Section A 6.1.1-01 **Annex Point IIA VI.6.1.1**		**Oral toxicity** LD50 study in the rat	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] 2002, Acute Oral Toxicity Study of ACITICIDE OIT 100%, [redacted] unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	THOR GmbH, Germany	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. OECD 423	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 of dossier.	
3.1.1	Lot/Batch number	ACTICIDE OIT 100%, [redacted]	
3.1.2	Specification	technical grade	
3.1.2.1	Description	Clear yellow brown liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Dosing solutions were freshly prepared on the day of application.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	[redacted]	X
3.2.5	Age/weight at study initiation	9-11 weeks 212-299 g	
3.2.6	Number of animals per group	3 per sex per dose level	X
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	oral	
3.3.1	Postexposure period	14 days	
3.3.2	Type	gavage	

Section A 6.1.1-01 **Annex Point IIA VI.6.1.1**		**Oral toxicity** LD50 study in the rat	
3.3.3	Concentration	Sarting dose: 200 mg/kg bw; followed by 2000 mg/kg bw; finally 500 mg/kg bw.	
3.3.4	Vehicle	water	
3.3.5	Concentration in vehicle		
3.3.6	Total volume applied	10 ml/kg bw	
3.3.7	Controls	no	
3.4	**Examinations**		
3.5	**Method of determination of LD_{50}**	Mortality, clinical signs	
3.6	**Further remarks**		

Section A 6.1.1-01 **Annex Point IIA VI.6.1.1**		**Oral toxicity** LD50 study in the rat	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	*100 per cent mortality was observed at the dose level of 2000 mg Acticide® OIT 100% /kg body weight. At the dose level of 500 mg Acticide® OIT 100% /kg body weight, one mortality was observed out of 6 animals (i.e. 16.7%). At the dose level of 200 mg Acticide® OIT 100% /kg body weight, no mortality was observed.* *Clinical signs observed in the treated animals were lethargy, abdominal breathing, gasping and piloerection. The cl signs were observed from the 1 hour alter dosing and persisted throughout the dosing day and up to 48 hours, the surviving animals were found normal an day 3 after dosing. Therfore, no abnormalities were observed.* *There was a normal increase in the body weight of all treated group animals except two male animals (N° 14 and 15) of group III (i.e. 500 mg/kg body weight) revealed reduction in body weight an day 7.*	X
4.2	**Pathology**	*External examination of the "found deadti` sacrificed animals of either sexes from treated groups did not reveal any lesion of pathological siginificance.* *Post mortem examination of "found dead" animals revealed lesions in lungs (congestion/hepatisation); liver (mottling); and stomach. (haerrhagic).* *Terminally sacrificed animals on necropsy revealed lesions as lungs (haemorrhages); liver (mottling); spieen (whitish foci) and congestion in kidneys.* *The mortality percentage in group G2 (100%) and lesions observed in different visceral organs could be correlated with the test substance administration.* *Terminally sacrificed animals from treated groups revealed insignificant vascular changes with low level of occurrence, hence it could be considered as unrelated with test substance administered.*	
4.3	**Other**		
4.4	**LD_{50}**	*As per the interpretation which states that when 2 to 3 mortality is observed at the dose level of 2000 mg/kg body weight and 0 to 1 at the dose level of 500 mg/kg body weight, then LD5o value is in between 500 and 2000 mg/kg body weight. Based an the mortality observed at 500 mg/kg body weight and 2000 mg/kg body weight dose levels, it is inferred that the acute oral median lethal dose (LD50) of Acticide® OIT 100% in [redacted] rats is in between 500 and 2000 mg/kg body weight by this test method.*	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	*Per-guideline study with technical grade test item [redacted]*	
5.2	**Results and discussion**	*Based on the mortality observed at 500 mg/kg body weight (1/6) and 2000 mg/kg body weight (3/3) dose levels, it is inferred that the acute oral median lethal dose (LD50) of Acticide® OIT 100% in [redacted] rats is in between 500 and 2000 mg/kg body weight by this test method.*	
5.3	**Conclusion**	The LD50 for both males and females and combined sexes was between 500 and 2000 mg/kg bw.	
5.3.1	Reliability	1	

