Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
Control	attractant			carried out on 4 poultry farms with the combination: muscalure/electro-cution traps/UV.	under local conditions of the farms.	farm, housefly pests could be more or less suppressed by the combination muscalure/ electrocution traps/UV.	van; Persoons, C.J. (1984a)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	Improvement of catch of flies on electrocution traps/UV light combinations.	Traps were hung in poultry stables under environmental conditions for ca. 1 week. Muscalure was applied in aerosol form in the traps. Every 2 min 10-100 µg muscalure was sprayed.	Improvement of fly catch was a factor 1.5 in the presence of muscalure.	Oosten, A.M. van; Persoons, C.J. (1984b)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	Improvement of catch of flies on electrocution traps/UV light combinations.	Traps were hung in poultry stables under environmental conditions for 4 weeks. Muscalure was applied in aerosol form in the traps (100 µL/3 min).	Improvement of fly catch was a factor 2.1-2.6 in the presence of muscalure.	Oosten, A.M. van; Kalisvaart, J.J.; Persoons, C.J. (1985a)
MG 03: Pest Control	PT 19: attractant	Muscalure (Technical)	*Musca domestica* (wild)	Catch of flies on glue plates and electrocution traps.	Traps were hung in poultry stables in combination with technical muscalure. Muscalure was applied in aerosol form in the traps (100 µL/3 min). Glue plates contained 250 mg muscalure in vermiculite/plate.	Improvement of fly catch was a factor 1.8 -1.6 in the presence of muscalure.	Oosten, A.M. van; Kalisvaart, J.J.; Persoons, C.J. (1985b)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	Several insecticides and baits were compared with respect to their attracting and killing activity.	Exposure for 24 h in poultry pens. 5 mL of wet baits were placed on egg cases. Killed flies were counted.	Best results were obtained with Bresmel (natural fly attractant) in combination with propoxur, and with Lurectron (= muscalure + methomyl).	Fujita, S.; Glatow, A.; Higuchi, S. (1995)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	Several insecticides and baits were compared with respect to their attracting and killing activity.	Exposure for 24 h in poultry pens. 5 mL of wet baits were placed on egg cases. Killed flies were counted.	Lurectron (methomyl + muscalure) attracted and killed the highest number of flies in pens, followed by the combination propoxur + Flylure (a.s.: muscalure) + Bresmel (natural fly attractant).	Fujita, S.; Glatow, A. (1995)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	Lurectron Fly-Bait (methomyl + muscalure) was tested outside in petri dishes, together with other fly baits.	Petri dishes containing the material (amount not given, milk or water added) were placed outside in the afternoon-evening for 10 hours.	Lurectron Fly-Bait was at least as effective in killing flies as other fly baits.	KenoGard (1993)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (non-resistant for carbamates)	Exposure of 200 individuals to Lurectron (methomyl + muscalure) and other fly baits in dishes.	22-25 g of test baits was placed in dishes in a room (30 m^3) and flies were exposed for 2 hours. Dead flies were counted.	Lurectron was the most effective product with 48% kill.	Fayette, J.-P. (1993)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	Flies were exposed to paperboard treated with Denka FLYLURE (muscalure + methomyl) in a cow stable.	FLYLURE was mixed with water (1:1) and 0.5 L painted on 5.4 m^2 paperboards. Exposure 1 day.	100% kill after 1 day on the paperboards.	Plüss-Staufer AG (1990)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	Flybait (muscalure + methomyl) was compared with Golden NT	Exposure in sheep stables. Several exposure times up to 18 hours. No further details given.	The products had similar efficacy.	S.I.A.P.A. (1989)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	Flybait (muscalure + methomyl) was	The product was painted on walls in sheep stables.	The products had more or less similar efficacy. RUBIDOR was	S.I.A.P.A. (1990)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
				compared with Golden NT in sheep stables and in the laboratory.	In the laboratory flies in cages were exposed to the product (1: 1 mixed with water, 100 mL) for upto 6 days.	less active. Mortality of Flybait was 100% after 3 days.	
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	The effect of Bayt (methomyl + muscalure) was investigated in 12 Danish animal farms during the whole fly season.	Different strategies of application were compared: strategic places and abundant application. The effect was compared with the effect of Baycidal WP 25 (triflumuron) applied to fly breeding sites.	The Bayt flybait applied as a paint-on bait in narrow bands was very attractive to the houseflies. No increase of resistance was observed in the trials, the abundant application scenario included, although a genetic resistance potential was present.	Knorr Lauridsen, M.; Jespersen, J.B. (1996)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (strain WHO (N), both sexes)	Exposure of flies (100) to 3 different test substances in cages.	Exposure to the wet bait for 20 hours at 25 °C, RH 60%.	Original Lurectron: 69% mortality after 20 h, red 1 experimental formulation (with 0.1% Bayferrox 130B): 73% mortality after 20 h, red 2 experimental formulation (with 0.5% Bayferrox 130B): 77% mortality after 20 h.	Nentwig, Dr. (1994)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* Strains: -Cooper susceptible -typical insecticide resistant -azamethiphos resistant - perethroid resistant	Exposure of flies (50) to five insecticidal baits, all containing muscalure. The baits were tested for their efficacy against several susceptible and resistant strains of flies.	Exposure in cages to the bait (sprinkled with carboxymethyl-cellulose solution) for 20 hours at 20 °C, RH 50%.	The bait containing methomyl showed the highest efficacy against the resistant strains tested. The bait containing dichlorvos alone, although less effective than methomyl, was more effective than permethrin. The 3 baits containing pyrethroids performed less well and there was some evidence that the pyrethroids in these formulations had a repellent action.	Pinniger, D.B. (1990)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	The following products were tested: Lurectron Granulé (product of Denka) and Golden Malrin Muscamone (product of Sanofi). Both products contained muscalure and methomyl.	Testing of the capacity to kill flies over a period of 28 days in 10 cow stables in France. 220-250 g product/100 m^2 was applied to paper. Temperature: 24 - 29 °C. Counting killed flies over a period of 8 hours.	The trials showed that Lurectron Granulé has insecticidal properties to houseflies of the same order as Golden Malrin Muscamone, or even slightly better than Golden Malrin Muscamone.	Société Somolog-France (1991)
MG 03: Pest Control	PT 19: attractant	Muscalure	*Musca domestica* (wild)	Golden Malrin fly bait (methomyl + muscalure) was tested against a housefly population in a chicken farm in Malaysia.	Application at 50 mg a.s./m^2	After application at 50 mg a.s./m^2 the test substance caused an almost 7-fold higher adult mortality than application at 10 mg a.s./m^2.	Sulaiman, S.; Omar, S. (1992)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Riebeek, W.M.; 1990. Determination of the acute oral toxicity of the compound "MUSCALURE" in rats. TNO-CIVO Institutes Laboratory report number: V90.356 (Project: B90-0060/028)	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Denka International B.V.	
1.2.2			
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 IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; - OECD401, - EPA Guideline 81-1 (Nov 1982), - MAFF Guideline, January 1985.	
2.2	**GLP**	Yes	
2.3	**Deviations**	It was decided not to conduct the preliminary study mentioned in the study protocol.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Muscalure	
3.1.1	Lot/Batch nr	31-90	
3.1.2	Specification	When this study was performed Denka based the 'purity' of the product on the content of both (Z)- en (E)-tricos-9-ene. Since then, the production methods for muscalure have not changed, which means that the muscalure used in this test had the same specification as given in Section 2.	
3.1.2.1	Description	Colourless to pale yellow liquid	
3.1.2.2	Purity	>98% (Tricos-9-ene)	
3.1.2.3	Stability	Stable at the temperatures and light circumstances in which the study was conducted.	
3.2	**Test Animals**		
3.2.1	Species	Wistar rats	
3.2.2	Strain	Crl:WI(WU)BR	
3.2.3	Source	Charles River Wiga GmbH, SULZFELD, Germany	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	The age of the animals was approximately 8 weeks by the time of the administration of the test material. M(Male) = 133 – 148 g, M(Females) = 100 – 115 g	
3.2.6	Number of animals per group	5 animals of each sex	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	**Oral**	
3.3.1	Post exposure period	14 days	
		Oral	
3.3.2	Type	Gavage	

