	0.17	0.16	0.15
	F	0.13	0.25	0.55	0.22	0.26
10. Tissues	M	Nd	1.12	0.55	0.60	0.63
	F	Nd	1.12	0.58	0.49	0.62
Sum of #4 – 9: blood, excreta, removed organs, remaining carcass **(= absorption)**						
Sum of all detected labelled compound (#1 – 9) (=recovery)						

Group 1 = Rats dosed orally with 2.5mg/kg of ^{14}C-acrolein dissolved in deionised water

Group 2 = Rats dosed intravenously with 2.5 mg/kg of ^{14}C-acrolein dissolved in saline

Group 3 = Rats dosed orally with 2.5 of ^{14}C-acrolein dissolved in deionised water

Group 4 = Rats dosed orally fourteen days with 2.5 mg/kg acrolein followed by an oral dose of 2.5 mg/kg of ^{14}C-acrolein dissolved in deionised water

Group 5 = Rats dosed orally with 15 mg/kg of ^{14}C-acrolein dissolved in deionised water

Table A6_2-2. Table for faecal elimination (in vivo test)

	Labelled compound									
	% of dose									
Compound applied	100									
Compartments with compound detected	1		2		3		4		5	
Sampling time (hours post dose)	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
0-4	-	-	<0.01	<0.01	ND	0.00	ND	0.03	ND	<0.01
4-8	-	-	0.05	0.10	NS	NS	<0.01	NS	<0.01	NS
8-12	17.1	3.58	0.08	0.27	4.95	3.31	2.93	2.24	<0.01	NS
12-24	11.9	16.4	0.49	0.30	7.29	6.54	6.69	6.34	2.23	5.84
24-48	1.97	2.83	0.40	0.29	1.62	2.12	2.18	2.06	21.1	18.5
48-72	0.39	0.41	0.20	0.16	0.47	0.38	0.42	0.65	4.14	5.24
72-96	0.17	0.27	0.19	0.07	0.19	0.24	0.14	0.27	0.48	0.60
96-120	0.05	0.18	0.21	0.07	0.22	0.05	0.05	0.14	0.22	0.23
120-144	0.05	0.15	0.17	0.07	0.05	0.02	0.11	0.11	0.14	0.15
144-168	0.03	0.06	0.11	0.09	0.04	0.03	0.03	0.10	0.08	0.08

Group 1 = Rats dosed orally with 2.5mg/kg of ^{14}C-acrolein dissolved in deionised water

Group 2 = Rats dosed intravenously with 2.5 mg/kg of ^{14}C-acrolein dissolved in saline

Group 3 = Rats dosed orally with 2.5 of ^{14}C-acrolein dissolved in deionised water

Group 4 = Rats dosed orally fourteen days with 2.5 mg/kg acrolein followed by an oral dose of 2.5 mg/kg of ^{14}C-acrolein dissolved in deionised water

Group 5 = Rats dosed orally with 15 mg/kg of ^{14}C-acrolein dissolved in deionised water

NS No sample

ND Not detectable

Table A6_2-3. Table for renal elimination (in vivo test)

	Labelled compound									
	% of dose									
Compound applied	100									
Compartments with compound detected	1		2		3		4		5	
Sampling time (hours post dose)	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
0-4	-	-	8.21	1.85	21.2	12.00	30.2	17.4	0.28	2.62
4-8	-	-	29.7	36.7	20.1	21.90	14.60	10.7	0.07	4.51
8-12	25.9	35.3	7.09	7.44	9.82	7.06	3.84	8.51	4.02	6.75
12-24	5.1	3.49	9.24	6.82	7.85	5.20	4.58	8.66	23.1	13.1
24-48	1.13	1.37	5.74	8.06	2.10	2.38	1.61	5.16	9.83	5.23
48-72	0.36	0.43	2.46	3.29	1.02	1.52	0.47	2.32	1.25	1.83
72-96	0.27	0.27	1.47	2.16	0.32	0.63	0.18	1.04	0.54	1.00
96-120	0.12	0.08	0.81	0.93	0.23	0.40	0.11	.052	0.27	0.60
120-144	0.08	0.09	0.56	0.68	0.12	0.32	0.07	0.45	0.15	0.33
144-168	0.08	0.02	0.40	0.52	0.12	0.21	0.05	0.25	0.10	0.17
168 cage wash	0.06	0.40	0.88	0.65	0.36	0.40	0.23	0.59	0.19	0.37
168 cage wipe	0.01	0.02	0.06	0.19	0.12	0.07	0.01	0.13	0.03	0.05