Section A 6.1.1-01 **Annex Point IIA VI.6.1.1**	**Oral toxicity** LD50 study in the rat	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*10/03/2009*	
Materials and Methods	*Group size/sex:* *200 mg/kg – 3 males and 3 females* *500 mg/kg – 3 males and 3 females* *2000 mg/kg – 3 females*	
Results and discussion	*500 mg/kg – No female deaths, 1/3 males deaths.*	
Conclusion		
Reliability	*1*	
Acceptability	*Acceptable*	
Remarks	*In agreement with the applicant's assessment.*	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A 6.1.1-1: Table for oral toxicity to rats

Group No.	Dose in mg/kg bw	Number of animals used	Mortality after dosing at							Total Mortality		
			1-3h	*	24h	48h	72h	4-7d	8-14d	M	F	%
1	200	3 M + 3 F	0	0	0	0	0	0	0	0	0	0
2	2000	3 M	2	0	1	0	0	0	0	3	-	100
3	500	3 M + 3 F	0	0	0	1	0	0	0	1	0	16.67

Key: M = male, F = female, bw = body weight, * = 5h 20 min – 7h

Section A 6.1.2-01 **Annex Point IIA VI.6.1.2**		**Dermal toxicity** Acute dermal toxicity – LD_{50} study in the rat.	
		6 REFERENCE	**Official use only**
6.1	**Reference**	[redacted] *1991, Acute Dermal Toxicity to the Rat of Acticide 45, unpublished*	
6.2	**Data protection**	*Yes*	
6.2.1	Data owner	*THOR GmbH, Germany*	
6.2.2	Companies with letter of access		
6.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.	
		7 GUIDELINES AND QUALITY ASSURANCE	
7.1	**Guideline study**	*Yes. EPA Fifra 81-2 equals OECD 402*	
7.2	**GLP**	*Yes*	
7.3	**Deviations**	*No*	
		8 MATERIALS AND METHODS	
8.1	**Test material**		
8.1.1	Lot/Batch number	ACTICIDE 45, [redacted]	
8.1.2	Specification	Other: technical grade product	
8.1.2.1	Description	Amber liquid	
8.1.2.2	Purity	[redacted]	
8.1.2.3	Stability	Stable during the study	
8.2	**Test Animals**		
8.2.1	Species	rat	
8.2.2	Strain	[redacted]	
8.2.3	Source	[redacted]	
8.2.4	Sex	Both sex	
8.2.5	Age/weight at study initiation	Age: 7-10 weeks 207-264 g	
8.2.6	Number of animals per group	Five/sex per group	
8.2.7	Control animals	no	
8.3	**Administration/ Exposure**		
8.3.1	Postexposure period	14 days	

Section A 6.1.2-01 **Annex Point IIA VI.6.1.2**		**Dermal toxicity** Acute dermal toxicity – LD_{50} study in the rat.	
8.3.2	Area covered	Approximately 50 x 50 mm (equivalent to ~10% of body surface)	
8.3.3	Occlusion	Yes, occlusive	
8.3.4	Vehicle	No further vehicle than the solvent propylene glycol	
8.3.5	Concentration in vehicle	Min. 45%	
8.3.6	Total volume applied	No data; dose: 2.0 g ACITICE 45 / kg bw. (900 mg a.i. /kg bw)	
8.3.7	Duration of exposure	24 hours	
8.3.8	Removal of test substance	With warm water	
8.3.9	Controls	no	
8.4	**Examinations**	Clinical signs: 5 times during dosing (on day one) and 2 times a day for the remaining study period dermal reactions body weight macroscopic post-mortem examination	
8.5	**Method of determination of LD_{50}**	On mortality	
8.6	**Further remarks**		
		9 RESULTS AND DISCUSSION	
9.1	**Clinical signs**	There were no deaths following a single dermal exposure of ACTICIDE 45 at 2.0 g/kg bw. There were no signs of systemic toxicity to treatment. Slight, or in two cases well-defined, oedema was recorded at sites of application of ACTICIDE 45 for all rats on Day 2. Reactions developed over the next few days via hardening of the dose site with localised serve damage in depth (chemical burn?) associated with well-defined to serve oedema to scabbing on the dose site for all animals bby Day 7; these responses prevent assessment of erythema and oedema. A gradual recovery was seen during the second week of the study with the appearance of a healing scab/skin from Day 10. At termination, the latter reaction was still recorded for the majority of animals although the skin of one male and one female rats appeared normal. Slightly low body weight gains were recorded for four males on Day 8 and for one of these rats on Day 15. The remaining male and all females achieved anticipated bodyweight gains throughout the study.	
9.2	**Pathology**	Terminal autopsy revealed no macroscopic abnormalities.	
9.3	**Other**		
9.4	**LD_{50}**	The acute lethal dose to rats of ACTICIDE 45 was geater than 2000 mg/kg bw (>900 mg a.i. /kg bw).	