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3.3.3	Concentration	5000 mg/kg bw	
3.3.4	Vehicle	Maize oil	
3.3.5	Concentration in vehicle	0.5 g test substance to each mL test suspension (50 % m/v)	
3.3.6	Total volume applied	10.0 mL / kg bw	
3.3.7	Controls	N.A.	
		Inhalation	
3.3.8	Concentrations		
3.3.9	Particle size		
3.3.10	Type or preparation of particles		
3.3.11	Type of exposure		
3.3.12	Vehicle		
3.3.13	Concentration in vehicle		
3.3.14	Duration of exposure		
3.3.15	Controls		
		Dermal	
3.3.16	Area covered		
3.3.17	Occlusion		
3.3.18	Vehicle		
3.3.19	Concentration in vehicle		
3.3.20	Total volume applied		
3.3.21	Duration of exposure		
3.3.22	Removal of test substance		
3.3.23	Controls		
		Intraperitoneal/Intravenous/Intratracheal instillation	
3.3.24	Vehicle		
3.3.25	Concentration in vehicle		
3.3.26	Total volume applied		
3.3.27	Controls		
3.4	**Examinations**	1) The following clinical properties were assessed at hour 1, 4, 24, 48 and 72: Sluggishness, exophtalmus, convulsions, tremors, ataxia, paralysis, lachrymation, emaciation, encrustations, piloerection, soiled fur, diarrhoea, dyspnoea and paleness. 2) Individual body weights were recorded on day 0, 3, 7 and 14. 3) At the end of the observation period, the rats were killed for macroscopic examination.	
3.5	**Method of determination of LD_{50}**	N.A. since no animal died during the 14 day observation period.	
3.6	**Further remarks**		

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		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	All clinical properties in all animals and at each moment of recording appeared normal.	
4.2	**Pathology**	No effects	
4.3	**Other**	No animals died, no signs of intoxication were recorded and all animals gained weight during the observation period.	
4.4	**LD_{50}**	No lethal effect at maximal dose of 5000 mg/kg bw.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The acute oral toxicity of muscalure was assessed at only the maximum dose of 5000 mg/kg bw according OECD-method 401 in 5 individuals of both male and female wistar rats. The method of dosing was oral gavage using maize oil as a vehicle. A variety of clinical properties were assessed during the first 3 days. After that individual body weights were recorded until day 14. At day 14 the animals were killed for macroscopic examination.	
5.2	**Results and discussion**	No animals died, no symptoms of intoxication were observed and all animals gained weight during the observation period.	
5.3	**Conclusion**	The LD_{50} in rats is higher than 5000 mg/kg bw.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

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		1 REFERENCE	
1.1	**Reference**	Riebeek, W.M.; 1990. Determination of the acute dermal toxicity of the compound "MUSCALURE" in rats. TNO-CIVO Institutes Laboratory report number: V90.359 (Project: B90-0084/006)	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Denka International B.V.	
1.2.2			
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 IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; - OECD402, - ECstandards mentioned in EC directive 84/449/EC (19 September 1984) - EPA Guideline 81-2 (Nov 1982), - MAFF Guideline, January 1985.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Muscalure	
3.1.1	Lot/Batch nr	31-90	
3.1.2	Specification	When this study was performed Denka based the 'purity' of the product on the content of both (Z)- en (E)-tricos-9-ene. Since then, the production methods for muscalure have not changed, which means that the muscalure used in this test had the same specification as given in Section 2.	
3.1.2.1	Description	Colourless to pale yellow liquid	
3.1.2.2	Purity	>98% (Tricos-9-ene)	
3.1.2.3	Stability	Stable at the temperatures and light circumstances in which the study was conducted.	
3.2	**Test Animals**		
3.2.1	Species	Wistar rats	
3.2.2	Strain	Crl:WI(WU)BR	
3.2.3	Source	Charles River Wiga GmbH, SULZFELD, Germany	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	The age of the animals was approximately 10 weeks by the time of the application of the test material. M(Male) = 220 – 258 g, M(Females) = 167 – 181 g	
3.2.6	Number of animals per group	5 animals of each sex	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	**Oral**	
3.3.1	Post exposure period		
3.3.2	Type		

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3.3.3	Concentration		
3.3.4	Vehicle		
3.3.5	Concentration in vehicle		
3.3.6	Total volume applied		
3.3.7	Controls		
		Inhalation	
3.3.8	Concentrations		
3.3.9	Particle size		
3.3.10	Type or preparation of particles		
3.3.11	Type of exposure		
3.3.12	Vehicle		
3.3.13	Concentration in vehicle		
3.3.14	Duration of exposure		
3.3.15	Controls		
		Dermal	
3.3.16	Area covered	Not reported	
3.3.17	Occlusion	Semi-occluded - occluded	
3.3.18	Vehicle	Maize oil	
3.3.19	Concentration in vehicle	0.2 g test substance to each mL test suspension (20 % m/v)	
3.3.20	Total volume applied	10 mL/kg bw	
3.3.21	Duration of exposure	24 hours	
3.3.22	Removal of test substance	With water	
3.3.23	Controls	N.A.	
		Intraperitoneal/Intravenous/Intratracheal instillation	
3.3.24	Vehicle		
3.3.25	Concentration in vehicle		
3.3.26	Total volume applied		
3.3.27	Controls		
3.4	**Examinations**	1) Immediately after removal of the test material and at day 3 and 7 the following skin readings were made: erythema, oedema, haemorrhages, scaliness, hyperaemia, and ischemia. 2) The following clinical properties were assessed during the first 4 post-treatment hours and later on, at least once daily: Sluggishness, exophtalmus, convulsions, tremors, ataxia, paralysis, lachrymation, emaciation, encrustations, piloerection, soiled fur, diarrhoea, dyspnoea and paleness. 3) Individual body weights were recorded on day 0, 3, 7 and 14. 4) At the end of the observation period, the rats were killed for macroscopic examination.	