Group 1 = Rats dosed orally with 2.5mg/kg of ^{14}C-acrolein dissolved in deionised water

Group 2 = Rats dosed intravenously with 2.5 mg/kg of ^{14}C-acrolein dissolved in saline

Group 3 = Rats dosed orally with 2.5 of ^{14}C-acrolein dissolved in deionised water

Group 4 = Rats dosed orally fourteen days with 2.5 mg/kg acrolein followed by an oral dose of 2.5 mg/kg of ^{14}C-acrolein dissolved in deionised water

Group 5 = Rats dosed orally with 15 mg/kg of ^{14}C-acrolein dissolved in deionised water

NS No sample

ND Not detectable

Section A6.2/05 Annex Point IIA6.2		Literature Review on Disposition and Metabolism of Acrolein in Various Species.	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Illing, H.P.A (2004) The disposition and metabolism of acrolein, hydroquinone and 3-hydroxypropenal: an updating literature review. PICS Paul Illing Consultancy Services.	
1.2	**Data protection**	No	
1.2.1	Data owner	n/a	
1.2.2	Criteria for data protection	n/a	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No. Guidelines not applicable to a literature review.	
2.2	**GLP**	n/a	
2.3	**Deviations**	n/a	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Acrolein, hydroquinone, 3-hydroxypropanal	
3.1.1	Lot/Batch number	n/a	
3.1.2	Specification	n/a	
3.1.2.1	Description	Liquid at room temperature	
3.1.2.2	Purity	Acrolein 96.3% water 2.98%, dimer 0.31%, hydroquinone 0.28%, acetone 0.09%, benzene 0.04%	
3.1.2.3	Stability	n/a	
3.1.2.4	Radiolabelling	^{14}C	
3.2	**Test Animals**		
3.2.1	Species	Rat, chicken and goat.	
3.2.2	Strain	Sprague Dawley, White leghorn, Nubian	
3.2.3	Source	n/a	
3.2.4	Sex	male/female	
3.2.5	Age/weight at study initiation	n/a	
3.2.6	Number of animals per group	n/a	
3.2.7	Control animals	n/a	
3.3	**Administration/ Exposure**	Oral and Intravenous	
3.3.1	Preparation of test site	n/a	

3.3.2	Concentration of test substance	n/a	
3.3.3	Specific activity of test substance	n/a	
3.3.4	Volume applied	n/a	
3.3.5	Size of test site	n/a	
3.3.6	Exposure period	n/a	
3.3.7	Sampling time	n/a	
3.3.8	Samples	n/a	
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	n/a	
4.2	**Dermal irritation**	n/a	
4.3	**Recovery of labelled compound**	Recovery of radioactive acrolein indicates that it is well absorbed when administered orally to rats, a lactating goat and laying hens.	
4.4	**Percutaneous absorption**	A QSAR study on the potential for skin absorption suggests that significant amounts of acrolein are likely to be absorbed through the skin.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The literature search is on the disposition, and metabolism of the components of acrolein.	
5.2	**Results and discussion**	***Acrolein*** Disposition: Comparisons of the amounts of radioactivity excreted following approximately equivalent oral and intravenous doses indicated that, in rats, approximately 85% is absorbed and approximately 80% of the absorbed radioactivity is excreted within 24 hours, principally in the urine but with some radioactive material being exhaled. Only very limited amounts of radioactivity were present in rat faeces and less than 2.5% of an oral dose was present, widely distributed, in the tissues at 7 days post dose. The study in lactating goat indicated that significant amounts of radiolabel (23%) could be found in milk, but the study in hen indicated that very little radioactivity was transferred to eggs. Metabolism: The studies in the rat identified the metabolites excreted in urine, faeces and exhaled air. The identified,metabolites were: N-acetyl-S-2-carboxy-2-hydroxyethylcysteine N-acetyl-S-3-hydroxypropylcysteine N-acetyl-2-carboxyethylcysteine 3-hydroxypropanoic acid	

