Section A6.1.1 Acute Toxicity

Annex Point IIA6.1.1 **Oral, Rats, Limit Test**

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] ACUTE ORAL TOXICITY STUDY OF Z,E-9,12-TETRADECADIEN-1-YL ACETATE IN CD RATS, [redacted] Dates of work: January 25, 2006 - March 2, 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Aeroxon Insect Control GmbH
1.2.2	Companies with letter of access	Not applicable
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EC method B.1 tris (2004/73/EC) and OECD guideline 423 (ATC method) - Limit Test -
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Z,E-9,12-Tetradecadien-1-yl acetate
3.1.1	Lot/Batch number	Batch no 2005340-0010
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Colourless liquid
3.1.2.2	Purity	99.5%
3.1.2.3	Stability	December 6, 2009 (expected shelf life)

Section A6.1.1 Acute Toxicity

Annex Point IIA6.1.1 **Oral, Rats, Limit Test**

3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	CD/ Crl: CD(SD)
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Males: 7 weeks, 214 – 240 g females: 8 weeks, 184 – 190 g
3.2.6	Number of animals per group	3 male and 3 female animals
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	**Oral**
3.3.1	Postexposure period	14 days
3.3.2	Type	Gavage
3.3.3	Concentration	2000 mg/kg bw
3.3.4	Vehicle	No vehicle (test item was used as supplied)
3.3.5	Concentration in vehicle	Not applicable
3.3.6	Total volume applied	2.25 mL/kg bw (as density was determined to be 0.89 g/mL)
3.3.7	Controls	None
3.4	**Examinations**	Test animals were observed for 14 days with respect to clinical signs (changes of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and nervous system, somatomotor activity), behaviour, bodyweight, mortality and necropsy, furthermore gross pathology.
3.5	**Method of determination of LD_{50}**	Estimated based on the absence of mortality.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	No effects
4.2	**Pathology**	No effects
4.3	**Other:**	
	body weight:	Males: slight body weight loss (-2%) during the first test week was observed. Females: slightly reduced body weight gain (+7.5%) during the first test week was observed. After two weeks recovery was complete and all animals gained the expected body weight.

Section A6.1.1 Acute Toxicity

Annex Point IIA6.1.1 **Oral, Rats, Limit Test**

4.4	**LD_{50}**	No mortality occurred following a single oral administration of 2000 mg Z,E-9,12-Tetradecadien-1-yl acetate/kg bw, i.e. oral LD_{50} >2000mg/kg bw.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Test guidelines: EC method B.1 tris (2004/73/EC) and OECD guideline 423 (ATC method) - Limit Test. No deviations from the guidelines Method: Limit test, 3 male and 3 female rats, one single oral administration of 2000 mg Z,E-9,12-Tetradecadien-1-yl acetate/kg bw, 14 days observation period.
5.2	**Results and discussion**	No mortality occurred. Rat, Z,E-9,12-Tetradecadien-1-yl acetate: oral LD_{50} >2000 mg/kg bw
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	

Section A6.1.2 Annex Point IIA6.1.2	Acute toxicity, dermal	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [x] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	An acute dermal toxicity study is considered to be not necessary, since (9Z,12E)-9,12-Tetradecadien-1-yl acetate is a member of a well characterised group (SCLP*) of low toxicity. Acute dermal toxicity studies in structurally similar SCLPs indicate no acute dermal toxicity with an LD_{50} skin >2000 mg/kg b.w. - EPA Category IV, non-toxic ([redacted]) The low toxicity is also demonstrated in different acute toxicity studies (oral, inhalation, dermal irritation) with (9Z,12E)-9,12-Tetradecadien-1-yl acetate where no adverse effects have been found (all: [redacted], refer to **Document III A6.1.1, A6.1.3 and A6.1.4**). Furthermore, the dermal route is not considered to be the important one, since (9Z,12E)-9,12-Tetradecadien-1-yl acetate is volatile. In addition, the end use product is formulated in a way that precludes direct contact to the pheromone. * Straight-Chained Lepidopteran Pheromone	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Evaluation of applicant's justification	Exposure to the a.s. via the application of the representative product (cardboard glue trap) is negligible, for exposure estimates please see Document II-B 4, for the intended use description see Document II-B 3. Agree with applicant's version
Conclusion	Agree with applicant's version
Remarks	

Section A6.1.3. Acute Toxicity

Annex Point IIA6.1.3 **Inhalation, Rats, Limit Test**

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], ACUTE INHALATION TOXICITY STUDY OF Z,E-9,12-TETRADECADIEN-1-YL ACETATE IN RATS, [redacted] Dates of work: January 25, 2006 - March 2, 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Aeroxon Insect Control GmbH
1.2.2	Companies with letter of access	Not applicable
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EC method B.2. (92/69/EEC) and OECD guideline 403 - Limit Test -
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Z,E-9,12-Tetradecadien-1-yl acetate
3.1.1	Lot/Batch number	Batch no. 2005340-0010
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Colourless liquid
3.1.2.2	Purity	99.5%
3.1.2.3	Stability	December 6, 2009 (expected shelf life)
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	CD/ Crl: CD(SD)
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Males: 52 days, 219 - 252g females: 63 days 211 - 211g
3.2.6	Number of animals per group	5 male und 5 female animals
3.2.7	Control animals	No

Section A6.1.3. Acute Toxicity

Annex Point IIA6.1.3 **Inhalation, Rats, Limit Test**

3.3	**Administration/ Exposure**	**Inhalation**		
3.3.1	Postexposure period	14 days		
3.3.2	Concentrations	Nominal concentration	33.3 µL/L air	
		Analytical concentration	5.20 ± 0.16 mg/L air	
3.3.3	Particle size	MMAD (mass median aerodynamic diameter) 1.864 µm ± GSD (geometric standard deviation) 4.02 µm		X
3.3.4	Type or preparation of particles	The aerosol of the test item was prepared using a spray-jet		
3.3.5	Type of exposure	Nose only		
3.3.6	Vehicle	No vehicle (test item was used as supplied)		
3.3.7	Concentration in vehicle	Not applicable		
3.3.8	Duration of exposure	4h		
3.3.9	Controls	Not applicable		
3.4	**Examinations**	After the exposure period, test animals were observed for 14 days with respect to clinical signs (changes of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and nervous system, somatomotor activity), behaviour, bodyweight, mortality and necropsy, furthermore gross pathology.		
3.5	**Method of determination of LD_{50}**	Estimated based on the absence of mortality		
3.6	**Further remarks**	None		
		4 RESULTS AND DISCUSSION		
4.1	**Clinical signs**	No effects		
4.2	**Pathology**	No effects		
4.3	**Other**	-		
4.4	**LD_{50}**	No LC_{50} was calculated as no mortality occurred, i.e. LC_{50} inhalation >5.2 mg/L air		
		5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1	**Materials and methods**	Test guidelines: EC method B.2. (92/69/EEC) and OECD guideline 403 - Limit Test. No deviations from the guidelines. Method: Limit test, 5 male and 5 female rats, 4 hour exposure to Z,E-9,12-Tetradecadien-1-yl acetate at a concentration of 5.2 mg/L air, 14 days observation period		
5.2	**Results and discussion**	No signs of toxicity under the present test conditions. No mortality occurred. No changes were noted at necropsy. Body weight and body weight gain were not affected. Rat, Z,E-9,12-Tetradecadien-1-yl acetate: LC_{50} inhalation >5.2 mg/L air		

Section A6.1.3. Acute Toxicity

Annex Point IIA6.1.3 **Inhalation, Rats, Limit Test**

5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Materials and Methods	**3.3.3. Particle size** The study report states: "The Geometric Standard Deviation is (GSD) of the MMAD was calculated as 4.02. The respirable amount (particle size ≤4µm) was 2.205 mg/L air. No smaller GSD could be achieved with the test item supplied." However, since no adverse effects were observed with the applied dose and it seems to be difficult to generate small particle aerosols it is reasonable to assume that the LC_{50} is quite higher than the applied dose, most probably above the classification limit of 5 mg/L.
Results and discussion	See above. Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	

Section A6.1.4/01 Acute Dermal Irritation

Annex Point IIA6.4 **Rabbit**

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], ACUTE DERMAL IRRITATION / CORROSION TEST (PATCH TEST) OF Z,E-9,12-TETRADECADIEN-1-YL ACETATE IN RABBITS [redacted] Dates of work: January 25, 2006 - February 16, 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Aeroxon Insect Control GmbH
1.2.2	Companies with letter of access	Not applicable
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EC method B.4. (2004/73/EC) OECD Guideline 404
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Z,E-9,12-Tetradecadien-1-yl acetate
3.1.1	Lot/Batch number	Batch no 2005340-0010
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Colourless liquid
3.1.2.2	Purity	99.5%
3.1.2.3	Stability	December 6, 2009 (expected shelf life)
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	Himalayan
3.2.3	Source	[redacted]
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Approx. 3.5 month animal no. 1: 2.2 kg animal no. 2: 2.1 kg animal no. 3: 2.0 kg

Section A6.1.4/01 Acute Dermal Irritation

Annex Point IIA6.4 **Rabbit**

3.2.6	Number of animals per group	3
3.2.7	Control animals	No (the surrounding untreated skin served as control)
3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	
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	Dorsal area of the trunk, shaved intact skin Approximately 24 hours before the test, the fur was removed from the dorsal area of the animals' trunks by shaving. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were taken.
3.3.2	Occlusion	Semi-occlusive
3.3.3	Vehicle	No vehicle
3.3.4	Concentration in vehicle	Not applicable
3.3.5	Total volume applied	0.5 mL/patch/animal
3.3.6	Removal of test substance	Not applicable (After application the test site was covered with a gauze patch. It was removed after 4 hours and the skin site was evaluated.)
3.3.7	Duration of exposure	4h
3.3.8	Postexposure period	14 days
3.3.9	Controls	None
3.4	**Examinations**	
3.4.1	Clinical signs	None of the animals died or showed any systemic toxicological symptoms. For individual results see **Table A6.1.4/01-1**.
3.4.2	Dermal examination	After the 4-hour exposure period the patch was removed and the skin sites were evaluated. Scores were taken 60 min, 24h, 48h, and 72h after patch removal. The observations were continued from the 4th to the 14th day after patch removal to determine the reversibility of the reactions.
3.4.2.1	Scoring system	Scoring system according to OECD 404
3.4.2.2	Examination time points	60 min, 24h, 48h, and 72h and 4 to 14 days after patch removal
3.4.3	Other examinations	Induration of the skin, laceration of the skin and scaling of the skin were observed from 72 hours on.
3.5	**Further remarks**	There were no systemic intolerance reactions.