Section A 6.1.2-01 **Annex Point IIA VI.6.1.2**	**Dermal toxicity** Acute dermal toxicity – LD_{50} study in the rat.	
	10 APPLICANT'S SUMMARY AND CONCLUSION	
10.1 Materials and methods	*Per-guideline study with technical grade product (45 % OIT in propylene glycol).*	
10.2 Results and discussion	No deaths. No signs of systemic reaction to treatment.	
10.3 Conclusion	The LD50 for the test substance is > 2000 mg/kg bw. The LD50 for the active substance is > 900 mg a.i./kg bw.	
10.3.1 Reliability	1	
10.3.2 Deficiencies	no	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*10/03/2009*	
Materials and Methods		
Results and discussion		
Conclusion		
Reliability	*1*	
Acceptability	*Acceptable*	
Remarks	*In agreement with the applicant's assessment.*	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A 6.1.2-1: Table for mortality following dermal application of OIT to SD rats

Dose [mg/kg]	Number of dead / number of investigated	Time of death (range)
900	0/5 males 0/5 females	

TABLE 1

Dermal reactions elicited by Acticide 45

Dose (g/kg)	Sex	Animal number & ear mark	E O	Day 2	3	4	5	6	7	8	9	10	11	12	13	14	15
2.0	♂	1 RP	E	0	α*	α*	β*	β*	β*	β*	β*	β*	γ*	γ*	γ*	γ*	γ*
			O	2	3	3	*	*	*	*	*	*	*	*	*	*	*
		2 LP	E	0	α*	α*	β*	β*	β*	β*	β*	β*	β*	γ*	0γ	0	0
			O	1	2	2	*	*	*	*	*	*	*	*	0	0	0
		3 RPLP	E	0	α*	α*	β*	β*	β*	β*	β*	γ*	γ*	γ*	γ*	γ*	γ*
			O	1	3	3	*	*	*	*	*	*	*	*	*	*	*
		4 RIRO	E	0	α*	α*	α*	β*	β*	β*	β*	β*	γ*	γ*	γ*	γ*	γ*
			O	1	2	2	3	*	*	*	*	*	*	*	*	*	*
		5 LILO	E	0	α*	α*	α*	β*	β*	β*	β*	β*	β*	γ*	γ*	γ*	γ*
			O	2	3	3	3	*	*	*	*	*	*	*	*	*	*
	♀	6 RP	E	0	α*	α*	α*	α*	β*	β*	β*	γ*	γ*	γ*	γ*	γ*	γ*
			O	1	2	2	3	4	*	*	*	*	*	*	*	*	*
		7 LP	E	0	α*	α*	α*	α*	β*	β*	β*	γ*	γ*	γ*	γ*	γ*	γ*
			O	1	2	2	4	4	*	*	*	*	*	*	*	*	*
		8 RPLP	E	0	α*	α*	β*	β*	β*	β*	β*	γ*	γ*	γ*	γ*	γ*	γ*
			O	1	2	2	*	*	*	*	*	*	*	*	*	*	*
		9 RIRO	E	0	α*	α*	α*	α*	β*	β*	β*	γ*	γ*	γ*	0γ	0	0
			O	1	3	3	4	4	*	*	*	*	*	*	0	0	0
		10 LILO	E	0	α*	α*	α*	α*	β*	β*	β*	γ*	γ*	γ*	γ*	γ*	γ*
			O	1	2	2	4	4	*	*	*	*	*	*	*	*	*

E Erythema
O Oedema
α Hardening of dose site with localised severe damage in depth (chemical burn?)
β Scabbing on dose site
γ Healing scab/skin
* Unable to assess for erythema and oedema

: 13 :