		malonic acid oxalic acid carbon dioxide In addition there was evidence that acrolein had polymerised during passage through the gastro-intestinal tract, although it is likely that the polymeric material present in the faeces was derived from unabsorbed material. In the studies in goat and chicken the animals were sacrificed 12 hours after the last dose of radioactivity and the nature of the radioactivity present in tissues was examined. The retained radioactivity was widely distributed and had been extensively incorporated into intermediary metabolism. The very limited information on human metabolites suggests that acrolein metabolism is likely to be very similar in rats and humans. ***Hydroquinone*** Disposition: Hydroquinone disposition was studied in rat following intratracheal, oral and dermal administration of radiolabelled hydroquinone. Following intratracheal or oral administration it is rapidly and completely absorbed and rapidly and almost completely excreted in the urine. Hydroquinone was incompletely absorbed following dermal administration, with most of the excreted radioactivity appearing in the urine. Significant amounts of radioactivity were still present in the carcass 7 days after the dermal dose was administered. There are two studies on humans examining the disposition of hydroquinone following dermal administration of 2% of the substance. Approximately half of the administered substance was recovered in the urine. Metabolism: Hydroquinone is excreted in urine, predominantly as the glucuronide and the sulphate conjugates. Some hydroquinone is excreted unchanged, and in limited amounts of the mercapturic acid conjugate and of p-benzoquinone have also been detected. ***3-hydroxypropanal*** There is no useful information published on the disposition and metabolism of 3-hydroxypropanal.	
5.3	**Conclusion**		
5.3.1	Reliability	The retained radioactivity was widely distributed and had been extensively incorporated into intermediary metabolism. The very limited information on human metabolites suggests that acrolein metabolism is likely to be very similar in rats and humans.	
5.3.2	Deficiencies	n/a	
		Evaluation by Competent Authorities	
		Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		*06/04/06*	
Materials and Methods		*As described by the Applicant.*	
Results and discussion		*As described by the Applicant.*	

Conclusion	*The very limited information on human metabolites suggests that acrolein metabolism is similar in rats and humans.*
Reliability	*N/A*
Acceptability	*As described by the Applicant.*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2/01 **Annex Point IIA6.2**		**Percutaneous absorption** Evaluation of percutaneous absorption potential using quantitative structure-activity relationships	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Barratt, M.D. (2004) Evaluation of the Percutaneous Absorption Potential of Acrolein Using Quantitative Structure-Activity Relationships. Marlin Consultancy.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No QSAR Assessment	
2.2	**Deviations**	Acrolein is corrosive. An *in-vitro* dermal study was not performed because there will be no human exposure to the acrolein or the biocidal product by this route of exposure. The study would be scientifically unjustified and technically unfeasible due to the high vapour pressure of the active substance. The active substance could not be applied to the skin to accurately measure dermal penetration therefore a QSAR assessment was calculated.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.2	**Method**	Method 1 This method is a refinement by Barratt of the models published by Potts and Guy, using human in vitro skin permeability data published by Flynn. The prediction model for log [permeability coefficient] was derived using multiple regression analysis for 47 low molecular weight organic chemicals from the Flynn data set and is based on their calculated log [octanol/water partition coefficient] (log P), their computed molecular volumes (MV) and melting points. Log P values used in the model are calculated from the chemical structure using the fragment-based method (CHEMICALC) of Suzuki and Kudo. Molecular volumes were calculated in A3 using TSAR from three-dimensional molecular structures generated by Sybyl 6.0 (Tripos Associates). Log PC = 0.921 log P – 0.0157 MW – 0.00729 mpt – 1.762 The Pearson correlation coefficient for the model is 0.954, and the maximum difference between actual and predicted values within the training set is about 0.5 log units. The parameter space of the training set, i.e. that within which the model is valid, covers chemicals with calculated log P values in the range -2.25 to 4.00, molecular volumes in the range 16.75 to 267.4 A3 and melting points up to 187°C.	X

Section A6.2/01 Annex Point IIA6.2	Percutaneous absorption Evaluation of percutaneous absorption potential using quantitative structure-activity relationships	
3.3	Method 2 This method is that of Potts and Guy and is derived from human in vitro skin permeability data. Log PC = -2.72 + 0.71Log P – 0.0061 MW This equation uses measured logP values from the Flynn dataset and covers chemicals with molecular weights from 18 to over 750 and logP values ranging from -3 to +6 with a Pearson coefficient of about 0.8. The uncertainty of the predicted log PC values is estimated to be within plus or minus one order of magnitude from the best-fit value.	
	4 RESULTS AND DISCUSSION	
4.1 **Calculation of Log[octanol/water partition coefficient] for acrolein**	Fragment: CH_2 = Number: 1 Contribution: 0.477 Total: 0.477 Fragment: =CH-(CO)- Number: 1 Contribution: 0.718 Total: 0.718 Fragment: (=C)-CH=O Number: 1 Contribution: -1.264 Total: -1.264 The calculated log P value of -0.069 is in good agreement with the experimental value of 0.04.	
4.2 **Calculation of log[skin permeability coefficient] for acrolein**	The log [permeability coefficient] (logPC) of acrolein for human skin *in vitro* calculated by method 1. Using the following parameters: Calculated: -0.069 Molecular volume: 46.08 A^3 Melting point: 25°C A value of -2.784 cm/hr was obtained. The log [permeability coefficient] (log PC) of acrolein for human skin *in vitro* calculated by method 2. Using the parameters: Measured Log P: 0.04 Molecular weight: 56.06 A value of -2.778 cm/hr was obtained.	X