4 RESULTS AND DISCUSSION

4.1 Average score

Section A6.1.4/01 Acute Dermal Irritation

Annex Point IIA6.4 **Rabbit**

4.1.1	Erythema	Very slight erythema (grade 1) was observed in all animals 60 minutes to 5 days after patch removal, in animal no. two until 7 days and in animal no. three until 13 days after patch removal. Average score for all animals at 24h: 1 48h: 1 72h: 1 For individual/further results see **Table A6.1.4/01-1.**
4.1.2	Edema	Very slight edema (grade 1) was observed in all animals 72 hours to 4 days after patch removal, in animal no. two from 48 hours to 6 days and in animal no. three from 48 hours to 5 days after patch removal. Average score for all animals at 24h: 0 48h: 0.67 72h: 1 For individual/further results see **Table A6.1.4/01-1**.
4.2	**Reversibility**	Yes All described reactions were completely reversible. <u>Erythema:</u> reversed after 6/8/14 days after patch removal. Average time: 9.33 days. <u>Edema:</u> reversed after 5/7/6 days after patch removal. Average time: 6 days.
4.3	**Other examinations**	Induration of the skin was observed in animal no. one 72 hours to 6 days after patch removal, in animal no. two 7 and 8 days after patch removal and in animal no. three 5 to 8 days after patch removal. Laceration of the skin was observed in animal no. one 72 hours and 5 to 6 days after patch removal, and in animal no. three 6 to 8 days after patch removal. Scaling of the skin was observed in animal no. one 4 to 6 days, and in animal no. three 9 to 13 days after patch removal.
4.4	**Overall result**	According to EC-Commission Directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions, Z,E-9,12-tetradecadien-1-yl acetate can be considered as non-irritating to skin.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test guidelines: EC method B.4. (2004/73/EC) and OECD guideline 404. No deviations from the guidelines. Method: 3 male rabbits, dermal application onto the shaved, intact dorsal skin of 0.5 mL Z,E-9,12-Tetradecadien-1-yl acetate/patch and animal for 4 hours, 14 days observation period.
5.2	**Results and discussion**	Z,E-9,12-tetradecadien-1-yl acetate was **non-irritating to skin,** hence, no labelling is required
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version, only slight irritation below the classification criteria of Directive 67/458/EEC
Reliability	1
Acceptability	acceptable
Remarks	

Table A6.1.4/01-1 Table for skin irritation study

Score	**Time**		Erythema			Edema		
			Animal no			Animal no		
		average score erythema // edema	one	two	three	one	two	three
Average score Draize scores (0 to maximum 4)	60 min	1 // 0	1	1	1	0*	0	0
	24h	1 // 0	1	1	1	0	0	0
	48h	1 // 0.67	1	1	1	0	1	1
	72h	1 // 1	1	1	1	$1_{a,b}$	1	1
	4 days	1 // 1	1	1	1	$1_{a,c}$	1	1
	5 days	1 // 0.67	1	1	1	$0_{a,b,c}$	1	1_{a}
	6 days	0.67 // 0.33	0	1	1	$0_{a,b,c}$	1	$0_{a,b}$
	7 days	0.67 // 0	0	1	1	0	0_{a}	$0_{a,b}$
	8 days	0.33 // 0	-	0	1	-	0_{a}	$0_{a,b}$
	9 days	0.33 // 0	-	0	1	-	0	0_{c}
	10 days	0.33 // 0	-	-	1	-	-	0_{c}
	11 days	0.33 // 0	-	-	1	-	-	0_{c}
	12 days	0.33 // 0	-	-	1	-	-	0_{c}
	13 days	0.33 // 0	-	-	1	-	-	0_{c}
	14 days	0 // 0	-	-	0	-	-	0
Average score	24h, 48h, 72h: **0.78**				**1**			**0.56**
Reversibility: *			cr	cr	cr	cr	cr	cr
Average time for reversibility			9.33 days			6 days		

* 0: no pathological findings
1: very slight erythema/edema
a: induration of the skin
b: laceration of the skin
c: scaling of the skin
cr: completely reversible

Average score: EU index score = total erythema and edema score at the 24, 48 and 72 hr intervals/ no of observation intervals

Section A 6.1.4/02 Acute Eye Irritation

Annex Point IIA6.1.4 **Rabbit**

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], ACUTE EYE IRRITATION / CORROSION TEST OF Z,E-9,12-TETRADECADIEN-1-YL ACETATE IN RABBITS [redacted] Dates of work: January 25, 2006 - February 7, 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Aeroxon Insect Control GmbH
1.2.2	Companies with letter of access	Not applicable
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EC method B.5. (2004/73/EC) OECD Guideline 405
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Z,E-9,12-Tetradecadien-1-yl acetate
3.1.1	Lot/Batch number	Batch no 2005340-0010
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Colourless liquid
3.1.2.2	Purity	99.5%
3.1.2.3	Stability	December 6, 2009 (expected shelf life)
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	Himalayan
3.2.3	Source	[redacted]
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Approx 4 months animal no. 1: 2.8 kg animal no. 2: 2.2 kg animal no. 3: 2.7 kg
3.2.6	Number of animals per group	3

Section A 6.1.4/02 Acute Eye Irritation

Annex Point IIA6.1.4 **Rabbit**

3.2.7	Control animals	No (left eye of each test animal remained untreated to serve as reference control)
3.3	**Administration/ Exposure**	
3.3.1	Preparation of test substance	Test substance was used as delivered
3.3.2	Amount of active substance instilled	0.1 mL of the test substance was placed into the conjunctival sac of the right eye
3.3.3	Exposure period	24h
3.3.4	Postexposure period	72h
3.4	**Examinations**	
3.4.1	Ophthalmoscopic examination	Yes Examination for ocular irritation reactions in the cornea, iris and conjunctivae
3.4.1.1	Scoring system	Scoring system according to OECD 405
3.4.1.2	Examination time points	1h, 24h, 48h, and 72h after administration
3.4.2	Other investigations	24 hours after administration the eyes were treated additionally with flourescein and examined.
3.5	**Further remarks**	Body weights of all animals were measured at the beginning of the study. Behaviour and food consumption were monitored.
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	None of the animals died or showed any systemic toxicological symptoms For individual results see **Table A6.1.4/02-1.** The fluorescein test performed 24 hours after instillation revealed no pathological findings.
4.2	**Average score**	Details on scores are given in **Table A6.1.4/02-1**. Average values are listed below.
4.2.1	Cornea	Average score for all animals at 24, 48, 72h: 0
4.2.2	Iris	Average score for all animals at 24, 48, 72h: 0
4.2.3	Conjunctiva	
4.2.3.1	Redness	Average score for all animals at 24h: 1 48h: 1 72h: 0
4.2.3.2	Chemosis	Average score for all animals at 24h: 0 48h: 0 72h: 0

Section A 6.1.4/02 Acute Eye Irritation

Annex Point IIA6.1.4 **Rabbit**

4.3	**Reversibility**	Yes **Conjunctival redness** (grade 1) was observed in all animals 1 hour to 48 hours after instillation, (reversed after 72h). **Chemosis** (grade 1) was observed in 1 animal, 1 hour after instillation (reversed after 24h)
4.4	**Other**	There were no systemic intolerance reactions.
4.5	**Overall result**	According to EC-Commission Directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions, Z,E-9,12-tetradecadien-1-yl acetate can be considered as non-irritating to eyes, hence no labelling is required.
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test guidelines: EC method B.5. (2004/73/EC) and OECD guideline 405. No deviations from the guidelines. Method: 3 male rabbits, instillation of 0.1 mL Z,E-9,12-Tetradecadien-1-yl acetate into the right conjunctival sac, left eye untreated to serve as control, 72-hour observation period.
5.2	**Results and discussion**	Z,E-9,12-tetradecadien-1-yl acetate was **non-irritating to eyes,** hence no labelling is required.
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version, only slight redness below the classification criteria of Directive 67/458/EEC
Reliability	1
Acceptability	acceptable
Remarks	

Section A 6.1.4/02 Acute Eye Irritation

Annex Point IIA6.1.4 **Rabbit**

Table A6.1.4/02-1. Results of eye irritation study

	Cornea	Iris	Conjunctiva	
			redness	chemosis
Score	0 to 4	0 to 2	0 to 3	0 to 4
60 min	0/0/0	0/0/0	1/1/1	0/0/1
24h	0/0/0	0/0/0	1/1/1	0/0/0
48h	0/0/0	0/0/0	1/1/1	0/0/0
72h	0/0/0	0/0/0	0/0/0	0/0/0
Average 24h, 48h, 72h	0	0	0.67	0
Area effected	n.a.	n.a.	no data	no data
Maximum average score (including area affected, max 110)	n.a.	n.a.	no data	no data
Reversibility*	n.a.	n.a.	c	c
average time for reversion	n.a.	n.a.	48 -72h	1-24h
Give method of calculation maximum average score. * *c: completely reversible* *n.a.: not applicable* *x/x/x: animal 1/ 2/ 3*				

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT), according to Magnusson and Kligman**

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], EXAMINATION OF Z,E-9,12-TETRADECADIEN-1-YL ACETATE IN GUINEA PIGS ACCORDING TO MAGNUSSON AND KLIGMAN (MAXIMISATION TEST) [redacted] Dates of work: January 25, 2006 - March 20, 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Aeroxon Insect Control GmbH
1.2.2	Companies with letter of access	Not applicable
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EC method B.6. (96/54/EC) OECD Guideline 406
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Z,E-9,12-Tetradecadien-1-yl acetate
3.1.1	Lot/Batch number	Batch no 2005340-0010
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Colourless liquid
3.1.2.2	Purity	99.5%
3.1.2.3	Stability	December 6, 2009 (expected shelf life)
3.1.2.4	Preparation of test substance for application	a) <u>for induction:</u> stage 1: diluted, 50% concentration in sesame oil b) <u>for induction:</u> stage 2: undiluted, used as delivered c) <u>for challenge:</u> diluted: 1% concentration in sesame oil
3.1.2.5	Pretest performed on irritant effects	Yes

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT), according to Magnusson and Kligman**

3.2	**Test Animals**	
3.2.1	Species	Guinea pigs
3.2.2	Strain	Dunkin-Hartley
3.2.3	Source	[redacted]
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	33 days 267 - 460 g (excluding positive reference group) positive reference group: 310 - 381 g
3.2.6	Number of animals per group	Preliminary study: 8 (6 for topical administration, 2 for intradermal administration) main study: 15
3.2.7	Control animals	Yes - Vehicle control group: sesame oil - Positive control group: Benzocaine solution (historical background group of 20 animals of the same origin strain from a study performed July 2005 by the same laboratory)
3.3	**Administration/ Exposure**	Adjuvant
3.3.1	Induction schedule	- day 0 : intradermal injection - day 7 : topical induction, patch-test technique, exposure 48h See **Table A6.1.5-1**
3.3.2	Way of induction	Intradermal and topical Occlusive

Section A6.1.5 **Annex Point IIA6.1.5**		**Skin sensitisation** **Guinea pig maximisation test (GPMT), according to Magnusson and Kligman**
3.3.3	Concentrations used for induction	Intradermal induction 3 injections of 0.1 mL - 50% FCA in physiological saline - 50% Z,E-9,12-Tetradecadien-1-yl acetate in sesame oil - 50% Z,E-9,12-Tetradecadien-1-yl acetate in FCA/ physiological saline Vehicle control group received sesame oil instead of the test item. Positive control group received - 2% benzocaine (dissolved in 40% ethanolic 0.9% NaCl solution) Topical induction - undiluted Z,E-9,12-Tetradecadien-1-yl acetate Vehicle control group received sesame oil instead of the test item. Positive control group received - 5% benzocaine (dissolved in 40% ethanolic 0.9% NaCl solution) Animals of the vehicle test group and animals of the positive control group were treated in the same way as the Z,E-9,12-Tetradecadien-1-yl acetate group, see **Table A6.1.5-1**
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	50% in physiological saline
3.3.5	Challenge schedule	Day 21 patch-test technique, exposure 24h. The left flank was treated with the test item, the right flank with the vehicle (as the vehicle test group).
3.3.6	Concentrations used for challenge	2 mL of - 1% Z,E-9,12-Tetradecadien-1-yl acetate in sesame oil Vehicle control group received sesame oil instead of the test item. Positive control group received - 5% benzocaine (dissolved in 40% ethanolic 0.9% NaCl solution), see **Table A6.1.5-1**
3.3.7	Rechallenge	No
3.3.8	Scoring schedule	24 and 48 hours after removal of the patches (= 48 resp. 72 hours from the start of challenge application) skin reaction was observed
3.3.9	Removal of the test substance	21h after removal of the patches the treated skin was cleaned
3.3.10	Positive control substance	Benzocaine
3.4	**Examinations**	
3.4.1	Pilot study	No
3.5	**Further remarks**	Clinical observations and morbidity/mortality checks were performed daily during the observation period, body weights were analysed statistically using Student's t-test at the beginning and at the end of the study.