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3.5	**Method of determination of LD_{50}**	N.A. since no animal died during the 14 day observation period.	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	All clinical properties in all animals and at each moment of recording appeared normal.	
4.2	**Pathology**	No effects	
4.3	**Other**	No animals died, no signs of intoxication were recorded and all animals gained weight during the observation period.	
4.4	**LD_{50}**	No lethal effect at maximal dose of 2000 mg/kg bw.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The acute dermal toxicity of muscalure was assessed at only the maximum dose of 2000 mg/kg bw according OECD-method 402 in 5 individuals of both male and female wistar rats. The method of dosing was 'occluded dermal' during 24 hours using maize oil as a vehicle. Skin readings were made and clinical properties were assessed. Individual body weights were recorded and at the end of the 14 day observation period, the rats were killed for macroscopic examination.	
5.2	**Results and discussion**	No animals died, no symptoms of intoxication were observed and all animals gained weight during the observation period.	
5.3	**Conclusion**	The LD_{50} in rats is higher than 2000 mg/kg bw.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	(No) The treated skin area in the animals was not recorded.	

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		1 REFERENCE	
1.1	**Reference**	Arts, J.H.E.; 1991 Acute (4-hour) inhalation toxicity study of muscalure in rats. TNO Nutrition and Food Research Laboratory report number: V91.375 (Project number: B90-8213)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Denka International B.V.	
1.2.2			
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 IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: - OECD403 (1981), - EPA Guideline 798.1150 (Sept. 1985), - Japanese MAFF Guideline of 1985.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes. 1) A second group of animals has been tested at also approximately the limit exposure concentration of 5 m/L because one animal died in the first group. 2) A high relative humidity (RH) of up to 85% during housing of the animals was recorded on day 7 due to cleaning activities. 3) Although the RH of the test atmosphere is not prescribed by OECD403 (1981), it is noted that the RH of the test atmosphere was below 1%.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Muscalure	
3.1.1	Lot/Batch number	HPI 240191	
3.1.2	Specification	When this study was performed Denka based the 'purity' of the product on the content of both (Z)- en (E)-tricos-9-ene. Since then, the production methods for muscalure have not changed, which means that the muscalure used in this test had the same specification as given in Section 2.	
3.1.2.1	Description	Colourless to pale yellow liquid	
3.1.2.2	Purity	>98% (Tricos-9-ene)	
3.1.2.3	Stability	Stable at the temperatures and light circumstances in which the study was conducted.	
3.2	**Test Animals**		
3.2.1	Species	Wistar rats	
3.2.2	Strain	Crl:WI(WU)BR	
3.2.3	Source	Charles River Wiga GmbH, SULZFELD, Germany	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Jus before exposure the mass of the animals was: Group A: M(Male) between 193 and 209 g, M(Females) between 151 and 165 g Group B: M(Male) between 233 and 239 g, M(Females) between 167 and 181 g	
3.2.6	Number of animals per group	GroupA: 5 animals of each sex GroupB: 5 animals of each sex	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Postexposure period	GroupA: 14 days GroupB: 18 days	

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		Oral	
3.3.2	Type		
3.3.3	Concentration		
3.3.4	Vehicle		
3.3.5	Concentration in vehicle		
3.3.6	Total volume applied		
3.3.7	Controls		
		Inhalation	
3.3.8	Concentrations	Nominal concentration: A : 5200 mg/m³ B : 6800 mg/m³	
3.3.2		Analytical (actual) concentration: A: 4910 mg/m³ B: 5710 mg/m³	
3.3.9	Particle size	Particle size distribution measurements were carried out once per exposure using an 11-stage cascade impactor. See table 1.	
3.3.10	Type or preparation of particles	The test atmosphere was generated by atomizing the test material into small droplets by using a compressed air driven nebulizer.	
3.3.11	Type of exposure	Nose only	
3.3.12	Vehicle	None	
3.3.13	Concentration in vehicle	N.A.	
3.3.14	Duration of exposure	4 hour	
3.3.15	Controls	N.A.	
		Dermal	
3.3.16	Area covered		
3.3.17	Occlusion		
3.3.18	Vehicle		
3.3.19	Concentration in vehicle		
3.3.20	Total volume applied		
3.3.21	Duration of exposure		
3.3.22	Removal of test substance		
3.3.23	Controls		
		Intraperitoneal/Intravenous/Intratracheal instillation	
3.3.24	Vehicle		
3.3.25	Concentration in vehicle		
3.3.26	Total volume applied		
3.3.27	Controls		
3.4	**Examinations**	1) The following standard clinical properties were assessed during the exposure period and once daily after treatment: Piloerection/hunch. app., clear restlessness,	

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		red nasal discharge, serous nasal discharge, salivation, visually increased breathing, visually decreased breathing, mouth breathing, laboured breathing, lachrymation, rales, wet head, wet fur. Other clinical signs were also examined during the period after exposure. 2) Individual body weights were recorded - group A: on day 0, 7 and 14 - group B: on day 0, 7, 14 and 18. 3) At the end of the observation period, the rats were killed for examination of gross pathological changes.	
3.5	**Method of determination of LD_{50}**	Not applicable.	
3.6	**Further remarks**	A second group of animals (group B) was tested as one animal in the first group (A) died shortly after the exposure. A higher concentration was applied to the second group. Yet, no animals died in the second group.	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	See table 2 for symptoms during and shortly after exposure. The following observations were made during the observation period after exposure: <u>Group A:</u> Although female rat with ear tag R1 in group A did not show severe symptoms it died on the first day after exposure. Male L2 was lethargic on day 2 – 4. Female rat L2 had a small left eye with corneal opacity on day 1-9 and 12-13. No abnormalities were observed on the other days or in the other animals. Body mass of all animals increased during the 14 day observation period. (Table 3) <u>Group B:</u> All animals had a dirty fur on day 1. No abnormalities were observed on the other days. In the observation period from day 7 to day 14 one male animal (R1) showed a decrease in body mass and all female animals showed a decrease in body mass. The observation period was therefore extended until day 18. In the additional 4 days all animals gained weight again. (Table 3)	
4.2	**Pathology**	Group A Female R1 (died on first day): dark discoloured and oedematous lungs, and hydrothorax. Female R2: Spotted lungs with irregular surface. Group B Abnormalities were observed in none of the animals of group B.	
4.3	**Other**	None	
4.4	**LC_{50}**	The LC_{50} is higher than 5710 mg/m^3	