Section A6.2/01 **Annex Point IIA6.2**	**Percutaneous absorption** Evaluation of percutaneous absorption potential using quantitative structure-activity relationships	
4.3 Calculation of skin permeation rate for acrolein	The permeation rate or flux of a chemical across a membrane in the steady state is proportional to its concentration differential across the membrane and to the area of the membrane (Fick's Law of Diffusion): dM/dt = PC.A.C Where M is the mass, t is time, A is area, C is the concentration differential across the membrane and PC is a proportionality constant called the permeability coefficient. The value of log PC calculated for acrolein (-2.78 cm/hr) by two methods gives a permeability coefficient of 1.66 x 10^{-3} cm/hr. For a steady state concentration differential of 10 mg/ml (1%), the flux of acrylate across human skin *in vitro* is calculated to be 16.6 µg/hr/cm^2. For 1ml of 1% acrolein solution applied to a 10 cm^2 area of skin, approximately 1.66% is expected to be absorbed over the first hour. Over a 24-hour period, between 30 and 40% of acrolein is expected to be absorbed.	
4.4 Discussion	Comparison of LD_{50} values for oral administration (26 mg/kg, rat) and dermal application (231.4 mg/kg, 4 hour exposure in rabbit) suggest that undiluted acrolein penetrated rabbit skin at a greater rate than that predicted from human skin for a 1% aqueous solution. This may be because undiluted acrolein is classified as a skin corrosive, and as such can destroy the permeability barrier of the skin, leading to a increased permeability rate. There is also evidence from other sources that the barrier to permeability of organic chemicals presented by human skin is greater than that of rat or rabbit skin.	
4.5 Conclusion	The log [permeability coefficient] of acrolein for human skin *in vitro* has been calculated to be -2.78 cm/hr by two different methods on physico/chemical parameters. Approximately 1.66% is expected to be absorbed over the first hour from 1 ml of 1% acrolein solution applied to a 10 cm^2 area of skin. Over a 24-hour period, between 30 - 40% of acrolein is expected to be absorbed.	X
4.5.1 Reliability	2 Calculation method.	
4.5.2 Deficiencies	Acrolein is corrosive. An *in-vitro* dermal study was not performed because there will be no human exposure to the acrolein or the biocidal product by this route of exposure. The study would be scientifically unjustified and technically unfeasible due to the high vapour pressure of the active substance. The active substance could not be applied to the skin to accurately measure dermal penetration therefore a QSAR assessment was calculated.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

Section A6.2/01 **Annex Point IIA6.2**	**Percutaneous absorption** Evaluation of percutaneous absorption potential using quantitative structure-activity relationships	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	06/04/06	
Materials and Methods	3.2 MW should be MV 4.2 The correct values for the melting point of acrolein (–87^{o}C) and the LogP (0.04) have not been used in the first calculation. The validity of the calculation based upon calculated values is questionable.	
Results and discussion	As described by the Applicant	
Conclusion	The value of 1.66% cannot be accepted. See below.	
Reliability	4	
Acceptability	Not acceptable The toxicokinetic dermal absorption assessment refers only to a 1% solution of acrolein. However, it is noted that the concentration of acrolein in the active substance and product is ~96%. Therefore, this assessment which indicates a dermal absorption of ~40% over a 24 hour period for a 1% acrolein solution is of limited value for this particular risk characterisation. Using the default values in the TGD, a dermal absorption value of 100% is predicted following exposure to acrolein (AS and product). This value is supported by the fact that acrolein is classified as a corrosive agent and a skin sensitiser.	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.3.1 **Annex Point IIA6.3**		**Repeated dose toxicity** 14-day Oral Toxicity Test in Mice	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Mansur, C.A.(1983b) 14-Day Oral Toxicity Test in Mice. Bioassay Systems Corporation. BSC Project No. 11496.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes	
		3 MATERIALS AND METHODS	
		.	
3.1	**Test material**	Acrolein	
3.1.1	Lot/Batch number	6288	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Liquid	
3.1.2.2	Purity	Greater than 96%	
3.1.2.3	Stability	Stable for five days at each dose solutions prepared	
3.2	**Test Animals**		
3.2.1	Species	Mice	
3.2.2	Strain	CD-1	
3.2.3	Source	Charles River Breeding Laboratory, Wilmington, Massachusetts	
3.2.4	Sex	Male & Female	
3.2.5	Age/weight at study initiation	Age: 7 weeks Weight: 22.3-37.0	
3.2.6	Number of animals per group	50 males, 50 females, 10 animals/sex/dose level	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	14 days	