4 RESULTS AND DISCUSSION

4.1	**Results of pilot studies**	Not applicable	X
4.2	**Results of test**		
4.2.1	24h after challenge	(= 24 hours after removal of the patches) The challenge with 2 mL of a 1% suspension of Z,E-9,12-Tetradecadien-1-yl acetate revealed no skin irritation in any animal → grade 0 The vehicle control group revealed no skin reactions per se, either (0). The positive control group exhibited a sensitizing reaction in all animals: discrete or patchy erythema, grade 1 (11 animals) resp. moderate and confluent erythema, grade 2 (9 animals), see **Table A6.1.5-2**.	
4.2.2	48h after challenge	(48 hours after removal of the patches): same results as in the 48 hour observation (see **Table A6.1.5-2**).	
4.2.3	Other findings	Body weight gains of the Z,E-9,12-Tetradecadien-1-yl acetate and benzocaine treated groups were within the range of the vehicle control group throughout the study. No necropsy was performed. Behaviour remained unchanged.	
4.3	**Overall result**	Z,E-9,12-Tetradecadien-1-yl acetate revealed no sensitising properties in guinea pigs in a test model according to Magnusson and Kligman.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test guidelines: EC method B.6. (96/54/EC) and OECD Guideline 406. No deviations from the guidelines. Method: 15 female guinea pigs, intradermal induction on day 0, topical induction on day 7, challenge on day 21, vehicle sesame oil, positive control benzocaine, 72-hour observation period.
5.2	**Results and discussion**	Z,E-9,12-Tetradecadien-1-yl acetate is not a dermal sensitizer.
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Materials and Methods	Agree with applicant's version
Results and discussion	**4.1. Results of pilot studies** Six concentrations were tested by intra-cutaneous injection: 0.01, 0.1, 1, 10, 50% suspensions in sesame oil and undiluted: A concentration of 50% revealed a moderate and confluent erythema 24 hours and an intense erythema and swelling 48 and72 hours after start of treatment. Six concentrations were tested by topical application: 1, 5, 10, 25, 50% suspension in sesame oil and undiluted: On depilated skin a concentration of 1% did not reveal any skin reactions. A concentration of 5% and above revealed increasing erythema and swelling. Since challenge exposure lasts for 24 hours, this is not in disagreement with the dermal irritation study that shows only slight irritation (after 4 hours of exposure).
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT), according to Magnusson and Kligman**

Table A6.1.5-1 Schedule and concentrations for skin sensitisation test

Group description	Number of animals	Concentration applied (%)		
		Induction		Challenge
		(intradermal)	(topical)	(topical)
		Day 0	Day 7	Day 21
Z,E-9,12-Tetradecadien-1-yl acetate test group	10	50	undiluted	1
Vehicle control group	5	0 (vehicle)	0 (vehicle)	0 (vehicle)
Positive control group: Benzocaine	20	2	5	5

Table A6.1.5-2 Summary of skin reactions following challenge

Group	No. animals tested	Time after challenge (hours)*	No. animals with skin reaction grade: 0	1	2	3	No. animals sensitized
Z,E-9,12-Tetradecadien-1-yl acetate	10	48 72	10 10	0 0	0 0	0 0	0
Vehicle control	5	48 72	5 5	0 0	0 0	0 0	0
Benzocaine control	20	48 72	0 0	11 11	9 9	0 0	20

*hours after start of application

Section A6.2 **Annex Point IIA6.2**	**Metabolism studies in mammals**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	SCLPs* like Z,E-9,12-Tetradecadien-1-yl acetate are biodegradable by enzyme systems present in most living organisms, and should not present problems with their normal physiology. It is reasonable to assume that these straight-chained compounds are metabolised similar to long-chain fatty acids. The alcohol moiety of the pheromone is closely related to the essential fatty acid linoleic acid - Z,Z-9,12-octadecadienoic acid. Because of this structural similarity the following metabolic pathway is proposed. The ester Z,E-9,12-Tetradecadien-1-yl acetate is hydrolysed chemically or via esterases to the corresponding alcohol. The alcohol then is oxidised by alcohol dehydrogenases to finally form the corresponding acid Z,E-9,12-Tetradecadienoic acid, which is degraded by β-oxidation to carbon dioxide like other fatty acids. The different configuration of the pheromone (Z,E) and linoleic acid (Z,Z) does not have an influence on metabolism (refer to *Coots R.H, (1964), A comparison of the metabolism of cis, cis-linoleic, trans,trans-linoleic, and a mixture of cis,trans- and trans,cis-linoleic acids in the rat. J. Lipid Res., 5: 473 – 476;* and *Bretillon et al., (2001) Isomerization increases the postprandial oxidation of linoleic acid but not α-linolenic acid in men. J. Lipid Res., 42: 995 - 997*). Thus it is reasonable to assume that Z,E-9,12-Tetradecadien-1-yl acetate is metabolised rapidly and efficiently in the body. This is in agreement with the assumption that SCLPs are metabolised either by β-oxidation yielding a series of paired carbon losses or by conjugation with glucuronide and excretion via the kidneys (Federal Register v. 60, Aug. 30/95). In conclusion, based on the considerations above and in the light of animal welfare, a metabolism study with (9Z,12E)-9,12-Tetradecadien-1-yl acetate is, therefore, not regarded as required. * Straight-Chained Lepidopteran Pheromone	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Evaluation of applicant's justification	Agree with applicant's version
Conclusion	The justification is acceptable.
Remarks	For further considerations of kinetics and metabolism see document II-A 3.1

Section A6.4.1 Annex Point IIA6.4	**Subchronic oral toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [x] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Straight-Chained Lepidopteran Pheromones are of low toxicity to mammals. Subchronic oral toxicity studies were not especially conducted for (9Z,12E)-9,12-Tetradecadien-1-yl acetate but for two structurally similar SCLPs. The US EPA has used the results of two subchronic toxicity studies as bridging data for the safety assessment of other structurally similar SCLP products submitted for registration. Published results of these studies indicated no significant health effects.	X
	A 90-day ~~feeding~~ gavage study (using rats) was conducted at doses up to 1 g/kg, of a commercial blend of branched acetates with an aliphatic chain length between C10 to C14. The results indicated no significant signs of toxicity other than those expected with longer term exposure to high dose of a hydrocarbon, namely, histopathologic evidence of nephropathy in males and increased liver and kidney weights in both sexes. *(Daughtrey, W.C., J.H. Smith, J.P. Hinz and R.W. Biles (1990). "Subchronic toxicity evaluation of tridecyl acetate in rats". Fundamental and Applied Toxicology 14: 104-11.)*	X
	Furthermore, the low toxicity of (9Z,12E)-9,12-Tetradecadien-1-yl acetate is also demonstrated in an acute oral toxicity study, where no adverse effects have been found (Please refer to **Section A6.1.1**).	
	In addition, pheromones are different from conventional chemical biocides: • They act by modifying the behaviour of the pest species rather than killing. • They are effective in very low concentrations. For purposes of pest control, releases of Pheromones have to be close to natural emissions because their effectiveness is dependent on arthropod olfactory systems that are tuned to natural emission rates. Male Lepidoptera typically respond to a discrete range in ambient pheromone concentration, with the consequence that a high rate of pheromone release may be less effective than an intermediate rate of release.	X
	For these reasons it is expected that most pheromones pose lower potential risk to human health and the environment than conventional biocides.	
	<u>Limited exposure:</u> Food or animal feeding stuff is unlikely to be contaminated with the active substance.	
	When lepidopteran pheromones were applied in retrievably-sized dispensers, food residues from airborne transfer of pheromone were not detected; in analyses of fruit treated with 325 to 350 g ai/ha (129 – 141 g ai/acre), no pheromone residues could be found with a detection limit of 2-5 µg/kg (or ppb) (Spittler *et al.*, 1988 and 1992).	
	If a cardboard glue trap is used in a kitchen or a pantry, there will probably be no residues on food either, since (9Z,12E)-9,12-Tetradecadien-1-yl acetate is volatile and will readily undergo photo-oxidation. Therefore the substance is unlikely to deposit on surfaces.	
	Exposure via water or soil is not to be expected because glue traps are made for indoor use.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2008
Evaluation of applicant's justification	We agree that exposure to the a.s. via the application of the representative product (cardboard glue trap) is negligible, for exposure estimates please see document II-B 4, for the intended use description see document II-B 3. The reference to the subchronic oral toxicity studies cited in the OECD Series of Pesticides No. 12 are Daughtrey et al 1990 and Nelson et al 1990. These are studies carried out with a blend of branched acetates with an aliphatic chain length between C10 to C14 (see Doc II-A. 1.5) and with long chain alcohols (see Doc II-A 1.8 and doc III-A 6.8.1), respectively. These substances are by definition not SCLPs.
Conclusion	The justification is acceptable.
Remarks	In order to provide more details from the publication of Daughtrey et al. 1990. (Subchronic toxicity evaluation of tridecyl acetate in rats. Fundamental and Applied Toxicology 14: 104-11) the RMS attached the tables from this publication below.

TABLE I

MEAN BODY WEIGHTS FOR RATS DOSED WITH TRIDECYL ACETATE

Treatment group (g/kg)	Time (days)						
	0	14	28	42	56	70	84
Males							
Control	227 ± 8[a]	330 ± 14	411 ± 21	462 ± 25	501 ± 29	531 ± 36	554 ± 42
0.1	226 ± 10	323 ± 20	394 ± 32	446 ± 39	485 ± 40	515 ± 43	536 ± 45
0.5	226 ± 10	321 ± 18	388 ± 25*	436 ± 32	479 ± 30	505 ± 33	525 ± 32
1.0	227 ± 9	324 ± 17	395 ± 23	443 ± 34	489 ± 36	516 ± 41	537 ± 46
Females							
Control	174 ± 6	210 ± 13	241 ± 15	267 ± 17	280 ± 21	292 ± 21	301 ± 27
0.1	173 ± 8	211 ± 10	244 ± 15	267 ± 16	282 ± 13	300 ± 16	310 ± 16
0.5	173 ± 8	214 ± 15	243 ± 17	267 ± 18	278 ± 17	292 ± 17	299 ± 18
1.0	174 ± 8	212 ± 15	240 ± 17	260 ± 16	274 ± 21	283 ± 24	293 ± 26

[a] Mean ± SD expressed in grams.

* Significantly different from control ($p \leq 0.05$).

Section A6.4.2 **Annex Point IIA6.4.**	**Subchronic dermal toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [x]**	
Detailed justification:	A subchronic dermal toxicity study is considered to be not necessary, since (9Z,12E)-9,12-Tetradecadien-1-yl acetate is a member of a well characterised group (SCLP*) of low toxicity. In an acute dermal toxicity study with E-11-tetradecenal, the LD50Skin/mg/kg was >5000/rabbit, all animals survived to the 14th day. ([redacted]). *According to the EPA Categories, SCLP are classified as non-toxic (Cat IV).* The low toxicity is also demonstrated in an acute oral toxicity study with (9Z,12E)-9,12-Tetradecadien-1-yl acetate where no adverse effects have been found (Please refer to **Section A6.1.1**). In addition, the end use product is a cardboard glue trap that precludes direct contact to the pheromone. * Straight-Chained Lepidopteran Pheromone	X
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Evaluation of applicant's justification	The EPA evaluations mentioned were not submitted by the applicant and not evaluated by the RMS Austria. Agree with applicant's version.
Conclusion	The justification is acceptable.
Remarks	Also no systemic effects and only minimal irritation was observed in the skin irritation test after 4 hours of exposure with 0.5 ml of (Z,E)-Tetradeca-9,12-dienyl-acetate.