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		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In 1991 the 4-hour acute inhalation toxicity of muscalure was determined under GLP and according OECD403 (1981). The study was done at only the limit concentration of approximately 5000 mg/m^3 in two groups of 5 male and 5 female rats. Although the rats in the first group (A) did not show severe signs of intoxication, one female died on the first day after treatment. Therefore a second group (B) was exposed to the test material at even a higher concentration (5710 mg/m^3). A variety of clinical properties were assessed in the animals of both groups (A and B), their body mass was recorded and they were sacrificed for examination on gross pathological changes.	
5.2	**Results and discussion**	Although the rats in the first group (A) did not show severe signs of intoxication, one female died on the first day after treatment. In the second group no animal died. All animals in the first group except for the one died, gained weight during the 14 day observation period. In the second group all females and one male rat showed a decrease in body weight in the period from day 7 to day 14. This was however, undone during 4 extra days of observation; the animals had gained weight. (Table 3) Considering that the animals in both groups did not show signs of severe intoxication the death of the one female must (mostly) be attributed to non-treatment related causes.	
5.3	**Conclusion**	It is concluded that the EC_{50} of the test material is higher than 5710 g/m^3.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	No	

Table 1: Aerodynamic particle size distribution

Aerodynamic diameter	Animal group	
	A	B
µm	Distribution in % of total mass	
< 1	3.6	3.8
1.0	13.1	12.1
1.4	18.7	14.0
1.8	15.0	19.5
2.4	18.4	17.6
2.8	10.0	11.2
3.1	9.7	9.0
3.4	7.4	8.3
3.8	3.2	2.9
4.2	0.2	0.5
> 4.2	0.8	1.1

Table 2a: Clinical signs in group A during 4 hours of exposure and shortly after.

Rat[1] >	MZ				MR1				MR2				ML1				ML2				FZ				FR1				F
Property[2]>	a	c	d	e	a	c	d	e	a	c	d	e	a	c	d	e	a	c	d	e	a	c	d	e	a	c	d	e	a	c
1st hour																														
2nd hour			1				1				1				1				1				1				1			
3rd hour		1	1		1	1	1			1	1			1	1			1	1		1	1	1			1	1		1	
4th hour		1	1			1	1			1	1			1	1			1	1			1	1			1	1		1	
After exp.			1				1				1				1				1				1				1			

Table 2b: Clinical signs in group B during 4 hours of exposure and shortly after.

Rat[1] >	MZ				MR1				MR2				ML1				ML2				FZ				FR1				
Property[2]>	b	c	d	e	b	c	d	e	b	c	d	e	b	c	d	e	b	c	d	e	b	c	d	e	b	c	d	e	b	c
1st hour										1												1				1				1
2nd hour		1	1			1	1			1	1			1	1			1	1			1	1			1	1			1
3rd hour		1	1			1	1			1	1			1	1			1	1			1	1	2		1	1			1
4th hour		1	1			1	1			1	1			1	1			1	1			1	1	2		1	1			1
After exp.			1				1				1				1				1				1	2			1			

1) A rat indicated by e.g. MR2 means: Male rat with ear tag R2.
2) The four clinical properties which deviated from normal are here indicated with a, b, c, and d.
a: clear restlessness (only in table 2)
b: red nasal discharge (only in table 3)
c: visually increased breathing
d: wet head
e: wet fur
The severity of the symptoms is indicated with a figure 1, 2 or 3:
No figure = no symptom
1 = slight
2 = moderate
3 = severe

Table 3: Individual body masses of the rats exposed to the muscalure aerosol

	Days of examinations in observation period							
Animal	0	7	14	18	0	7	14	18
	Body mass males (g)				Body mass females (g)			
Group A								
Z	202	226	249		165	177	190	
R1	209	233	259		154	---	---	
R2	213	232	257		161	160	167	
L1	193	211	238		156	169	183	
L2	195	211	240		158	165	178	
Mean	202	223	249		159	168	180	
Group B								
Z	234	259	260	293	176	193	186	204
R1	239	250	247	276	173	182	169	188
R2	233	240	250	280	169	180	169	188
L1	233	239	245	270	181	178	168	191
L2	235	243	246	283	167	168	166	182
Mean	235	246	250	280	173	180	172	190

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			Official use only
		1 REFERENCE	
1.1	**Reference**	Daamen, P.A.M.; 1990. Primary skin irritation/corrosion study with muscalure in the rabbit (4-hour semi-occlusive application). RCC NOTOX B.V. Laboratory project number: 038576	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Denka International B.V.	
1.2.2			
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 IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; - OECD 404, - EC Directive 67/548/EC, Annex V, B.4 (1984) - EPA Guideline 81-5 (Nov 1982), - MAFF Guideline, January 1985.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No deviations from guideline.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Muscalure	
3.1.1	Lot/Batch nr	I/12-09-1990	
3.1.2	Specification	When this study was performed Denka based the 'purity' of the product on the content of both (Z)- en (E)-tricos-9-ene. Since then, the production methods for muscalure have not changed, which means that the muscalure used in this test had the same specification as given in Section 2.	
3.1.2.1	Description	Colourless to pale yellow liquid	
3.1.2.2	Purity	>98% (Tricos-9-ene)	
3.1.2.3	Stability	Stable at the temperatures and light circumstances in which the study was conducted.	
3.2	**Test Animals**		
3.2.1	Species	Rabbits,	
3.2.2	Strain	New Zealand White, (SPF-quality)	
3.2.3	Source	Broekman Institute, Someren, The Netherlands	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	The age of the animals was approximately 12 weeks by the start of the treatment. Mass: 2163 – 2615 g	
3.2.6	Number of animals per group	6 females	
3.2.7	Control animals	No. The contralateral flank of the test animals was used as "procedural control".	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Application		
3.3.1.1	Preparation of test substance	The test substance was used as delivered.	

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3.3.1.2	Test site and preparation of test site	Approximately 24 hours before treatment, the dorsal fur was shaved with electric clippers, exposing an area of approximately 100 square centimetres (10cm x10cm). The contralateral flank (used as "procedural control") of the test animals were similarly prepared.	
3.3.2	Occlusion	Surgical gauze 2x3 cm^2 mounted on Micropore tape (3M St. Paul, U.S.A.). Dressing was wrapped around the abdomen and secured with an elastic bandage (Coban, 3M, St. Paul, U.S.A.).	
3.3.3	Vehicle	None	
3.3.4	Concentration in vehicle	The test substance was applied undiluted.	
3.3.5	Total volume applied	0.5 mL	
3.3.6	Removal of test substance	Gauze and dressings were removed. The remaining test article was removed using a tissue moistened with tap-water and subsequently a dry tissue.	
3.3.7	Duration of exposure	Four (4) hours.	
3.3.8	Post exposure period	72 hours.	
3.3.9	Controls	No (Just the dry surgical gauze on the contralateral flank.)	
3.4	**Examinations**		

			Official use only
3.4.1	Clinical signs	Yes	
3.4.2	Dermal examination	Yes	
3.4.2.1	Scoring system	Erythema and eschar formation No erythema....0 Very slight erythema (barely perceptible)....1 Well defined erythema....2 Moderate to severe erythema....3 Severe erythema (beet redness) to slight eschar formation (injuries in depth)....4 Oedema formation No oedema....0 Very slight oedema (barely perceptible)....1 Slight oedema (edges of area well defined by definite raising)....2 Moderate oedema (raised approximately 1 mm)....3 Severe oedema (raised more than 1 mm and extending beyond area of exposure)....4 A primary irritation index was calculated by combining the average skin irritation scores for erythema and oedema after 24 and 72 hours and the following table was used to obtain the degree of irritation (Draize, J.H., Woodward, G. and Calvery, H.O., 1944).	