Section A6.3.1 **Annex Point IIA6.3**		**Repeated dose toxicity** 14-day Oral Toxicity Test in Mice	
3.3.2	Frequency of exposure	daily	
3.3.3	Postexposure period	14 days, 4 weeks or other	X
3.3.4	**Oral**		
3.3.4.1	Type	gavage	
3.3.4.2	Concentration	gavage 0.0, 4.6, 5.8, 7.2, 9.0mg/kg bw	
3.3.4.3	Vehicle	Deionised water	
3.3.4.4	Concentration in vehicle	Dose formulations within 4% the target dose level	
3.3.4.5	Total volume applied	10 ml/kg	
3.3.4.6	Controls	vehicle, plain diet or other	X
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, twice daily	
3.4.1.2	Mortality	Yes daily	X
3.4.2	Body weight	Yes, Day 1, 8, 15	
3.4.3	Food consumption	Yes, Day 1, 8, 15	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	No	
3.4.6	Haematology	no	
3.4.7	Clinical Chemistry	no	
3.4.8	Urinalysis	no	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	no	
3.5.2	Gross and histopathology	Yes all dose groups, stomach and lungs.	
3.5.3	Other examinations	no	
3.5.4	Statistics		
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	All ten males at high doses, 9mg/kg, showed rough coats. Other signs	

Section A6.3.1 Annex Point IIA6.3		Repeated dose toxicity 14-day Oral Toxicity Test in Mice	
		in some males at high dose were pilo erection, reddening of the tip of the tail, bite wounds, closed eyes, and the presence of exudate around the eyes. Some males receiving 7.2mg/kg of acrolein showed hunching, lethargy, rough coats and squinted eyes. One female receiving 5.8mg/kg of acrolein displayed lethargy, one female at 7.2mg/kg and two females at 9.0mg/kg showed reddened tail tips.	
4.1.2	Mortality	A total of 4 mice died during the study. Two males at 7.2mg/kg dose level of acrolein died on day 3 and 4, and one male at 9.0mg/kg died on day 4. One female at 5.8mg/kg died on day 6.	
4.2	**Body weight gain**	There were no evident signs of toxicity based on body weights	
4.3	**Food consumption and compound intake**	There were no evident signs of toxicity based on food consumption.	
4.4	**Ophtalmoscopic examination**		
4.5	**Blood analysis**		
4.5.1	Haematology	n/a-observation not carried out	
4.5.2	Clinical chemistry	n/a-observation not carried out	
4.5.3	Urinalysis	n/a-observation not carried out	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	n/a-observation not carried out	
4.6.2	Gross and histopathology	One level II male, two level III males, nine level IV and six level IV females showed white and thickened gastric mucosa in the squamous portion of the stomach. Five level III males and two level VI females had pinpoint raised foci or nodules in the squamous portion of the stomach. Other lesions in the stomach include ulcers, black flecks in the gastric contents, black pinpoint foci, and red foci or reddened appearance in thee squamous portion of the stomach. Other observations include hemorrhagic lungs and tails darkened near the tip.	
4.7	**Other**	none observed.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	All animals were dosed orally by gavage using a syringe and stainless steel ball tip gavage tube. The dose volume was 10ml/kg with dose levels of 4.6, 5.8, 7.2 and 9.0 mg/kg. Each animal was dosed once daily for 14 consecutive days. All animals were subjected to gross necropsy.	
5.2	**Results and discussion**	The most common findings at necropsy were in the squamous portion of the gastric mucosa. Other lesions in the stomach included ulcers, black flecks in the gastric contents, black pinpoint foci, and red foci or reddened appearance in the squamous portion of the stomach. Other observations included hemorrhagic lungs and tails darkened near the tip. There was no effect on food consumption and the bodyweight of	