Section A6.4.3 Annex Point IIA6.4	Subchronic inhalation toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	A study of repeated dose toxicity - inhalation - is considered to be not necessary, since the substance is a member of a well characterised group (SCLP*) of low toxicity. The low toxicity is also confirmed in a new limit test ([redacted], refer to Section A6.1.5) that indicates acute inhalation toxicity LC_{50} for (9Z,12E)-9,12-Tetradecadien-1-yl acetate is greater than 5.2 mg/L air. According to the EPA Categories (III -IV) this is practically non-toxic. Furthermore, subchronic oral toxicity studies conducted for two structurally similar SCLP indicated no significant health effects. *(Daughtrey, W.C., J.H. Smith, J.P. Hinz and R.W. Biles (1990) "Subchronic toxicity evaluation of tridecyl acetate in rats". Fundamental and Applied Toxicology 14: 104-11.)* Therefore, there is no concern from toxicological profile.	X
	<u>Application: Low concentration</u> The end use product is a cardboard glue trap. The active substance ((9Z,12E)-9,12-Tetradecadien-1-yl acetat) is completely covered with glue and dissipates over time to a negligible level, primarily by volatilisation and degradation. Saturated vapour concentration calculations by the IBMA (based on the results of Hirooka and Suwanai, 1976), carried out using the worst case scenario vapour pressure found for 12 carbon sex pheromones, gave the following results: 2 mg of (9Z,12E)-9,12-Tetradecadien-1-yl acetate dispersed in 1 m³ gives a concentration of 0.18 ppm. Instead of 2 mg being released instantaneously, a more typical release would be over a 30 day period, therefore the likely concentration is 1/30 of 0.18 ppm, i.e. 0.006 ppm, which is insignificant. *(IBMA Specific Paper on the Four Notified Pheromones On the Attractants and Repellents a.i. List Biocide Directive 98/8/EC, p. 10)*	X
	Due to their biological function, pheromones degrade rapidly. Persistence would be counterproductive to an olfactory system of communication. Therefore no increasing concentrations are considered in the following calculations of a 30 day period. The cardboard glue trap contains 1.2 mg of (9Z,12E)-9,12-Tetradecadien-1-yl acetat). According to the IBMA calculations, this would give the following concentrations: If 1.2 mg a.i. were released in a cupboard of 1 m³ instantaneously, the concentration would be 0.11 ppm.(This is only a theoretical idea, because the active substance is covered by glue and therefore diffuses slowly.) If released over a 30 day period, the concentration would be 0.004 ppm. If the trap were placed in a kitchen of 30 m³ (3 m x 4 m x 2.5 m) instead and the active substance were released over a 30 day period, the concentration would be 0.0001ppm. In addition, the trap is supposed to be effective for at least 6 weeks = 42 days, therefore the concentration would be even lower.	X
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Evaluation of applicant's justification	We agree that exposure to the a.s. via the application of the representative product (cardboard glue trap) is negligible, for exposure estimates please see document II-B 4, for the intended use description see document II-B 3. The reference to the subchronic oral toxicity studies for two structurally similar SCLP is cited in the OECD Series of Pesticides No. 12, but the original studies were not submitted and evaluated by the RMS. The results of the oral 90 day study with a blend of branched acetates with an aliphatic chain length between C10 to C14 are described in document II-A. 1.5. *Daughtrey, W.C., J.H. Smith, J.P. Hinz and R.W. Biles (1990) "Subchronic toxicity evaluation of tridecyl acetate in rats". Fundamental and Applied Toxicology 14: 104-11* Agree with applicant's version.
Conclusion	The justification is acceptable.
Remarks	Also no systemic effects and only minimal irritation was observed in the skin and eye irritation tests. The efficacy was proven only for 1 week, see document III-A.5 and III-B.5 and Appendix II to Document I (list of intended uses).

Section A6.5 **Annex Point IIA6.5**	**Chronic toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	No adverse effects have been found neither in mutagenicity studies (Please refer to 6.6.1-6.6.3) nor in subchronic oral toxicity studies with structurally related compounds (Daughtrey, W.C., J.H. Smith, J.P. Hinz and R.W. Biles 1990). The alcohol moiety of the pheromone Z,E-9,12-tetradecadien-1-yl acetate is closely related to the essential fatty acid linoleic acid - Z,Z-9,12-octadecadienoic acid. Because of this structural similarity the following metabolic pathway is proposed. The ester Z,E-9,12-tetradecadien-1-yl acetate is hydrolysed chemically or via esterases to the corresponding alcohol. The alcohol then is oxidised by alcohol dehydrogenases to finally form the corresponding acid Z,E-9,12-tetradecadienoic acid, which is degraded by β-oxidation like other fatty acids. Thus, it is reasonable to assume that Z,E-9,12-tetradecadien-1-yl acetate is metabolised rapidly and efficiently in the body and no toxic metabolites are expected. Moreover, the application rate is typically very low and likely comparable to natural emissions. Thus the exposure to humans is nearly negligible and far below the recommended dose of the structural closely related essential fatty acid linoleic, which is in the g/day range for an adult. In addition, results of acute toxicity studies (please refer to A6.1.1 and A6.1.3) confirmed the low toxicity of (9Z,12E)-9,12-Tetradecadien-1-yl acetate. Therefore, no adverse effects are to be expected and there is no concern from toxicological profile. It should also be taken into consideration that there are inherent differences between pheromones and conventional chemical biocides. Pheromones act by modifying the behaviour of the pest species rather than killing, are more target specific than conventional insecticides, are used at concentrations close to those in nature, and dissipate rapidly. For these reasons it is expected that most pheromones, for instance SCLPs*, pose much lower potential risk to human health and the environment than conventional biocides. Furthermore, the US EPA has received no reports of adverse effects to	

	humans or the environment arising from their policy concerning pheromones: In 1994 the US Environmental Protection Agency (US EPA) exempted arthropod pheromones from the requirement of an experimental use permit for trials on up to 250 acres, at a rate of up to 375 g ai/ha/yr (150 g ai/acre/yr). A threshold of 375 g ai/ha/yr was established as high enough to accommodate the maximum reasonable use level that companies would require for testing. As this level is comparable to naturally occurring emissions of pheromones during an infestation, it is expected to have no impact on public health, non-target organisms, or the environment. In conclusion, based on the considerations above and in the light of animal welfare, a chronic toxicity study with (9Z,12E)-9,12-Tetradecadien-1-yl acetate is, therefore, not regarded as required. * Straight-Chained Lepidopteran Pheromone	x
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2008	
Evaluation of applicant's justification	The calculation or measurement of the potential natural background level of 375 g ai/ha/y is not traceable in the dossier. However, due to the low toxicity within the studies and literature data submitted and due to the low expected exposure the justification is considered as acceptable.	
Conclusion	The non-submission of the chronic toxicity study is acceptable.	
Remarks	The arguments for waiving the chronic/carcinogenicity and the reproductive toxicity studies are summarized in doc IIA 3.7 and 8.	

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1

Gene mutation in bacteria

Mutagenicity Study in the Salmonella Typhimurium Reverse Mutation Assay in vitro

Official use only

1 REFERENCE

1.1	**Reference**	Leuschner, J. (2006), MUTAGENICITY STUDY OF Z,E-9,12-TETRADECADIEN-1-YL ACETATE IN THE *SALMONELLA TYPHIMURIUM* REVERSE MUTATION ASSAY (*IN VITRO*) LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany, LPT Report No. 19785/06 Dates of work: January 25, 2006 - March 2, 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Aeroxon Insect Control GmbH
1.2.2	Companies with letter of access	Not applicable
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EC Method B.13/14. (2000/32/EC) and OECD Guideline 471
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

4.1	**Test material**	Z,E-9,12-Tetradecadien-1-yl acetate
3.1.1	Lot/Batch number	Batch no. 2005340-0010
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Colourless liquid
3.1.2.2	Purity	99.5%
3.1.2.3	Stability	December 6, 2009 (expected shelf life)
4.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	*S. typhimurium*: TA 98, TA 100, TA 102, TA 1535, TA 1537
3.2.2	Deficiencies / Proficiencies	Histidine-auxotrophic strains
3.2.3	Metabolic activation system	S9 mix Post-mitochondrial fraction (S9 fraction) from rat liver treated with Aroclor 1254 was prepared according to MARON and AMES (1983). S9 was collected from 20 - 30 rats.
3.2.4	Positive control	See **Table A6.6.1-1**
4.3	**Administration / Exposure; Application of test substance**	

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1

Gene mutation in bacteria

Mutagenicity Study in the Salmonella Typhimurium Reverse Mutation Assay in vitro

3.3.1	Concentrations	Main study: plate incorporation test: 31.6, 100, 316, 1000 and 3160 µg Z,E-9,12-tetradecadien-1-yl acetate /plate each carried out without and with metabolic activation. preincubation test: 0.316, 1.0, 3.16, 10 and 31.6 µg Z,E-9,12-tetradecadien-1-yl acetate /plate each carried out without and with metabolic activation. Note: Z,E-9,12-tetradecadien-1-yl acetate was examined in two preliminary cytotoxicity tests (plate incorporation test and preincubation test) without metabolic activation in test strain TA 100. Ten concentrations ranging from 0.316 to 5000 µg Z,E-9,12-tetradecadien-1-yl acetate/plate were tested. Cytotoxicity (scarce background lawn and reduction of the number of revertants) was noted at concentrations of 3160 and 5000 µg/plate or from concentrations of 31.6 µg/plate onwards in the plate incorporation test or in the preincubation test, respectively. Hence, 3160 µg/plate was chosen as the top concentration for the main study for the plate incorporation test and 31.6 µg/plate for the preincubation test.
3.3.2	Way of application	The test components were mixed and then poured onto a minimal agar plate. Z,E-9,12-tetradecadien-1-yl acetate was dissolved in ethanol.
3.3.3	Pre-incubation time	20 minutes at 37°C
3.3.4	Other modifications	Quality criteria The genotypes of the test strains were confirmed for each batch in the following way: a) Histidine and biotin requirement ((his^-) (bio^-)): Each of the five strains was streaked onto two Vogel-Bonner medium E plates in the following way: After incubation at 37°C for 24 hours, none of the strains should have grown on plate 2; all strains should have shown excessive growth on plate 1. b) (rfa^-) deep rough character: 10 µL of 0.1% crystal violet applied with a paper disc should have given zones of inhibition in all test strains after incubation at 37°C for 24 hours. c) UV-sensitivity ($uvr\ B^-$): Plates were covered partly with black paper and placed under germicidal UV-irradiation. After incubation at 37°C for 24 hours, all strains except TA 102 should have grown only under the covered portion of each plate. TA 102 should also have grown under the uncovered area. d) Ampicillin-resistance (pKM 101): 0.8 mg ampicillin/plate was placed onto plates seeded with bacteria: Absence of zones of inhibition around the discs indicate resistance to ampicillin (TA 98, TA 100 and TA 102), whereas strains TA 1535 and TA 1537 show zones of inhibition.
4.4	**Examinations**	See tables in appendix for examinations and results

Section A6.6.1

Annex Point IIA6.6.1

Genotoxicity in vitro

Gene mutation in bacteria

Mutagenicity Study in the Salmonella Typhimurium Reverse Mutation Assay in vitro

3.4.1	Number of cells evaluated	The revertant colonies on the test plates and on the control plates were counted with a colony counter, and the presence of the background lawn on all plates was confirmed. See **Table A6.6.1-2**
		RESULTS AND DISCUSSION
4.5	**Genotoxicity**	
3.5.1	without metabolic activation	No
3.5.2	with metabolic activation	No
4.6	**Cytotoxicity**	Pre-tests revealed cytotoxicity in strain TA 100 when tested in the absence of S9 at concentrations of 3160 and 5000 µg/plate or from concentrations of 31.6 µg/plate onwards in the plate incorporation test or in the preincubation test, respectively
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	EC Method B.13/14. (2000/32/EC) and OECD Guideline 471 Method: In vitro bacterial reverse mutation assay in Salmonella typhimurium, two independent experiments (plate incorporation and preincubation test) without and with metabolic activation, solvent and positive control. No deviations from the guidelines
5.2	**Results and discussion**	No mutagenic effect (no increase in revertant colony numbers as compared with control counts) was observed for Z,E-9,12-tetradecadien-1-yl acetate tested up to cytotoxic concentrations of 3160 or 31.6 µg/plate in any of the 5 test strains in two independent experiments without and with metabolic activation (plate incorporation and preincubation test), respectively.
5.3	**Conclusion**	Z,E-9,12-tetradecadien-1-yl acetate tested up to cytotoxic concentrations caused no mutagenic effect in the Salmonella typhimurium strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 neither in the plate incorporation test nor in the preincubation test each carried out without and with metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2008
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	