Primary irritation index	Degree of irritation
0	Non-irritating
0.1 – 2.0	Mildly irritating
2.1 – 5.0	Moderately irritating
5.1 – 8.0	Severely irritating

3.4.2.2	Examination time points	40 min, 24h, 48h and 72 hours after removal of dressing and test substance.	
3.4.3	Other examinations	The viability of the animals was examined on a daily basis. Signs of toxicity were also examined on a daily basis.	
3.5	**Further remarks**	Observations of the control (contralateral) flank of the test animals are not specifically reported.	
		4 RESULTS AND DISCUSSION	
4.1	**Average score**		
4.1.1	Erythema	Scores were zero (0) to all animals at all time points	
4.1.2	Oedema	Scores were zero (0) to all animals at all time points	
4.2	**Reversibility**	Not applicable (No effects observed.)	
4.3	**Other examinations**	No staining (colouration) of the treated skin was observed. There was no evidence of a corrosive effect on the skin. Scaliness was observed in three of the six animals only at 72 hours. No symptoms of systemic toxicity were observed and no mortality occurred.	
4.4	**Overall result**	Degree of irritation: non-irritating to skin.	

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		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Six female New Zealand White Rabbits were used. 100 square cm of the dorsal fur of each animal was shaved and 0.5 mL of undiluted test material was applied to the skin. The material was kept in place by a surgical gauze of 2x3cm^2 The contralateral flank of the test animals was similarly prepared as "procedural control". After four hours the material was removed. Observations were made 40 minutes, 24 hours, 48 hours and 72 hours after removal of the test substance. The effects were 'scored' according the system discussed in section 3.4.2.1.	
5.2	**Results and discussion**	Erythema scores were zero (0) to all animals at all time points. Oedema scores were zero (0) to all animals at all time points. No staining (colouration) of the treated skin was observed. There was no evidence of a corrosive effect on the skin. Scaliness was observed in three of the six animals only at 72 hours. No symptoms of systemic toxicity were observed and no mortality occurred.	
5.3	**Conclusion**	The material is not a skin irritant.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No (Except that observations of the control (contralateral) flank of the test animals are not specifically reported.)	

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			Official use only
		1 REFERENCE	
1.1	**Reference**	Daamen, P.A.M.; 1990. Acute eye irritation/corrosion study with muscalure in the rabbit. RCC NOTOX B.V. Laboratory project number: 038587	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Denka International B.V.	
1.2.2			
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 IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; - OECD 405 (1987), - EC Directive 67/548/EC, Annex V, B.5 (1984) - EPA Guideline 81-4 (Nov 1982) - Japanese MAFF test guidelines 1982.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No deviation from guideline.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Muscalure	
3.1.1	Lot/Batch nr	I/12-09-1990	
3.1.2	Specification	When this study was performed Denka based the 'purity' of the product on the content of both (Z)- en (E)-tricos-9-ene. Since then, the production methods for muscalure have not changed, which means that the muscalure used in this test had the same specification as given in Section 2.	
3.1.2.1	Description	Colourless to pale yellow liquid	
3.1.2.2	Purity	>98% (Tricos-9-ene)	
3.1.2.3	Stability	Stable at the temperatures and light circumstances in which the study was conducted.	
3.2	**Test Animals**		
3.2.1	Species	Rabbits,	
3.2.2	Strain	New Zealand White, (SPF-quality)	
3.2.3	Source	Broekman Institute, Someren, The Netherlands	
3.2.4	Sex	Females	
3.2.5	Age/weight at study initiation	The age of the animals was approximately 13 weeks by the start of the treatment. Mass: 2377 – 2602 g	
3.2.6	Number of animals per group	6	
3.2.7	Control animals	No (The 'other' eye served as control.)	

			Official use only
3.3	**Administration/ Exposure**		
3.3.1	Preparation of test substance	The test substance was used as delivered.	
3.3.2	Amount of active substance instilled	0.1 mL	
3.3.3	Exposure period	At the 24h-point a solution of 2% fluorescein in water (adjusted to pH = 7) was applied to both eyes of each animal.	
3.3.4	Post exposure period	48 hours after application of the fluorescein solution. (Total observation period: 24 + 48 = 72 hours.	
3.4	**Examinations**		

			Official use only
3.4.1	Ophthalmoscopic examinations	No	
3.4.1.1	Scoring system	**Cornea** Opacity; degree of density (Area most dense taken for reading.) No ulceration or opacity ..... 0 Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible ..... 1 Easily discernible translucent area; details of iris slightly obscured ..... 2 Nacreous areas; no details of iris visible; size of pupil barely discernable ..... 3 Opaque cornea, iris not discernible trough the opaque ..... 4 Area of cornea affected No ulceration or opacity ..... 0 One quarter or less but not zero ..... 1 Greater than one quarter, but less than half ..... 2 Greater than half, but less than three quarter ..... 3 Greater than three quarter, up to whole area ..... 4 **Iris** Normal ..... 0 Markedly deepened rugae; congestion; swelling; moderate circumcorneal hyperaemia, or injection. Any of these or any combination thereof; iris still reacting to light (sluggish reaction is positive) ..... 1 No reaction to light; heaorrhage; gross destruction (any or all of these) ..... 2 **Conjuctivae** Redess (Refers to palperbral and bulbar conjunctivae, excluding cornea and iris) Blood vessels normal ..... 0 Some blood vessels definitely hyperaemic (injected) ..... 1 Diffuse, crimson colour, individual vessels not easily discernible ..... 2 Diffuse beefy red ..... 3 Chemosis; lids and/or nictating membrane No swelling ..... 0 Any swelling above normal (includes nictating membrane) ..... 1 Obvious swelling with partial eversion of lids ..... 2 Swelling with lids about half closed ..... 3 Swelling with lids more that half closed ..... 4 Discharge No discharge ..... 0 Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) ..... 1 Discharge with moistening of the lids and hairs, just adjacent to lids ..... 2 Discharge with moistening of the lids and hairs, in a considerable area around the eye ..... 3 A Draize score was calculated for each moment of observation, using the following formula: 5(corneal opacity grade)•(area of opacity rade) + 5(iridial injury grade) +	

2(conjunctival redness grade + chemosis grade + discharge grade).

With the maximum score of the study (i.e. Draize score), a Kay and Calandra interpretation was obtained, using the following table and taking into account the time needed for healing (J. Society of Cosmetic Chemists, Vol. B no. 6, 1962).