Section A6.3.1 **Annex Point IIA6.3**	**Repeated dose toxicity** 14-day Oral Toxicity Test in Mice	
	the animals.	
5.3 Conclusion		
5.3.1 LO(A)EL	Not calculated	
5.3.2 NO(A)EL	Not calculated	
5.3.3 Other	n/a	
5.3.4 Reliability	2	
5.3.5 Deficiencies	Yes, the study does not follow any specific guidelines. As it does comply with GLP it is still a valid study.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	03/05/06	
Materials and Methods	3.3.3 Animals were observed for the duration of the study (14 days) only. 3.3.4.6 The control was a vehicle control. 3.4.1.2 Mortality examinations were performed twice daily.	
Results and discussion		
Conclusion	LO(A)EL: 5.8 mg/kg NO(A)EL: 4.6 mg/kg	
Reliability	2	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	

Section A6.3.1 **Annex Point IIA6.3**	**Repeated dose toxicity** 14-day Oral Toxicity Test in Mice	
Remarks		

Table A6_3-1. Results *(specify)* of repeated dose toxicity study

Parameter	**Control**		**4.6 mg/kg**		**5.8 mg/kg**		**7.2 mg/kg**		**9.0 mg/kg**		**dose-response +/-**	
	m[a]	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m**	**f**
number of animals examined	10	10	10	10	10	10	10	10	10	10	50	50
Mortality	0/10	0/10	0/10	0/10	0/10	1/10	2/10	0/10	1/10	0/10	+	+
Hunched	0/10	0/10	0/10	0/10	0/10	0/10	2/10	0/10	0/10	0/10	+	-
Lethargy	0/10	0/10	0/10	0/10	0/10	1/10	3/10	0/10	0/10	0/10	+	+
Rough coat	0/10	0/10	0/10	0/10	0/10	0/10	3/10	0/10	10/10	0/10	+	-
Pilo erection	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/9	0/10	+	-
Squinted eyes	0/10	0/10	0/10	0/10	0/10	0/10	3/10	0/10	0/10	0/10	+	-
Tip of tail red	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	3/9	2/10	+	+
Bite wounds	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10	0/10	+	-
Eye closed	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	+	-
Exudate around eye(s)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10	0/10	+	-
Blood around mouth after dosing	0/10	0/10	0/10	0/10	0/10	0/10	1/8	0/10	0/10	0/10	-	-
body weight change	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	-	-
food consumption	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	-	-
clinical chemistry*	-	-	-	-	-	-	-	-	-	-	-	-
haematology*	-	-	-	-	-	-	-	-	-	-	-	-
urinalysis*	-	-	-	-	-	-	-	-	-	-	-	-
<u>Stomach</u>												
White thickened gastric mucosa (squamous portion)	0/10	0/10	0/10	0/10	1/10	0/10	2/10	0/10	9/10	6/10	+	+
Ulcers	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10	0/10	+	-
Black flecks in gastric contents	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10	1/10	+	+
Black pinpoint foci over mucosal surface	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	+	-

Pinpoint raised foci or nodules on squamous portion	0/10	0/10	0/10	0/10	0/10	0/10	5/10	1/10	0/10	2/10	+	+
Cardia portion reddened or red foci	0/10	0/10	0/10	0/10	0/10	0/10	2/10	0/10	1/10	0/10	+	-
Tail darkened near tip	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	+	-
Lungs haemorrhagic	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	+	-

[a] *number of animals affected/total number of animals*

- not measured

Section A6.3.2 Annex Point IIA6.3		Repeated dose toxicity 21-Day dermal study in rabbits	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Muni, I.A.(1982) 21-Day Dermal Test of Acrolein in Rabbits. Bioassay Systems Corporation. BSC Project No. 10258.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes FIFRA, 43 CFR, Section 163.82-2	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	SFSL-5993	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2	
3.1.2.2	Purity	See 3.1.2	
3.1.2.3	Stability	See 3.1.2	
3.2	**Test Animals**		
3.2.1	Species	Rabbits	
3.2.2	Strain	New Zealand White	
3.2.3	Source	H.A.R.E. Rabbits for Research, Hewitt, New Jersey, USA.	
3.2.4	Sex	Male and Female	
3.2.5	Age/weight at study initiation	Age: 10-12 weeks (males) 11-13 weeks (females) Bodyweight: 2.2-3.2 kg (males and females)	
3.2.6	Number of animals per group	10 animals/sex/group Vehicle control: 6 animals/sex Untreated control: 4 animals/sex	