Table A6.6.1-1 Positive control substances and dose levels

Strain	Without metabolic activation (µg/plate):		With metabolic activation (µg/plate):	
TA 98	2-nitro-fluorene in DMSO*	10	2-amino-anthracene in DMSO	2
TA 100	sodium azide in H_2O	10	cyclophosphamide in *aqua ad iniectabilia*	1500
TA 102	methyl methane sulfonate (MMS)in DMSO	1300	2-amino-anthracene in DMSO	2
TA 1535	sodium azide in H_2O	10	cyclophosphamide in *aqua ad iniectabilia*	1500
TA 1537	9-amino-acridine in ethanol, abs.	100	2-amino-anthracene in DMSO	2

* dimethyl sulfoxide

Table A6.6.1-2

Summarised data Preincubation test/ Plate incorporation test with and without metabolic activation

Plate incorporation test

***without* metabolic activation**

test item (µg/plate)		Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Z,E-9,12-tetradecadien-1-yl acetate						
3160	mean	23,0 #	112,3 #	217,3 #	12,3 #	6,0 #
	SD	4,4	6,4	18,5	2,5	1,0
1000	mean	27,7	120,3	260,3	14,7	4,3
	SD	3,1	5,0	13,9	0,6	1,2
316	mean	32,0	125,7	263,3	14,0	5,3
	SD	1,7	4,0	4,0	1,7	2,1
100	mean	24,7	128,7	272,0	13,7	5,0
	SD	1,2	3,5	7,0	1,5	1,7
31,6	mean	32,3	127,3	263,0	13,0	7,0
	SD	1,5	4,0	5,3	1,7	2,6
Negative reference item 50 µL/plate						
	mean	33,3	163,7	276,3	16,3	9,0
	SD	6,4	4,5	18,5	2,1	1,0
Positive reference item		2-Nitro-fluorene	Sodium azide	Methyl-methane sulfonate	Sodium azide	9-Amino-acridine
Concentration µg/plate		10	10	1300	10	100
	mean	577,7	878,7	1254,3	590,7	579,0
	SD	9,9	14,7	6,5	2,3	4,4

Preincubation test

***without* metabolic activation**

test item (µg/plate)		Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Z,E-9,12-tetradecadien-1-yl acetate						
31,6	mean	15,0 #	130,3 #	232,7 #	10,0 #	2,7 #
	SD	2,6	11,0	3,2	1,0	0,6
10	mean	22,3	152,3	266,3	14,0	5,3
	SD	2,5	16,2	4,9	1,7	0,6
3,16	mean	27,0	137,7	288,0	13,7	6,0
	SD	2,6	5,5	8,7	2,1	1,0
1	mean	23,3	146,0	278,0	15,0	5,3
	SD	0,6	20,2	4,0	1,0	0,6
0,316	mean	32,7	160,0	272,3	14,7	5,3
	SD	2,5	5,6	7,6	0,6	1,5
Negative reference item 50 µL/plate						
	mean	41,0	126,3	277,7	17,3	8,0
	SD	1,7	12,7	7,6	2,1	1,0
Positive reference item		2-Nitro-fluorene	Sodium azide	Methyl-methane sulfonate	Sodium azide	9-Amino-acridine
Concentration µg/plate		10	10	1300	10	100
	mean	444,3	820,3	1294,3	359,3	243,7
	SD	13,6	4,0	55,1	52,6	15,9

# = scarce background lawn
SD = standard deviation

Plate incorporation test
with **metabolic activation**

test item (µg/plate)		Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Z,E-9,12-tetradecadien-1-yl acetate						
3160	mean	9,7 #	77,0 #	194,3 #	11,7 #	7,0 #
	SD	2,1	2,0	5,9	0,6	1,0
1000	mean	12,7	129,0	253,3	13,7 #	6,0 #
	SD	3,1	13,1	3,8	1,5	1,0
316	mean	21,0	147,3	280,3	14,0	7,3
	SD	1,0	5,5	18,6	1,0	0,6
100	mean	21,0	140,7	270,0	14,0	6,7
	SD	1,0	23,9	11,4	1,7	1,5
31,6	mean	21,3	139,7	265,3	13,7	7,7
	SD	1,2	11,4	3,1	1,5	0,6
Negative reference item 50 µL/plate						
	mean	34,0	152,7	267,3	19,3	9,7
	SD	8,7	2,1	22,7	1,5	0,6
Positive reference item		2-Amino-anthracene	Cyclophos-phamide	2-Amino-anthracene	Cyclophos-phamide	2-Amino-anthracene
Concentration µg/plate		2	1500	2	1500	2
	mean	509,7	867,0	1045,0	578,0	581,7
	SD	6,8	13,5	49,6	6,6	1,2

Preincubation test
with **metabolic activation**

test item (µg/plate)		Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Z,E-9,12-tetradecadien-1-yl acetate						
31,6	mean	15,3 #	92,0 #	225,0 #	10,7 #	2,3 #
	SD	2,1	4,0	4,0	1,2	0,6
10	mean	26,0	135,7	253,7	15,3	4,7
	SD	1,7	4,0	1,5	0,6	0,6
3,16	mean	26,7	133,3	261,0	15,7	5,0
	SD	0,6	1,2	5,0	2,1	1,0
1	mean	29,7	135,0	265,3	16,0	5,7
	SD	1,5	6,6	6,7	1,0	1,2
0,316	mean	24,7	134,0	269,0	15,7	6,0
	SD	5,0	2,6	9,8	1,2	1,0
Negative reference item 50 µL/plate						
	mean	29,0	144,3	281,7	17,7	8,3
	SD	6,2	7,6	4,0	1,5	0,6
Positive reference item		2-Amino-anthracene	Cyclophos-phamide	2-Amino-anthracene	Cyclophos-phamide	2-Amino-anthracene
Concentration µg/plate		2	1500	2	1500	2
	mean	735,7	921,3	1291,3	408,0	236,3
	SD	19,9	8,4	54,0	15,4	15,7

# = scarce background lawn
SD = standard deviation

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**

Cultured human peripheral lymphocytes

			Official use only
		1 REFERENCE	
1.1	**Reference**	Leuschner, J. (2006), IN VITRO ASSESSMENT OF THE CLASTOGENIC ACTIVITY OF Z,E-9,12-TETRADECADIEN-1-YL ACETATE IN CULTURED HUMAN PERIPHERAL LYMPHOCYTES LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany, LPT Report No. 19786/06 Dates of work: January 25, 2006 - April 10, 2006	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Aeroxon Insect Control GmbH	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EC method B.10. (2000/32/EC) and OECD guideline 473	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Z,E-9,12-Tetradecadien-1-yl acetate	
3.1.1	Lot/Batch number	Batch no. 2005340-0010	
3.1.2	Specification	As given in section 2:	
3.1.2.1	Description	Colourless liquid	
3.1.2.2	Purity	99.5%	
3.1.2.3	Stability	December 6, 2009 (expected shelf life)	
3.2	**Study Type**	In Vitro mammalian chromosome aberration test	
3.2.2	Organism/cell type	Human peripheral lymphocytes (from healthy donors without any medication) were established in complete culture medium containing small inocula of whole blood. <u>primary cultures:</u> lymphocytes	
3.2.3	Deficiencies / Proficiencies	Not applicable	
3.2.4	Metabolic activation system	S9 mix Post-mitochondrial fraction (S9 fraction) from rat liver treated with Aroclor 1254 was prepared according to MARON and AMES (1983). S9 was collected from 20 - 30 rats.	

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**

Cultured human peripheral lymphocytes

3.2.5	Positive control	Mitomycin C was used as the positive control for the study in the absence of metabolic activation, and cyclophosphamide as the positive control for the study in the presence of metabolic activation.
3.3	**Administration / Exposure; Application of test substance**	
3.3.2	Concentrations	0 (solvent, i.e. ethanol) 312.5, 625, 1250, 2500, 5000 µg Z,E-9,12-tetradecadien-1-yl acetate/mL in the experiments with 4-h exposure (without and with metabolic activation) 6.3, 12.5, 25, 50, 100 µg Z,E-9,12-tetradecadien-1-yl acetate/mL in the experiment with 24-h exposure (without metabolic activation) Note: A preliminary toxicity study was conducted at concentrations of 0 to 5000 µg/mL to establish the top concentration for the main cytogenetic test. At least three analysable concentrations were used for the main test. These concentrations covered a range from the maximum to little or no toxicity; this usually means that the concentrations are separated by no more than a factor between 2 and √10. At the time of harvesting, the highest concentration showed a significant reduction in the mitotic index (greater than 50%). For relatively non-cytotoxic compounds the maximum concentration would be 5 µL/mL or 5 mg/mL, or 0.01 M, whichever is lowest. In this preliminary experiment, pronounced cytotoxicity in form of destroyed cells was noted at concentrations of 100 µg Z,E-9,12-tetradecadien-1-yl acetate/mL and above in the experiments without activation (24-h exposure). Hence, the top concentration employed in the main study was 5000 µg Z,E-9,12-tetradecadien-1-yl acetate/mL in the experiments with 4-h exposure (without and with metabolic activation) and 100 µg Z,E-9,12-tetradecadien-1-yl acetate/mL in the experiment with 24-h exposure (without metabolic activation). See **Table A6.6.2-6**
3.3.3	Way of application	Z,E-9,12-tetradecadien-1-yl acetate was dissolved in ethanol to the appropriate concentrations prior to the treatment of the cells. After 48 hours of culture in complete medium the cell pellet was resuspended to 4.5 mL (for S9 mix addition) or 5.0 mL with treatment medium including test item at the final concentrations. S9 mix (0.5 mL) was added to the appropriate cultures. Treatments were added at a volume 50 µL.

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**

Cultured human peripheral lymphocytes

3.3.4	Incubation time	The cultures were incubated at 37°C for 4 hours with shaking, after which the cultures were washed, resuspended in a volume of 5 mL of complete medium and again incubated for 20 hours. (The incubation procedure took place in the dark). Two hours before termination the cell division was arrested by the addition of 0.5 mL of a 10 µg/mL solution of colcemid to each culture. The cultures were left to incubate for a further2 hours. The cells were harvested by low speed centrifugation and the pellets of cells thus collected were resuspended in hypotonic potassium chloride solution (0.56%) and later fixed in freshly prepared methanol: glacial acetic acid fixative (4:1, v/v). See **Table A6.6.2-1.**
3.3.5	Other modifications	Not applicable
3.4	**Examinations**	
3.4.2	Number of cells evaluated	The tubes were centrifuged, the fixative removed and the cell pellet resuspended in a few drops of 60% acetic acid. Single drops of the cell suspension were spread on clean, grease-free glass slides, and the slides were left to air-dry. Two slides were made from each culture, stained for 30 minutes in Giemsa stain (1:10 in WEISE's buffer pH 6.8), washed in buffer and left to air-dry on a hot plate (approx. 50°C). The slides received code numbers randomly chosen by a computer. The slides were examined under low power (x 100 objective) and those areas judged to be of sufficient technical quality were located and examined under high power (x 1000, oil immersion objective). For each treatment and culture, 100 metaphases were examined, if possible. Observed abberations were noted and scored according to J.R.K. SAVAGE (1975). In addition, the total number of gaps was recorded in 100 metaphases for each culture. Metaphases which differed from the normal diploid complement (46) were excluded from evaluation. However, test item-related variations of the normal chromosome number were noted (polyploidy / endoreduplication). To examine the toxicity of the test item, 1000 cells were scored and the mitotic index was calculated as the percentage of cells in metaphase.