Section A 6.1.3-01 Annex Point IIA VI.6.1.3	Inhalation toxicity Acute inhalation toxicity – LC_{50} study in the rat	
	11 REFERENCE	**Official use only**
11.1 Reference	[redacted] 1992, Acticide 45 Acute Inhalation Toxicity to the Rat, [redacted] unpublished	
11.2 Data protection	Yes	
11.2.1 Data owner	THOR GmbH, Germany	
11.2.2 Companies with letter of access		
11.2.3 Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.	
	12 GUIDELINES AND QUALITY ASSURANCE	
12.1 Guideline study	Yes. OECD 403, EPA 81-3	
12.2 GLP	Yes	
12.3 Deviations	A vehicle control group was not included.	
	13 MATERIALS AND METHODS	
13.1 Test material	Other: ACTICIDE 45	
13.1.1 Lot/Batch number	[redacted]	
13.1.2 Specification	[redacted]	
13.1.2.1 Description	Amber liquid	
13.1.2.2 Purity		
13.1.2.3 Stability	[redacted]	
13.2 Test Animals		
13.2.1 Species	Rat	
13.2.2 Strain	[redacted]	
13.2.3 Source	[redacted]	
13.2.4 Sex	Both sex	
13.2.5 Age/weight at study initiation	6-8 weeks old ca. 200 g	
13.2.6 Number of animals per group	5 per sex / group	
13.2.7 Control animals	Yes	
13.3 Administration/ Exposure		
13.3.1 Postexposure period	14 days	

Section A 6.1.3-01 **Annex Point IIA VI.6.1.3**	**Inhalation toxicity** Acute inhalation toxicity – LC_{50} study in the rat	
13.3.2 Concentrations	Group ACTICIDE 45 in air mg/l SD 1 Control (air) 2 0.734 0.013 3 0.256 0.05 4 0.498 0.012	
13.3.3 Particle size	Overal Means Cut-off size µm Cumulative % less than stated size 9.8 98.8 6.0 95.3 3.5 84.7 1.55 47.0 0.93 34.9 0.52 5.5 Mass median aerodynamic diameter 1.58 µm, Standard geometrc deviation 2.21	
13.3.4 Type or preparation of particles	Aerosol generator	
13.3.5 Type of exposure	Whole body exposure to liquid droplet aerosol	
13.3.6 Vehicle	Propylene glycol (55%)	
13.3.7 Concentration in vehicle	45% ai	
13.3.8 Duration of exposure	4 hours	
13.3.9 Controls	Air only	
13.4 Examinations		
13.5 Method of determination of LD_{50}	Log pobit method of Miller and Tainter Standard error of LC50 = $2s / (2N)^{-2}$	
13.6 Further remarks		

Section A 6.1.3-01 **Annex Point IIA VI.6.1.3**	**Inhalation toxicity** Acute inhalation toxicity – LC_{50} study in the rat	
	14 RESULTS AND DISCUSSION	
14.1 Clinical signs	The signs seen during exposure were considered to be consistent with inhalation of an irritant aerosol and included partial closing of the eyes, wetness around the snout and eyes, prone posture and disturbances to respiration. During the observation period signs evident in rats exposed to ACTICIDE 45 included gasping and other disturbances to respiration, immobility and staining of the body fur. Subsequently, signs indicative of an effect on the respiratory tract, including noisy and exaggerated respiration persisted for up to 12 days post exposure. By the end of the observation period, all rats that survived exposure to ACTICIDE 45 were of normal appearance and behaviour. The mortality is summarised below: Group — Male — Deaths Female — Total 1 (Control) — 0/5 — 0/5 — 0/10 2 (0.734 mg/l) — 4/5 — 3/5 — 7/10 3 (0.256 mg/l) — 0/5 — 0/5 — 0/10 4 (0.498 mg/l) — 1/5 — 2/5 — 3/10 In Group 2 (0.734 mg/l) 2 male rats (112, 113) were found dead 50 minutes post exposure, 4 rats (111d, 115d, 1179, 1189) were found dead on Day 1 and 1 female rat (120) was found dead on Day 2. In Group 4 (0.498 mg/1) 3 rats (14d, 169, 209) were found dead on Day 1 of the observation period.	
14.2 Pathology	The macroscopic pathological findings for rats that died as a result of exposure to ACTICIDE 45 were typified by congestion of the lungs. The stomachs of a number of decedents were found to be gas-filled. This finding is often seen in rats that die as a result of respiratory distress and is due to swallowing air during attempts to breath. No treatment-related abnormalities were seen in rats that survived exposure to ACTICIDE 45. The lung weight to bodyweight ratios were higher than control values in most rats that died as a result of exposure to ACTICIDE 45. Lung weights for 9/10 rats that survived exposure at 0.256 mg/1 were within normal limits but 1 female rat had a high lung weight. Lung weights for female rats that survived exposure at 0.498 mg/1 were higher than control values but those for male rats surviving exposure at 0.498 mg/1 and for all rats surviving exposure at 0.734 mg/1 were within normal limits.	
14.3 Other	For rats that survived, there were moderate to marked decreases of bodyweight or reductions in the rate of bodyweight gain for up to 6 days days following exposure. Subsequently the rate of weight gain for rats that survived exposure to ACTICIDE 45 was generally similar to or greater than that of the control rats. Following exposure to ACTICIDE 45 the rats consumed little or no food overnight. Food consumption was moderately or markedly reduced for several further days, returning to normal values during the latter part of the observation period. The degree and duration of the effect an food consumption was generally related to the exposure level. Rats exposed to ACTICIDE 45 consumed little water overnight	