Section A6.3.2 Annex Point IIA6.3		Repeated dose toxicity 21-Day dermal study in rabbits	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Duration of treatment	21 days	
3.3.2	Frequency of exposure	5 days per week	
3.3.3	Postexposure period		
3.3.4	**Dermal**		
3.3.4.1	Area covered	10% of body surface area	
3.3.4.2	Occlusion	Semi-occlusive	
3.3.4.3	Vehicle	50:50 (v:v) solution of deionised water and absolute ethanol	
3.3.4.4	Concentration in vehicle	3.5, 10.5, 31.5 mg/ml	X
3.3.4.5	Total volume applied	2 ml/kg	
3.3.4.6	Duration of exposure	6 hours	
3.3.4.7	Removal of test substance		
3.3.4.8	Controls	Deionised water, absolute ethanol	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, once daily	
3.4.1.2	Mortality	Yes, once daily	
3.4.2	Body weight	Yes, every four days	
3.4.3	Food consumption	Yes, every four days	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	No	
3.4.6	Haematology	Yes Number of animals: 5 males and 5 females from each group. Time points: once prior to initial dosing and once shortly prior to terminal sacrifice. Parameters: Haemoglobin, haematocrit, erythrocyte, total and differential leucocyte counts and platelet counts.	

Section A6.3.2 Annex Point IIA6.3		Repeated dose toxicity 21-Day dermal study in rabbits	
3.4.7	Clinical Chemistry	Yes Number of animals: 5 males and 5 females from each group Time points: once prior to initial dosing and once shortly prior to terminal sacrifice Parameters: Calcium, potassium, serum lactate dehydrogenase, serum glutamate pyruvatetransaminase, serum glutamine oxaloacetic transaminase, glucose, blood urea nitrogen, direct and total bilirubin, alkaline phosphatase, cholesterol, total protein, albumin, globulin	
3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes Organs: Brain, pituitary, heart, thyroids/parathyroids, adrenals, liver, kidneys, testes, ovaries	
3.5.2	Gross and histopathology	Yes all dose groups organs: brain, pituitary, heart, thyroid, parathyroid, adrenals, liver, kidneys, ovaries or testes, eyes, lungs, trachea, skin from treated and untreated areas	
3.5.3	Other examinations		
3.5.4	Statistics		
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Clinical signs such as slight to moderate conditions of nasal mucus (discharge), lethargy, and apparent weight loss, were seen more frequently in the acrolein treated animals than in the controls.	
4.1.2	Mortality	All males survived to the end of the study. One female in dose group III, 63 mg/kg, (unabraded) was found dead on day 4 and one female in dose group two, 21 mg/kg, (abraded) was found dead on day 5 of the treatment period. A female at dose group III, 63 mg/kg, (unabraded) and another female at dose group one, 7.0 mg/kg (abraded) was sacrificed moribund on day 5 of the treatment period. The animals which were sacrificed moribund were found to have suffered broken backs attributable to hyperactivity behaviour following dosing.	
4.2	**Body weight**	Acrolein treated males exhibited lower (but not significant) final body weight than the control animals. The mean body weight changes in males revealed statistically significant differences. Bodyweight changes for the females were significantly lower in the	X

Section A6.3.2 Annex Point IIA6.3		Repeated dose toxicity 21-Day dermal study in rabbits	
		acrolein-treated groups when compared to the controls.	
4.3	**Food consumption and compound intake**	A statistically significant effect of the test substance on food consumption was found among male animals. The effect was attributed to the lower intake found animals in dose groups I and III.	
4.4	**Opthalmoscopic examination**		
4.5	**Blood analysis**		
4.5.1	Haematology	No significant haematological effects were seen in male or female rabbits when treated with acrolein	
4.5.2	Clinical chemistry	There were no significant differences in the blood chemistry amongst all the groups.	
4.5.3	Urinalysis	Not applicable	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Results for male and female rabbit organ weights were similar among all groups	
4.6.2	Gross and histopathology	At the lowest dose level, acrolein produced epidermal necrosis, at the next dose level necrotising dermatitis was accentuated. At higher dose levels, chronic topical administration of acrolein produced severe necrotising, ulcerative dermatitis, (which was not fatal), and resulted in healing with marked dermal fibrosis, hyperkeratosis acanthosis and occasionally pseudoepitheliomatous hyperplasia. These were expected reactions of the integument to severe but reversible injury. In addition to integumentary lesions, as the dose level increases, toxicity was observed in kidneys and lungs characterised by multifocal to diffuse interstitial nephritis and interstitial pneumonia. Mesangioproliferative glomerulopathies frequently observed in higher doses levels was a secondary immune complex complication of non-specific inflammatory process and cannot be directly implicated in the toxicity of acrolein. Hepatic lesions in all cases may be compatible with infestation by *Eimeria steidiae*, therefore the toxicity of acrolein on the liver is impossible to evaluate.	
4.7	**Other**	Slight to moderate erythema and edema of the skin of almost all rabbits treated with acrolein were found and all groups after first dose administration. The erythema and edema conditions became more pronounced in all groups treated with acrolein, particularly at 63 mg/kg. Skin damage was similar between animals whose skin was abraded those animals whose skin was not abraded. General appearance of the treatment site for both male and female are as follows: **7 mg/kg dose group** – Slight to moderate reddening with swelling and firmness of test site. Scab formation with peeling of the test site was apparent. **21 mg/kg dose group** – Moderate to severe reddening with swelling	