Section A6.6.2 Annex Point II6.6.2	Genotoxicity in vitro Cytogenicity in mammalian cells Cultured human peripheral lymphocytes	
6.4.6 Statistical evaluation	It is generally accepted that chromatid gaps are not examples of true chromosomal aberrations. In this study, therefore only the total numbers of cells with aberrations exclusive of gap damage were analysed. However, the numbers of cells with aberrations including gap damage are also tabulated. The following concentrations were so far not evaluated, as it was thought that they would provide no further information: 312.5 µg Z,E-9,12-tetradecadien-1-yl acetate/mL (in the experiments without and with metabolic activation, 4-h exposure) 0.1 µg Mitomycin C/mL (in the experiment without metabolic activation, 4-h exposure) 0.2 µg Mitomycin C/mL (in the experiment without metabolic activation, 24-h exposure) 100 µg Z,E-9,12-tetradecadien-1-yl acetate/mL (in the experiment without metabolic activation, 24-h exposure) 10 µg cyclophosphamide/mL (in the experiments with metabolic activation, 4-h exposure)	
	RESULTS AND DISCUSSION	
3.5 Genotoxicity		
3.5.2 without metabolic activation	The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with Z,E-9,12-tetradecadien-1-yl acetate at concentrations from 625 to 2500 µg/mL (4-h exposure) or 6.3 to 50 µg/mL medium (24-h exposure) in the absence of metabolic activation ranged from 0.0% to 3.5%. The results obtained are considered to be within the normal range of the solvent control where a mean incidence of chromosomal aberrations (excluding gaps) of 3.0% or 0.5% was observed after a 4-hour or 24-hour exposure respectively. Only at the pronounced cytotoxic concentration of 5000 µg/mL medium (4-h exposure), a marginal, though not significant, increase was noted in the number of aberrations to 4.3%. It is known that high cytotoxicity causes artefacts in the form of aberrations in in vitro chromosomal tests. Hence, the increase at the concentration of 5000 µg Z,E-9,12-tetradecadien-1-yl acetate/mL medium is considered as artefact and not test item-related. See **Table A6.6.2-3** and **Table A6.6.2-5**	

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**

Cultured human peripheral lymphocytes

3.5.3	with metabolic activation	Test with metabolic activation (4-hour exposure) The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with Z,E-9,12-tetradecadien-1-yl acetate at concentrations from 625 to 5000 µg/mL medium in the presence of metabolic activation ranged from 0.0% to 2.5%. The results obtained are considered to be within the normal range of the solvent control where a mean incidence of chromosomal aberrations (excluding gaps) of 2.0% or 0.5% was observed in the first and second experiment respectively. See **Table A6.6.2-4** and **Table A6.6.2-5**
3.6	**Cytotoxicity**	The concentrations employed were chosen based on the results of a cytotoxicity study. In this preliminary experiment, pronounced cytotoxicity was noted at concentrations of 100 µg Z,E-9,12-tetradecadien-1-yl acetate/mL onwards in the experiment without metabolic activation (24-h exposure). No cytotoxicity was noted after the 4-hour exposure in the preliminary experiment with metabolic activation. Hence, the top concentrations employed in the main study were 5000 µg Z,E-9,12-tetradecadien-1-yl acetate/mL in the experiments without and with metabolic activation (4-h exposure) and 100 µg Z,E-9,12-tetradecadien-1-yl acetate/mL in the experiment without metabolic activation for an exposure time of 24 hours. Cytotoxicity was noted in the main study at the top concentration of 5000 µg Z,E-9,12-tetradecadien-1-yl acetate/mL for the 4-h exposure at 25 and 50 µg/mL for the 24-h exposure in the experiments without metabolic activation and at the top concentration of 5000 µg/mL in the second experiment with metabolic activation (4-h exposure). See **Table A6.6.2-2.**

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**

Cultured human peripheral lymphocytes

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

EC method B.10. (2000/32/EC) and OECD guideline 473

Method: In Vitro mammalian chromosome aberration test, two sets of experiment: two exposure times (without metabolic activation) and one exposure time (with metabolic activation); solvent and positive control.

No deviations from the guidelines.

4.2 Results and discussion

Tests without metabolic activation (4- and 24-hour exposure)

The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with Z,E-9,12-tetradecadien-1-yl acetate at concentrations from 625 to 2500 µg/mL (4-h exposure) or 6.3 to 50 µg/mL medium (24-h exposure) in the absence of metabolic activation ranged from 0.0% to 3.5%. The results obtained are considered to be within the normal range of the solvent control where a mean incidence of chromosomal aberrations (excluding gaps) of 3.0% or 0.5% was observed after a 4-hour or 24-hour exposure respectively.

Test with metabolic activation (4-hour exposure) The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with Z,E-9,12-tetradecadien-1-yl acetate at concentrations from 625 to 5000 µg/mL medium in the presence of metabolic activation ranged from 0.0% to 2.5%. The results obtained are considered to be within the normal range of the solvent control, where a mean incidence of chromosomal aberrations (excluding gaps) of 2.0% or 0.5% was observed in the first and second experiment respectively.

In the same test, Mitomycin C and cyclophosphamide induced significant damage.

4.3 Conclusion

Z,E-9,12-tetradecadien-1-yl acetate tested up to cytotoxic concentrations in the absence and in the presence of metabolic activation employing two exposure times (without S9) and one exposure time (with S9) revealed no indications of mutagenic properties with respect to chromosomal or chromatid damage.

4.3.2 Reliability: 1

4.3.3 Deficiencies: No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2008
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Cultured human peripheral lymphocytes

Table A6.6.2-1 Timetable for Treatments in Experiments 1 and 2

Experiment 1:

Hours	Absence of S9 4-h exposure / 24-h sampling	Presence of S9 4-h exposure / 24-h sampling
0	commence treatment	commence treatment
+ 4	remove treatment medium, wash and add recovery medium	remove treatment medium, wash and add recovery medium
+ 22	add colcemid	add colcemid
+ 24	harvest, prepare slides	harvest, prepare slides

Experiment 2:

Hours	Absence of S9 24-h exposure / 24-h sampling	Hours	Presence of S9 4-h exposure / 24-h sampling
0	commence treatment	0	commence treatment
	24-hour continuous treatment	+4	remove treatment medium, wash and add recovery medium
+ 22	add colcemid	+22	add colcemid
+ 24	harvest, prepare slides	+24	harvest, prepare slides

Section A6.6.2 **Genotoxicity in vitro**

Annex Point II6.6.2 **Cytogenicity in mammalian cells**

Cultured human peripheral lymphocytes

Table A6.6.2-2 Table Cytotoxicity

TABLE 1 **Estimation of the cytotoxicity of Z,E-9,12-tetradecadien-1-yl acetate in human peripheral lymphocytes**

Concentration Z,E-9,12-tetradecadien-1-yl acetate (µg/mL medium)	S9 mix	Metaphases/ 1000 cells	Mitotic index#
		24-hour exposure	
0	-	22	1.00
10	-	32	1.45
25	-	27	1.23
100	-	12 d	0.55
250	-	0 d	0.00
1000	-	4 d	0.18
2500	-	11 d	0.50
5000	-	15 d	0.68
		4-hour exposure	
0	+	47	1.00
10	+	41	0.87
25	+	41	0.87
100	+	43	0.91
250	+	12	0.26
1000	+	40	0.85
2500	+	54	1.15
5000	+	59	1.26

\# mitotic index: number of metaphases/1000 cells; solvent control = 1.00

\+ with metabolic activation

\- without metabolic activation

d destroyed cells

Table A6.6.2-3. Chromosome Analysis: Without Metabolic Activation

Z,E-9,12-tetradecadien-1-yl acetate

Table 2a

Summary: Chromosome analysis in human peripheral lymphocytes in vitro

without metabolic activation

	1st experiment			2nd experiment			
	4-h exposure			24-h exposure			
Treatment (µg/mL medium)	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps	Treatment (µg/mL medium)	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps
Ethanol							
0	1.00	200	3.0	0	1.00	200	0.5
Z,E-9,12-tetradecadien-1-yl acetate							
625	0.85	200	3.5	6.3	1.14	200	0.5
1250	0.89	200	3.5	12.5	1.30	200	0.0
2500	0.60	200	3.5	25	0.89	187 ##	1.6
5000	0.73	47 ##	4.3	50	0.50	100 ##	0.0
Mitomycin C							
0.2	0.90	200	14.0 s.	0.1	1.07	200	10.0 s.

\# = mitotic index: number of metaphases/1000 cells; negative control = 1.00

s. = significantly different from negative control ($p \leq 0.05$)

= no more metaphases of sufficient quality for evaluation due to cytotoxicity of Z,E-9,12-tetradecadien-1-yl acetate

Table A6.6.2-4. Table Chromosome Analysis with Metabolic Activation

Z,E-9,12-tetradecadien-1-yl acetate

Table 2b

Summary: Chromosome analysis in human peripheral lymphocytes in vitro

with metabolic activation

	1st experiment			2nd experiment			
	4-h exposure			4-h exposure			
Treatment (µg/mL medium)	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps	Treatment (µg/mL medium)	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps
Ethanol							
0	1.00	200	2.0	0	1.00	200	0.5
Z,E-9,12-tetradecadien-1-yl acetate							
625	1.08	200	2.0	625	1.37	200	1.0
1250	1.28	200	2.5	1250	1.45	200	1.0
2500	0.84	200	1.5	2500	1.30	200	0.0
5000	1.11	200	1.5	5000	0.98	142 ##	0.7
Cyclophosphamide							
20	0.82	200	13.5 s.	20	0.76	200	12.0 s.

\# = mitotic index: number of metaphases/1000 cells; negative control = 1.00

s. = significantly different from negative control ($p \leq 0.05$)

= no more metaphases of sufficient quality for evaluation due to cytotoxicity of Z,E-9,12-tetradecadien-1-yl acetate

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Cultured human peripheral lymphocytes

Table A6.6.2-5. Table Background data: Solvent controls, Positive controls

Background data

The incidence of chromosomal aberrations (excluding gaps) of the solvent controls and positive controls Mitomycin C and cyclophosphamide without and with metabolic activation for the last 39 experiments (most recent background data, not audited by the QAU-department) are given as follows:

	Solvent controls#			
	Without activation		**With activation**	
	1st experiment 4-h exposure	**2nd experiment 24-h exposure**	**1st experiment 4-h exposure**	**2nd experiment 4-h exposure**
	% of cells with aberrations excluding gaps			
mean	1.0	1.2	1.2	1.2
SD	1.0	1.0	0.9	1.0
range	0.0 – 4.0	0.0 – 4.0	0.0 – 3.0	0.0 – 4.0

	Positive controls#			
	Without activation (Mitomycin C)		**With activation (cyclophosphamide)**	
	1st experiment 4-h exposure	**2nd experiment 24-h exposure**	**1st experiment 4-h exposure**	**2nd experiment 4-h exposure**
	% of cells aberrations excluding gaps			
mean	10.4	12.9	12.1	11.4
SD	2.7	4.5	3.6	3.3
range	6.0 – 18.0	7.0 – 29.0	6.0 – 26.0	5.0 – 20.0

data obtained from analysis of 100 metaphases per plate in 39 experiments performed during October 2003 to December 2004; the control data of this study are not included in the background data.