Draize score	Tentative eye irritation rating to Kay and Calandra	
0 - 0.5	Non-irritating	N
0.5 - 2.5	Practically non-irritating	PN
2.5 - 15	Minimally irritating	M1
15 - 25	Mildly irritating	M2
25 - 50	Moderately irritating	M3
50 - 80	Severaly irritating	S
80 - 100	Extremely irritating	E
100 - 110	Maximally irritating	Mx

For borderline scores the higher rating is chosen.

Tentative rating	Requirement for maintenance
N	$MTS_{24} = 0$; for $MTS_{24} > 0$, raise one level.
PN	As for "N".
M1	$MTS_{48} = 0$; for $MTS_{48} > 0$, raise one level.
M2	$MTS_{96} = 0$; for $MTS_{96} > 0$, raise one level.
M3	1) $MTS_f \leq 20$; for $MTS_f > 20$, raise one level. 2) $ITS_f \leq 10$ (60%); if not true then no rabbit may show $ITS_f > 30$; otherwise raise one level.
S	1) As for M3 except use $MTS_f \leq 40$. 2) As for M3 except use $ITS_f \leq 30$ (60%) and 60 for high.
E	1) As for M3 except use $MTS_f \leq 80$. 2) As for M3 except use $ITS_f \leq 60$ (60%) and 100 for high.
Mx	1) $MTS_f > 80$ (60%); for $MTS_f \leq 80$, lower one level. 2) $ITS_f > 60$ (60%); otherwise lowerone level.

Symbols: MTS = Mean total score; ITS = Individual rabbit score
Subscribts denote scoring interval: 24, 48 or 96 hrs.
f = final score (7 days)

3.4.1.2	Examination time points	1, 24, 48 and 72 hours after instillation of test substance.
3.4.2	Other investigations	The viability of the animals was examined on a daily basis. Signs of toxicity were also examined on a daily basis.
3.5	**Further remarks**	-

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4 RESULTS AND DISCUSSION

4.1 Clinical signs

No toxic symptoms were observed in the animals during the test period and no mortality occurred.

4.2 Average score

4.2.1 Cornea

Time point	Average opacity scores	Average area scores
24h	0	0
48h	0	0
72h	0	0

4.2.2 Iris

Average iris score: 24h: 0 / 48h: 0 / 72h: 0

4.2.3 Conjunctiva

4.2.3.1 Redness

Average redness score: 24h: 0 / 48h: 0 / 72h: 0

4.2.3.2 Chemosis

Average chemosis score: 24h: 0 / 48h: 0 / 72h: 0

4.2.4 Discharge

Average discharge score: 24h: 0 / 48h: 0 / 72h: 0

4.3 Reversibility

Instillation of muscalure into one of the eyes of each of six animals affected the conjunctivae (see 4.4). The irritation of the conjunctivae was reversible within 24 hours in all six animals.

4.4 Other examinations

1) Lacrimation was observed in all animals at time point '1 hour'. This subsided before the 24 hour time point.
2) Chemosis grade 1 for eyelids was observed in 3 of 6 animals at time point '1 hour'. This subsided before the 24 hour time point.
3) Treatment of the eyes with 2% fluorescein, 24 hours after test substance instillation revealed no corneal epithelial damage in any of the animals.
4) No staining by the test substance was observed.
5) There was no evidence of ocular corrosion.
6) See 4.1

4.5 Overall result

The study director concludes that the degree of irritation is: "Minimally irritating (M1) to the rabbit eye. (Kay and Calandra interpretation of the Draize score : 3.)" (This is based on the observations from the 1h-timepoint.)

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		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Six female New Zealand White Rabbits (approximately 13 weeks old) were used. 0.1mL of muscalure was instilled in one of the eyes of each animal. The other eye was used as a control; it was left untreated. At the 24h-point a solution of 2% fluorescein in water (adjusted to pH = 7) was applied to both eyes of each animal. Observations were made 1, 24, 48 and 72 hours after the instillation of the test substance. The following was scored: - effects on cornea (opacity and area); - effects on iris; - conjunctivae (redness and chemosis) and - discharge. Ophthalmoscopic examinations were not made. The viability of the animals and signs of toxicity were examined on a daily basis. A Draize score was calculated for each moment of observation, using the following formula: 5(corneal opacity grade)•(area of opacity rade) + 5(iridial injury grade) + 2(conjunctival redness grade + chemosis grade + discharge grade). With the maximum score of the study (i.e. Draize score), a Kay and Calandra interpretation was obtained, taking into account the time needed for healing (J. Society of Cosmetic Chemists, Vol. B no. 6, 1962).	
5.2	**Results and discussion**	1) Lacrimation was observed in all animals at time point '1 hour'. This subsided before the 24 hour time point. 2) Chemosis grade 1 for eyelids was observed in 3 of 6 animals at time point '1 hour'. This subsided before the 24 hour time point. 3) Treatment of the eyes with 2% fluorescein, 24 hours after test substance instillation revealed no corneal epithelial damage in any of the animals. 4) No staining by the test substance was observed. 5) There was no evidence of ocular corrosion. 6) No toxic symptoms were observed in the animals during the test period and no mortality occurred. 7) At the observation time points 24, 48 and 72 hours, all irritation effects (cornea, iris, conjunctivae and discharge) were scored "0". Based on the 1 hour observations the Draize score is calculated to be "3". According to the Kay and Calandra interpretation the degree of irritation is: "Minimally irritating (M1) to the rabbit eye. Although it is not required according OECD guideline 405, it would have been better if the 'control eye' was also treated but then with 0.1mL of a non-irritating liquid (isotonic solution in water). By doing this it could be excluded that the irritation scores observed for the treated eye were not the result of the handling of the eye: Opening the eye by hand, putting a droplet into it and holding the eye closed for a second. No observations are recorded on the control eye. The draize scores for the other observation time points is "0". Thus the degree of	

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		irritation according to the Kay and Calandra interpretation for those time points is "Non-irritating".	
5.3	**Conclusion**	The material is not an eye irritant to the rabbit eye.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

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			Official use only
		1 REFERENCE	
1.1	**Reference**	Daamen, P.A.M.; 1991. Contact hypersensitivity to muscalure in the Albino Guinea Pig (Maximization test). RCC NOTOX B.V. Laboratory project number: 051637	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Denka International B.V.	
1.2.2			
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 IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; - OECD 406 (1981), - EC Directive 67/548/EC, Annex V, B.6 (1984) - EPA Guideline 81-6 (Nov 1982)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No deviation from guideline.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Muscalure	
3.1.1	Lot/Batch nr	31-90	
3.1.2	Specification	When this study was performed Denka based the 'purity' of the product on the content of both (Z)- en (E)-tricos-9-ene. Since then, the production methods for muscalure have not changed, which means that the muscalure used in this test had the same specification as given in Section 2.	
3.1.2.1	Description	Colourless to pale yellow liquid	
3.1.2.2	Purity	>98% (Tricos-9-ene)	
3.1.2.3	Stability	Stable at the temperatures and light circumstances in which the study was conducted.	
3.1.2.4	Preparation of test substance for application	a) For induction: b) For challenge:	
3.1.2.5	Pretest performed on irritant effects	Yes	
3.2	**Test Animals**		
3.2.1	Species	Guinea pig	
3.2.2	Strain	Himalayan, albino (SPF-quality)	
3.2.3	Source	BRL Ltd Basel, Switzerland	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	355 – 470 grams	
3.2.6	Number of animals per group	20 in experimental group 10 in control group	
3.2.7	Control animals	Yes, treated the same way, but without test substance	