Section A6.3.2 **Annex Point IIA6.3**		**Repeated dose toxicity** 21-Day dermal study in rabbits	
		more pronounced and firmness of test site. Scab formation with peeling of the test site was apparent. **63 mg/kg dose group** – Severe reddening with marked swelling. Firmness of the test site and scab formation with cracking and peeling of the test area was also observed.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In accordance to FIFRA guidelines, 43 CFR section 163.82-2. 5 rabbits per group were dosed at 7, 21 and 63 mg of acrolein per kilogram of body weight. Absorbent gauze material was placed over the clipped area (flank). Teflon sheeting was wrapped around the trunk of the animal, holding the gauze material in place. Elasticized tubular stockinette was placed over the sheeting and taped in position. The dose was then administered by injecting the acrolein or vehicle formulation through the wrappings, onto the gauze pad. The dose pad and wrappings were removed after 6 hours of contact with skin. The compound was administered for three weeks, five days per week.	
5.2	**Results and discussion**	An evaluation of the data collected over the course of the study suggests evidence of toxicity resulting from the repeated application of acrolein at 7, 21, and 63 mg/kg. These effects were manifested in slight to significant reduction in body weight gain, more frequent occurrence of some clinical signs such as nasal mucus discharge and lethargy, slight to moderately lowered food consumption values, and moderate to severe skin irritation. Furthermore, histopathologic lesions in the skin and lungs were seen more frequently in those animals treated with acrolein. Slight mortality was observed in the 21 and 63 mg/kg groups of female rabbits. There were no notable effects observed in haematology, blood chemistry, organ weights, or organ weight ratios.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	7 mg/kg	
5.3.2	NO(A)EL	<7 mg/kg	
5.3.3	Other		
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	
		Evaluation by Competent Authorities	
		Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		03/05/06	

Section A6.3.2 **Annex Point IIA6.3**	**Repeated dose toxicity** 21-Day dermal study in rabbits	
Materials and Methods	2.1 OECD guideline 410 3.3.4.4 The doses used were: 7, 21 and 63 mg/kg.	
Results and discussion	4.2 A significant decrease in body weight gain was observed (70/31, 66/28 and 91/77% reduction for males/females for 7, 21 and 63 mg/kg bw/d, respectively, compared with controls).	
Conclusion	LO(A)EL: 7 mg/kg NO(A)EL: not determined	
Reliability	1	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_3-1. Results of repeated dose toxicity study

Parameter	**Control**		**low dose**		**medium dose**		**high dose**		**dose-response +/-**	
	m[a]	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m**	**f**
number of animals examined	10	10	10	10	10	10	10	10		
Mortality				1/10		1/10		2/10		
clinical signs										
Nasal mucus	1/10	4/10	1/10	1/10	6/10	3/10	2/10	4/10	+/-	+
Lethargy		1/10		1/10	1/10	1/10	4/10	1/10	+	+/-
Apparent weight loss			1/10		1/10		1/10	1/10	+/-	+
body weight			↓ *	↓ *	↓ *	↓ *	↓ *	↓ *	+	+
food consumption			↓ *				↓ *		+/-	
clinical chemistry										
haematology										
Organ x organ weight*										
gross pathology Skin lesions			↑	↑	↑	↑	↑↑	↑↑	+	+
microscopic pathology* Kidney Lung								 ↑ ↑		

* = P 0.01 significance level

Section A6.3.3 Annex Point IIA VI.6.3	Repeated Dose Toxicity (Inhalation)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	As a subchronic inhalation study has been carried out on the active substance (Section A6.4.3, Annex Point IIA, VI.6.3.), studies for repeat dose toxicity (inhalation) are considered to be unjustified. Also, the use pattern of acrolein would lead to minimal exposure from the inhalation route. Therefore it is considered that an additional inhalation study is not required and is not in the interests of animal welfare.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	05/10/07	
Evaluation of applicant's justification	Applicant's justification is acceptable	
Conclusion	Applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		