SD standard deviation

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**

Cultured human peripheral lymphocytes

Table A6.6.2-6 Concentrations

The following dose levels (concentrations in the medium) were established:

Culture number	Compound	Concentration (µg/mL medium)	S9 mix
4-hour exposure			
	conduct: February 20 to February 23, 2006		
1, 9	ethanol	0	-
6, 14	Z,E-9,12-tetradecadien-1-yl acetate	312.5	-
5, 13	Z,E-9,12-tetradecadien-1-yl acetate	625	-
4, 12	Z,E-9,12-tetradecadien-1-yl acetate	1250	-
3, 11	Z,E-9,12-tetradecadien-1-yl acetate	2500	-
2, 10	Z,E-9,12-tetradecadien-1-yl acetate	5000	-
8, 16	Mitomycin C	0.1	-
7, 15	Mitomycin C	0.2	-
	conduct: February 20 to February 23, 2006		
1, 9	ethanol	0	+
6, 14	Z,E-9,12-tetradecadien-1-yl acetate	312.5	+
5, 13	Z,E-9,12-tetradecadien-1-yl acetate	625	+
4, 12	Z,E-9,12-tetradecadien-1-yl acetate	1250	+
3, 11	Z,E-9,12-tetradecadien-1-yl acetate	2500	+
2, 10	Z,E-9,12-tetradecadien-1-yl acetate	5000	+
8, 16	Cyclophosphamide	10	+
7, 15	Cyclophosphamide	20	+
	conduct: February 27 to March 2, 2006		
1, 9	ethanol	0	+
6, 14	Z,E-9,12-tetradecadien-1-yl acetate	312.5	+
5, 13	Z,E-9,12-tetradecadien-1-yl acetate	625	+
4, 12	Z,E-9,12-tetradecadien-1-yl acetate	1250	+
3, 11	Z,E-9,12-tetradecadien-1-yl acetate	2500	+
2, 10	Z,E-9,12-tetradecadien-1-yl acetate	5000	+
8, 16	Cyclophosphamide	10	+
7, 15	Cyclophosphamide	20	+
24-hour exposure			
	conduct: February 27 to March 2, 2006		
1, 9	ethanol	0	-
6, 14	Z,E-9,12-tetradecadien-1-yl acetate	6.3	-
5, 13	Z,E-9,12-tetradecadien-1-yl acetate	12.5	-
4, 12	Z,E-9,12-tetradecadien-1-yl acetate	25	-
3, 11	Z,E-9,12-tetradecadien-1-yl acetate	50	-
2, 10	Z,E-9,12-tetradecadien-1-yl acetate	100	-
8, 16	Mitomycin C	0.1	-
7, 15	Mitomycin C	0.2	-

S9 mix: + with metabolic activation
- without metabolic activation

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3

In vitro gene mutation in mammalian cells

Mouse lymphoma L5178 cells/TK Locus

Official use only

1 REFERENCE

1.1	**Reference**	Stien, J. (2006), MUTAGENICITY STUDY OF Z,E-9,12-TETRADECADIEN-1-YL ACETATE IN THE MOUSE LYMPHOMA FORWARD MUTATION ASSAY LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany, LPT Report No. 19786/06 Dates of work: January 25, 2006 - April 27, 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Aeroxon Insect Control GmbH
1.2.2	Companies with letter of access	Not applicable
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EC method B.17. (2000/32/EC) and OECD guideline 476
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	2005340-0010
3.1.2	Specification	As given in section 2:
3.1.2.1	Description	Colourless liquid
3.1.2.2	Purity	99.5%
3.1.2.3	Stability	Stable at room temperature
3.2	**Study Type**	
3.2.2	Organism/cell type	Mouse lymphoma L5178Y cells
3.2.3	Deficiencies / Proficiencies	TK+/- genotype
3.2.4	Metabolic activation system	S9 mix. S9 Fraction: microsomal fraction S9, derived from homogenates of livers from male Wistar rats induced with Aroclor 1254
3.2.5	Positive control	Methylmethanesulfonate (-S9) and 3-methylcholanthrene (+S9)
3.3	**Administration / Exposure; Application of test substance**	

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **In vitro gene mutation in mammalian cells**

Mouse lymphoma L5178 cells/TK Locus

3.3.2	Concentrations	Trial 1: The concentrations 0, 3.9, 7.81, 15.63, 31.25, 62.5 µg/mL were incubated for 3h in the absence of S9-mix. Trial 2: The concentrations 0, 1.95, 3.90, 7.81, 15.63, 31.25 µg/mL were incubated for 24h in the absence of S9-mix. Trial 3: The concentrations 0, 7.81, 15.63, 31.25, 62.5, 125 µg/mL were incubated for 3h in the presence of S9-mix. Trial 4: The concentrations 0, 1.95, 3.90, 7.81, 15.63, 31.25 µg/mL were incubated for 3h in the presence of S9-mix
1.	Way of application	Dissolved in medium (solvent: dimethylsulfoxide)
2.	Incubation time	a) Cell treatment without metabolic activation Cells were exposed to the test substance, solvent and positive control for 3h and 24h at 37°C. b) Cell treatment with metabolic activation (S9 cofactor mix) Cells were exposed to the test substance, solvent and positive control twice for 3h at 37°C.
3.3.3	Other modifications	Not relevant
3.4	**Examinations**	The mutagenicity of the test substance was determined by the mutant frequency (MF), the ratio of mutant cloning efficiency and cloning efficiency. The minimum criterion considered necessary to demonstrate mutagenesis for any given treatment is a mutant frequency that is ≥2 times the concurrent background mutant frequency. The test substance was considered to be mutagenic if a concentration-related increase in MF was observed or if a reproducible positive response for at least one of the test substance concentrations was observed. A test item is evaluated as non-mutagenic in a single assay only if the minimum increase in mutant frequency is not observed for a range of applied concentrations that extends to toxicity causing 10% to 20% relative growth or in the case of relatively non-toxic items, a range or applied concentrations extending to the maximum of 5 mg/mL (or 5 µL/mL) or in the case of non-toxic, insoluble materials, a range of applied concentrations extending to at least twice the solubility limit in culture media.
3.4.2	Number of cells evaluated	Not applicable. Mutant colonies were counted. The mutant frequency is expressed as mutants/10^6 viable cells.
3.4.3	Statistical evaluation	The data were not evaluated for statistical significance.

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3

In vitro gene mutation in mammalian cells

Mouse lymphoma L5178 cells/TK Locus

RESULTS AND DISCUSSION

3.5	**Genotoxicity**	
3.5.2	without metabolic activation	The mutation frequencies of the cultures treated with Z,E-9,12-tetradecadien-1-yl acetate ranged from 19.56 to 43.64 per 10^6 clonable cells in the experiments without metabolic activation. These results were within the range of the solvent controls and, hence, no mutagenicity was observed according to the criteria for assay evaluation.
3.5.3	with metabolic activation	The mutation frequencies of the cultures treated with Z,E-9,12-tetradecadien-1-yl acetate ranged from 14.98 to 39.82 per 10^6 clonable cells in the experiments with metabolic activation. These results were within the range of the solvent controls and, hence, no mutagenicity was observed according to the criteria for assay evaluation.
3.6	**Cytotoxicity**	Cytotoxicity (decreased survival) was noted in a preliminary experiment from concentrations of 100 or 250 µg/mL onwards in the experiments without or with metabolic activation, respectively.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Gene mutations in mammalian cells OECD guideline 476 Deviations: none The test substance Z,E-9,12-tetradecadien-1-yl acetate was examined for its potential to induce gene mutations at the TK-locus of cultured mouse lymphoma L5178Y cells in both the absence and presence of an S9-activation system. Four independent trials in both the absence and the presence of S9-mix activation system were conducted.
4.2	**Results and discussion**	Under the present test conditions, Z,E-9,12-tetradecadien-1-yl acetate, tested up to cytotoxic concentrations in the absence and presence of metabolic activation in two independent experiments, was negative with respect to the mutant frequency in the LK5178Y TK+/- mammalian cell mutagenicity test. Under the same conditions, the positive controls exerted potent mutagenic effects. No change was noted in the ratio of small to large mutant colonies.
4.3	**Conclusion**	It is concluded that Z,E-9,12-tetradecadien-1-yl acetate did not exhibit mutagenic and clastogenic potential at the concentration range investigated.
4.3.2	Reliability	1
4.3.3	Deficiencies	No

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **In vitro gene mutation in mammalian cells**
Mouse lymphoma L5178 cells/TK Locus

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version See tables with mutation frequency below (copied from study report)
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	

Section A6.7 **Annex Point IIA6.7**	**Carcinogenicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [x]**	
Detailed justification:	A carcinogenicity study is considered to be not necessary, since adverse effects have neither been found in repeated dose studies nor in mutagenicity studies. (*Daughtrey et al. (1990). "Subchronic toxicity evaluation of tridecyl acetate in rats". Fundamental and Applied Toxicology 14: 104-11; Leuschner, 2006*). No mutagenicity was observed in genotoxicity assays. Thus there is no hazard for cell transformation by mutations. The substance is a member of a well characterised group (SCLP = Straight-Chained Lepidopteran Pheromone) of low toxicity. Pheromones act by modifying the behaviour of the pest species rather than killing, Therefore it is expected that most pheromones pose much lower potential risk to human health and the environment than conventional biocides. The alcohol moiety of the pheromone Z,E-9,12-Tetradecadien-1-yl acetate is structurally closely related to the essential fatty acid linoleic acid - Z,Z-9,12-octadecadienoic acid. Because of this structural similarity the following metabolic pathway is proposed. The ester Z,E-9,12-tetradecadien-1-yl acetate is hydrolysed chemically or via esterases to the corresponding alcohol. The alcohol is then oxidised by alcohol dehydrogenases to finally form the corresponding acid Z,E-9,12-tetradecadienoic acid, which is degraded by β-oxidation to carbon dioxide like other fatty acids. The different configuration of the pheromone (Z,E) and linoleic acid (Z,Z) does not have an influence on metabolism (please refer to A6_02). Thus it is reasonable to assume that Z,E-9,12-Tetradecadien-1-yl acetate is metabolised rapidly and efficiently in the body. Long term exposure has to be expected. However, considering a standard room of 58 m^3, concentration in air will be below 1 $\mu g/m^3$ and thus result in very low exposure figures. The application rate is very low and likely comparable to natural emissions, and degradation is fast. The exposure to humans is therefore almost negligible and far below the recommended dose of the structurally closely related essential fatty acid linoleic, which is in the g/day range for an adult.	x

	The Z,E-9,12-Tetradecadien-1-yl acetate pheromone is not a bulk chemical. It is used in low quantities in the EU. Following these considerations and in the light of animal welfare, a carcinogenicity study with Z,E-9,12-Tetradecadien-1-yl acetate is not regarded necessary.	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Evaluation of applicant's justification	We agree that exposure to the a.s. via the application of the representative product (cardboard glue trap) is negligible, for exposure estimates please see Document II-B 4, for the intended use description see Document II-B 3. The justification is acceptable.
Conclusion	The non-submission of the carcinogenicity study is acceptable.
Remarks	The arguments for waiving the chronic/carcinogenicity and the reproductive toxicity studies are summarized in doc IIA 3.7 and 8.