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3.3	**Administration/ Exposure**	State study type: skin sensitisation, maximization test	
3.3.1	Induction schedule	Day 1: Three pairs of intradermal injections (0.1 mL/site) Epidermal application: 7 days after intradermal injections	
3.3.2	Way of induction	Intradermal injection followed by epidermal application	
3.3.3	Concentrations used for induction	Intradermal injection of Muscalure 5 % w/w in corn oil Epidermal application of 0.5 mL of undiluted test substance. After 48 hours, the dressings and residual test substance were removed.	
3.3.4	Concentrations FCA	50:50 with distilled water	
3.3.5	Challenge schedule	Two weeks after epidermal induction	
3.3.6	Concentrations used for challenge	25% test substance in corn oil 10% test substance in corn oil 5% test substance in corn oil Corn oil	
3.3.7	Rechallenge	Not reported	
3.3.8	Scoring challenge	24 and 48 hours after removal of the dressings	
3.3.9	Removal of the test substance	Yes	
3.3.10	Positive control substance	Formaldehyde	
3.4	**Examinations**		
3.4.1	Pilot study	Primary irritation study on one animal	
3.5	**Further remarks**	Not applicable	
		4 RESULTS AND DISCUSSION	
4.1	**Results of pilot studies**	Based on the results of the pilot study, the concentrations for the main study were chosen.	
4.2	**Results of test**		
4.2.1	24h after challenge	One experimental animal showed red spots (score 1) at the 25% test site.	
4.2.2	48h after challenge	Five experimental animals and three control animals showed red spots (score 1) at the 25% test site. Six experimental animals showed red spots at the 10% test site and three animals showed red spots at the 5% test site. Seven of the 20 experimental animals were sensitised.	
4.2.3	Other findings	No symptoms of systemic toxicity or mortality were observed and the average body weight gain was similar for experimental and control animals.	
4.3	**Overall results**	The results lead to a sensitisation rate of 30%, indicating that muscalure has moderate sensitizing properties.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The skin sensitisation test was performed in accordance with the method described by Magnusson and Kligman and performed according to OECD 406.	
5.2	**Results and discussion**	No symptoms of systemic toxicity or mortality were observed and the average body weight gain was similar for experimental and control animals. Five experimental animals and three control animals showed red spots (score 1) at the 25% test site. Six experimental animals showed red spots at the 10% test site and three animals showed red spots at the 5% test site. Seven of the 20 experimental animals were sensitised.	
5.3	**Conclusion**	The results of this test indicate that muscalure has moderate sensitizing properties.	
5.3.1	Reliability	1	

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5.3.2	Deficiencies	None	

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Section A6 Annex Point IIA6.2	Metabolism	
	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:	According to the 'draft guidance document for waiving of data requirements for pheromones for inclusion in Annex I/IA of Directive 98/8/EC' (further referred to as 'Guidance for waiving') data on metabolism are only required when triggered by adverse effects or toxicological concerns arising form other data points for health risk. The available information on the toxicology of muscalure does not give rise to concern for the human health (see Verberk et al., 2004 and De Raat, 2006). Being a higher linear mono-alkene, there are no structural alerts for specific toxic effects. Moreover, the human exposure to muscalure resulting form the use of the attractant is very low (see document IIB), even much lower than the designated threshold of toxicological concern. Waiving is further justified by the fact that humans are exposed to very similar compounds via their food and otherwise at levels exceeding the estimated exposure to muscalure (see De Raat, 2006). No further details on metabolism of muscalure are available in the secondary literature.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6 Annex Point IIA6.3	Short-term repeated dose 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:	According to the 'Guidance for waiving' data on short-term (sub-acute) toxicity are only required if (1) there is a significant exposure potential e.g. above background levels (depending on level, frequency and duration of exposure), or (2) a tolerance/MRL will be set. The available information on the toxicology of muscalure does not give rise to concern for the human health (see Verberk et al., 2004 and De Raat, 2006). Being a higher linear mono-alkene, there are no structural alerts for specific toxic effects. Moreover, the human exposure to muscalure resulting form the use of the attractant is very low (see document IIB), even much lower than the designated threshold of toxicological concern. Waiving is further justified by the fact that humans are exposed to very similar compounds via their food and otherwise at levels exceeding the estimated exposure to muscalure (see De Raat, 2006).	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6 **Annex Point IIA6.4**	**Subchronic 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:	According to the 'Guidance for waiving' data on sub-chronic toxicity (90-day) are normally not required if there is no concern from toxicological profile and depending on the level, frequency and duration of exposure. As for muscalure there is no concern from toxicological profile and the exposure will be low, this data requirement can be waived.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6 **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 [ ]** **Other justification [x]**	
Detailed justification:	According to the 'draft guidance document for waiving of data requirements for pheromones for inclusion in Annex I/IA of Directive 98/8/EC' (further referred to as 'Guidance for waiving') data on chronic toxic are normally not required if there is no concern from toxicological profile and depending on the level, frequency and duration of exposure. Data can be required when triggered by adverse effects in mutagenicity or short-term studies. Data can be waived if long-term exposure above background can be excluded. As for muscalure there is no concern from toxicological profile and the exposure will be low, this data requirement can be waived. Moreover, the results of the mutagenicity test were negative.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.6.1/6.6.2/ 6.6.3

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3

Genotoxicity in vitro

Salmonella typhimurium reverse mutation assay

Escherichia coli reverse mutation assay

Official use only

1 REFERENCE

1.1	**Reference**	Verspeek-Rip, C.M. (2006) Evaluation of the mutagenic activity of muscalure technical in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat) NOTOX B.V. Project no. 456457, 27 March 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Denka International B.V.
1.2.2		
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 IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD Guideline 471; EEC 2000/32/EC B.13/14
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

Section A6.6.1/6.6.2/ 6.6.3	**Genotoxicity in vitro** *Salmonella typhimurium* reverse mutation assay
Annex Point IIA6.6.1 / 6.6.2 / 6.6.3	*Escherichia coli* reverse mutation assay