Section A6.8.1 **Annex Point IIA6.8.1**	**Teratogenicity Study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [x] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	A developmental toxicity study (using rats), involving inhalation exposure to unbranched, primary alcohols with chain lengths C8 to C10, indicated no detectable developmental toxicity (Nelson et al. 1990). No mutagenicity was observed in genotoxicity assays. Thus there is no hazard for teratogenicity by mutations. The active substance is a member of a well characterised group (SCLP = Straight-Chained Lepidopteran Pheromone) of known low toxicity. Pheromones act by modifying the behaviour of the pest species rather than killing. Therefore it is expected that most pheromones pose much lower potential risk to human health and the environment than conventional biocides. The alcohol moiety of the pheromone Z,E-9,12-Tetradecadien-1-yl acetate is closely related to the essential fatty acid linoleic acid - Z,Z-9,12-octadecadienoic acid. Because of this structural similarity the following metabolic pathway is proposed. The ester Z,E-9,12-Tetradecadien-1-yl acetate is hydrolysed chemically or via esterases to the corresponding alcohol. The alcohol is then oxidised by alcohol dehydrogenases to finally form the corresponding acid Z,E-9,12-Tetradecadienoic acid, which is degraded by β-oxidation to carbon dioxide like other fatty acids. Thus it is reasonable to assume that Z,E-9,12-tetradecadien-1-yl acetate is metabolised rapidly and efficiently in the body. No metabolites with teratogenic activity are expected. In addition, there is no significant exposure potential. Due to their biological function, pheromones are most effective in low concentrations and degrade rapidly. Persistence would be counterproductive to an olfactory system of communication. They act by modifying the behaviour of the pest species rather than killing. The exposure to humans is therefore almost negligible and far below the recommended dose of the structurally closely related essential fatty acid linoleic, which is in the g/day range for an adult. Following these considerations and in the light of animal welfare, teratogenicity studies with (9Z,12E)-9,12-Tetradecadien-1-yl acetate are not regarded necessary.	X
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2008
Evaluation of applicant's justification	Reference is made to the publication from Nelson et al. 1990. (*Developmental toxicology of industrial alcohols: a summary of 13 alcohols administered by inhalation to rats. Toxicology and Industrial Health 6: 3/4, 373-387.)* This review summarizes developmental rat studies carried out with an exposure of 7 hours/day for 19 days of gestation. For the longer chain alcohols 1-octanol, 1-nonanol, 1-decanol no maternal and no fetal effects were observed at 350, 150, 100 mg/m^3, respectively which would support a safe use with low dose exposure. Due to the low vapor pressure of these substances no higher vapor exposure concentrations were achievable.
Conclusion	The non-submission of the developmental toxicity studies is acceptable in case of low exposure.
Remarks 1	The arguments for waiving the chronic/carcinogenicity and the reproductive toxicity studies are summarized in doc IIA 3.7 and 8.
Remarks 2	In order to make results of Nelson et al 1990 more transparent the RMS attached the respective abstract and results table.

NELSON ET AL. 1990

ABSTRACT: The developmental toxicology of 13 industrial alcohols (methanol, ethanol, 1-propanol, isopropanol, 1-butanol, 2-butanol, tertiary-butanol, 1-pentanol, 1-hexanol, 2-ethyl-1-hexanol, 1-octanol, 1-nonanol, and 1-decanol), and the behavioral teratogenicity of 4 of these alcohols, were assessed in a series of experiments. The results of individual alcohols have been published previously, but the present paper summarizes the results in view of structure-activity relationships among these alcohols. The alcohols were administered by inhalation for 7 hours per day (6 hours/day for 1-decanol) on gestation days 1-19 to groups of approximately 15 pregnant Sprague-Dawley rats. For developmental toxicology evaluations, dams were sacrificed on gestation day 20. Fetuses were serially removed, weighed, sexed, and examined for external malformations. The frequency of visceral malformations and variations was determined in one-half of the fetuses, and the frequency of skeletal deviations was determined in the other half. Behavioral teratology endpoints were investigated in groups of 15 pregnant rats exposed to one of four alcohols (ethanol, 1-propanol, 1-butanol, and tertiary-butanol) and also involved groups of 18 male rats which were exposed to the same concentrations of each alcohol for 6 weeks, and then mated to untreated females. In the behavioral teratology evaluations, all litters were culled to eight pups and fostered to unexposed mothers. Offspring were tested from days 10-90 on a series of behavioral tests designed to evaluate neuromotor integrity, activity levels, learning, and memory. Additionally, brains were removed from 10 offspring per group at 21 days of age, and were dissected into cerebrum, cerebellum, brainstem, and midbrain; these samples were assayed for steady-state levels of protein and the neurotransmitters acetylcholine, dopamine, norepinephrine, 5-hydroxytryptamine (serotonin), substance P, B-endorphin, and met-enkephalin. Congenital malformations were noted for methanol, 1-propanol, isopropanol, and 1-butanol, but only at concentrations in excess of 5000 ppm. These concentrations also produced toxicity in the maternal animals; thus, there was little evidence of selective developmental toxicity among the alcohols. Although sporadic behavioral and neurochemical deviations were detected, no consistent pattern of effects was seen for any of the alcohols we tested. It should be noted that alcohols with chain lengths longer than the butyl series could not be generated as vapors at sufficiently high concentrations to produce observable toxicity in the maternal animals. This limits the generality of these findings to the possible developmental effects of these alcohols when taken through other routes of exposure.(ABSTRACT TRUNCATED AT 400 WORDS)

Section A6.8.2 **Annex Point IIA6.8.2**	**Fertility Study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Results of recently conducted studies (see acute toxicity oral, inhalation and gene mutation, please refer to 6.1.1, 6.1.3, 6.6.1-6.1.3), of subchronic oral toxicity studies and of a developmental toxicity study demonstrate the low toxicity of Straight-Chained Lepidopteran Pheromone (SCLP). (*Daughtrey, W.C., J.H. Smith, J.P. Hinz and R.W. Biles 1990; Nelson et al. 1990*). Sex organs are not target for fatty alcohols like (9Z,12E)-9,12-Tetradecadien-1-yl acetate mediated toxicity. The alcohol moiety of the pheromone Z,E-9,12-Tetradecadien-1-yl acetate is closely related to the essential fatty acid linoleic acid - Z,Z-9,12-octadecadienoic acid. Because of this structural similarity the following metabolic pathway is proposed. The ester Z,E-9,12-tetradecadien-1-yl acetate is hydrolysed chemically or via esterases to the corresponding alcohol. The alcohol then is oxidised by alcohol dehydrogenases to finally form the corresponding acid Z,E-9,12-tetradecadienoic acid, which is degraded by β-oxidation to carbon dioxide like other fatty acids. Thus it is reasonable to assume that Z,E-9,12-tetradecadien-1-yl acetate is metabolised rapidly and efficiently in the body. No metabolites are expected, which may be associated with an impact on reproduction. In addition, there is no significant exposure potential. Due to their biological function, pheromones are most effective in low concentrations and degrade rapidly. Persistence would be counterproductive to an olfactory system of communication. They act by modifying the behaviour of the pest species rather than killing. Thus, human exposure to (9Z,12E)-9,12-Tetradecadien-1-yl acetate will be minimal. In the light of these considerations and animal welfare, a fertility study with (9Z,12E)-9,12-Tetradecadien-1-yl acetate is regarded as not required.	x
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Evaluation of applicant's justification	Reference is made to developmental studies published by Nelson et al. 1990. (*Developmental toxicology of industrial alcohols: a summary of 13 alcohols administered by inhalation to rats. Toxicology and Industrial Health 6: 3/4, 373-387.)* This review summarizes developmental rat studies carried out with an exposure of 7 hours/day for 19 days of gestation. For the longer chain alcohols 1-octanol, 1-nonanol, 1-decanol no maternal and no fetal effects were observed at 350, 150, 100 mg/m^3, respectively which would support a safe use with low dose exposure. Due to the low vapor pressure of these substances no higher vapor exposure concentrations were achievable.
Conclusion	The non-submission of the developmental toxicity studies is acceptable in case of low exposure.
Remarks	The arguments for waiving the chronic/carcinogenicity and the reproductive toxicity studies are summarized in doc IIA 3.7 and 8.

Section A6.12.1/2/3/5/6/7/8 Annex Point IIA6.9	Human Case Reports	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [x]**	
Detailed justification:	• Adverse effects are not to be expected, since arthropod semiochemicals are inherently different from conventional pesticides in their nontoxic, target-specific mode of action and natural occurrence. They are generally effective at very low rates, comparable to levels that occur naturally. They are generally volatile and dissipate rapidly in the environment. In addition, this end use product is a cardboard glue trap that presents little direct exposure to humans. All these factors minimise the risk of adverse effects from the use of semiochemicals. • The US EPA has received no reports of adverse effects to humans In 1994 the US Environmental Protection Agency (US EPA) exempted arthropod pheromones from the requirement of an experimental use permit for trials on up to 250 acres, at a rate of up to 375 g ai/ha/yr (150 g ai/acre/yr). A threshold of 375 g ai/ha/yr was established as high enough to accommodate the maximum reasonable use level that companies would require for testing. As this level is comparable to naturally occurring emissions of pheromones during an infestation, it is expected to have no impact on public health, non-target organisms, or the environment. The US EPA has received no reports of adverse effects to humans or the environment arising from this policy.*(OECD Series on Pesticides No. 12, 26-Feb-2002, ENV/JM/MONO(2001)12, page12)*	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Evaluation of applicant's justification	Medical data have to be submitted only if available.
Conclusion	The justification is acceptable.
Remarks	

Section 6.15 Food and feedingstuffs
Annex Point IIIA VI.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [x]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	The application rate of Z,E-9,12-Tetradecadien-1-yl acetate is typically very low (below the 1 µg/m^3 range) and likely comparable to natural emissions. Moreover, its degradation is fast. Occurrence in high concentrations or persistence would be counterproductive to an olfactory system of communication. This pheromone is closely related to the essential fatty acid linoleic acid - Z,Z-9,12-octadecadienoic acid- which occurs in food and is taken up by humans in the g/day range. Due to its structural similarity to linoleic acid it is highly reasonable to assume that Z,E-9,12-tetradecadien-1-yl acetate is metabolised rapidly and efficiently in the body to carbon dioxide (please refer to A6_02) or to conjugates of its acid derivative. Therefore no tests according to Section A6.15 are required.	x

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2008
Evaluation of applicant's justification	We agree that exposure to the a.s. via the application of the representative product (cardboard glue trap) is negligible, for exposure estimates please see Document II-B 4, for the intended use description see Document II-B 3, for discussion or risk from exposure to residues on food and feeding stuff see document II-C 1. Agree with applicant's version.
Conclusion	The justification is acceptable.

Remarks	See Document III-A 6.2. The "Lebensmittelmotten-Falle" is used in cupboards and rooms to protect food and feed by preventing and reducing infestations with moths. However, no relevant food and feed stuff exposure is to be expected since the "Lebensmittelmotten-Falle" contains only 2 mg of ZE-TDA and should only be applied where food and feed-stuff is stored in closed or re-closed package. Furthermore, in analogy to literature data for the structurally related very long chain (C24 to C34) esters (waxes) (Hargrove et al. 2004), it is expected that ZE-TDA (C16) is easily catabolised by hydrolysis to the free alcohol, dehydrogenation to the acid and further ß-oxidation or glucuronide conjugation and excreted via the kidneys. It is also known that higher alcohols occur either free or bound in plant and animal tissues and free higher alcohols, including Cetyl alcohol (C16H33OH), Stearyl alcohol (C18H37OH) and Oleyl alcohol (C18H35OH) are abundant in fish oil (Berlitz et Grosch 1999). C14 to C24 fatty acids are – bound as esters within phoshpholipids and glycolipids - the major component of cell membranes and a relevant part of our natural diet. Natural intake of the structurally related very long chain (C24 to C34) alcohols, aldehydes, acids and esters (waxes) thereof is estimated to be about 2 g/day as part of our natural diet including cereal grains, bran, germ, leaves, seeds, nuts and unrefined oils (Hargrove et al. 2003). Furthermore, on the basis of a conservative AEL of 1 mg/kg bw day derived from a sub-chronic rat study, even the risk for immediate uptake of the total amount of the active substance (2 mg) within a single trap is acceptable, also for infants. Thus, the risk from residues from ZE-TDA on food/feeding stuff is considered to be negligible.