3.1	**Test material**	As given in section 2
3.1.1	Batch number	05.079
3.1.2	Specification	As given in section 2 (Muscalure Technical) When this study was performed Denka based the 'purity' of the product on the content of both (Z)- en (E)-tricos-9-ene. Since then, the production methods for muscalure have not changed, which means that the muscalure used in this test had the same specification as given in Section 2.
3.1.2.1	Description	Clear colourless to light-yellow liquid
3.1.2.2	Purity	94.6% tricos-9-ene (sum of both (Z)- en (E)-tricos-9-ene)
3.1.2.3	Stability	Stable
3.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	*S. typhimurium*: TA 1535, TA 1537, TA 98, TA 100 *E. coli:* WP_2uvrA
3.2.2	Deficiencies / Proficiencies	Not applicable
3.2.3	Metabolic activation system	S9 mix from rat liver
3.2.4	Positive control	TA 1535: sodium azide TA 1537: 9-aminoacridine TA 98: 2-nitrofluorene TA 100: methylmethanesulphonate WP_2uvrA: 4-nitroquinoline N-oxide With S9 mix: all strains 2-aminoanthracene
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Range finding (TA 100 and WP_2uvrA only): 3, 10, 33, 100, 333, 1000, 3330 and 5000 µg/plate + solvent control and positive control (the test substance did not dissolve in the ≥ 1000 µg/plate test plates). Definitive experiment: 10, 33, 100, 333 and 1000 µg/plate + solvent control and positive control. 3 replicates. Two independent tests.
3.3.2	Way of application	Test substance was dissolved in ethanol and 0.1 mL of the solution was added to molten top agar together with bacteria and, if needed, S9 mix. The mixed molten top agar was then poured onto a selective agar plate.
3.3.3	Pre-incubation time	No pre-incubation
3.3.4	Other modifications	None
3.4	**Examinations**	

Section A6.6.1/6.6.2/ 6.6.3 — **Genotoxicity in vitro**

Salmonella typhimurium reverse mutation assay

Escherichia coli reverse mutation assay

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3

3.4.1	Number of cells evaluated	Not applicable

RESULTS AND DISCUSSION

3.5	**Genotoxicity**	
3.5.1	without metabolic activation	No, see Table A6_6_1-1
3.5.2	with metabolic activation	No, see Table A6_6_1-1
3.6	**Cytotoxicity**	No

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	An evaluation of the mutagenic activity of muscalure technical in the *Salmonella typhimurium* and *E. coli* reverse mutation assay was performed. The test was carried out with 4 histidine–requiring strains of *S. typhimurium* and one tryptophane-requiring strain of *E. coli*. Two independent tests were carried out in the presence and absence of S9-mix from rat liver. The range of concentrations tested was 10-1000 µg/plate. The guidelines OECD Guideline 471 and EEC 2000/32/EC B.13/14 were followed. There were no relevant deviations from the guidelines.
4.2	**Results and discussion**	The solvent control and the positive controls fulfilled the requirements of the guidelines. Muscalure technical did not induce a dose-related, two-fold increase in the number of revertant (His+) colonies in each of the four tester strains of *S. typhimurium* and in the number of revertant (Trp+) colonies of *E. coli* both in absence or presence of S9-metabolic activation.
4.3	**Conclusion**	It is concluded that muscalure technical is not mutagenic in the applied assays.
4.3.1	Reliability	1
4.3.2	Deficiencies	No

Section A6.6.1/6.6.2/ 6.6.3	**Genotoxicity in vitro** *Salmonella typhimurium* reverse mutation assay
Annex Point IIA6.6.1 / 6.6.2 / 6.6.3	*Escherichia coli* reverse mutation assay

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_6_1-1. Results of S. typhimurium/E. coli reverse mutation assay

Dose (µg/plate)	**Mean number of revertants/plate (± SD)[1]**

	Without metabolic activation		With metabolic activation (S9 mix rat)	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**
***S. typhimurium*, strain TA 1535**				
Positive control	1483 ± 71	1426 ± 35	160 ± 37	89 ± 11
0 (solvent control)	12 ± 4	11 ± 3	14 ± 5	16 ±3
10	11 ± 3	12 ± 2	15 ± 3	14 ± 3
33	12 ± 7	11 ± 3	12 ± 2	21 ± 1
100	11 ± 3	14 ± 2	13 ± 5	16 ± 3
333	14 ± 5	17 ± 6	11 ± 3	19 ± 5
1000 [1]	12 ± 2	12 ± 2	13 ± 2	16 ± 4
S. *typhimurium*, strain TA 1537				
Positive control	479 ± 86	287 ± 32	368 ± 37	206 ± 20
0 (solvent control)	6 ± 2	8 ± 2	6 ± 3	12 ± 4
10	6 ± 1	8 ± 2	5 ± 1	10 ± 4
33	5 ± 2	8 ± 4	5 ± 1	8 ± 4
100	6 ± 5	7 ± 5	4 ± 2	7 ± 3
333	4 ± 3	6 ± 1	5 ± 2	9 ± 3
1000 [1]	3 ± 1	7 ± 3	3 ± 2	11 ± 6
S. *typhimurium* strain TA 98				
Positive control	741 ± 158	1077 ± 38	628 ± 81	320 ± 47
0 (solvent control)	23 ± 5	19 ± 4	20 ± 8	25 ± 3
10	18 ± 4	25 ± 3	21 ± 4	36 ± 14
33	18 ± 5	17 ± 4	26 ± 4	31 ± 12
100	20 ± 6	20 ± 4	24 ± 6	32 ± 5
333	16 ± 1	19 ± 2	25 ± 2	33 ± 2
1000 [1]	17± 4	15 ± 2	27 ± 4	30 ± 11
S. *typhimurium* strain TA 100 [2]				
Positive control	1282 ± 47	1152 ± 77	1142 ± 18	1217 ± 73
0 (solvent control)	122 ± 9	144 ± 12	140 ± 26	113 ± 20
10	125 ± 10	150 ± 0	129 ± 9	102 ± 3
33	126 ± 5	153 ± 4	120 ± 8	118 ± 9
100	112 ± 10	152 ± 1	115 ± 8	107 ± 11
333	109 ± 13	156 ± 9	115 ± 5	100 ± 11
1000 [1]	117 ± 9	150 ± 18	116 ± 3	107 ± 20
E. *coli* strain $WP_2uvrA117$ [2]				

Table A6_6_1-1. Results of S. typhimurium/E. coli reverse mutation assay

Dose (µg/plate)	Mean number of revertants/plate (± SD)[1]			
	Without metabolic activation		With metabolic activation (S9 mix rat)	
	Trial 1	Trial 2	Trial 1	Trial 2
Positive control	796 ± 63	415 ± 29	349 ± 18	243 ± 18
0 (solvent control)	24 ± 5	11 ± 2	22 ± 9	23 ± 3
10	23 ± 3	9 ± 2	26 ± 4	18 ± 8
33	26 ± 2	10 ± 5	22 ± 4	14 ± 1
100	21 ± 3	11 ± 1	25 ± 9	21 ± 4
333	24 ± 2	12 ± 3	27 ± 5	18 ± 4
1000 [1]	25 ± 3	15 ± 4	26 ± 4	17 ± 4

[1] Slight precipitation visible

[2] *S. typhimurium* strain TA 100 and *E. coli* strain WP_2uvrA117: data of trial 1 are from the 10 -1000 µg/plate part of the range-finding test