Section A 6.1.3-01 **Annex Point IIA VI.6.1.3**	**Inhalation toxicity** Acute inhalation toxicity – LC_{50} study in the rat	
	following exposure. In rats exposed to ACTICIDE 45 at 0.256 mg/l, water consumption returned to normal by Day 7 of observation. In rats exposed at 0.498 mg/1 water consumption returned to normal within 4 days (males) or 8 days (females). Water consumption by the rats exposed at 0.734 mg/1 was reduced and variable for 8 days following exposure. Subsequently values were often higher than control values.	
14.4 LD_{50}	From the mortality data for all groups the LC_{50} (4-hour) for ACTICIDE 45 was established at: 0.60 mg per litre of air The standard error (SE) of the estimate was 0.084 mg/l. There was no evidence of a sex-related difference in mortality.	
	15 APPLICANT'S SUMMARY AND CONCLUSION	
15.1 Materials and methods	*Per-guideline study with technical grade product (ACTICIDE 45) containing 45% of the test item in propylene glycol.*	
15.2 Results and discussion	The LC50 for the test substance ACTICIDE 45 is 0.6 mg/l.	
15.3 Conclusion	The LC50 for ACTICIDE OIT is 0.27 mg/l.	
15.3.1 Reliability	2	
15.3.2 Deficiencies	No vehicle control	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*10/03/2009*	
Materials and Methods		
Results and discussion		
Conclusion		
Reliability	*1*	
Acceptability	*Acceptable*	
Remarks	*In agreement with the applicant's assessment.*	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

TABLE 1

Concentrations of ACTICIDE 45

Group	Sample	Time	Amount in air (mg/l)
2	2.1	0h : 30m	0.734
	2.2	1h : 00m	0.718
	2.3	2h : 00m	0.735
	2.4	3h : 00m	0.755
	2.5	3h : 55m	0.729
		Mean	0.734
3	3.1	0h : 30m	0.253
	3.2	1h : 00m	0.259
	3.3	2h : 00m	0.262
	3.4	3h : 00m	0.249
	3.5	3h : 55m	0.259
		Mean	0.256
4	4.1	0h : 30m	0.496
	4.2	1h : 00m	0.505
	4.3	2h : 00m	0.480
	4.4	3h : 00m	0.496
	4.5	3h : 55m	0.512
		Mean	0.498

TABLE 2

Particle size distribution of ACTICIDE 45

Group 2

Sample	Time taken	Stage	Cut-off size (µm)	Amount collected (mg)	% of total
PSD 1	0h : 45m	3	9.8	0.02779	0.6
		4	6.0	0.08857	2.0
		5	3.5	0.28182	6.5
		6	1.55	1.57265	36.0
		7	0.93	0.68023	15.6
		8	0.52	1.38243	31.6
		Filter	0.0	0.33474	7.7
			Totals	4.36823	100.0
PSD 2	1h : 45m	3	9.8	0.03811	1.2
		4	6.0	0.09175	2.9
		5	3.5	0.26621	8.5
		6	1.55	1.18195	37.8
		7	0.93	0.33586	10.8
		8	0.52	0.97878	31.3
		Filter	0.0	0.23010	7.4
			Totals	3.12276	99.9

Overall means	Cut-off size (µm)	Cumulative % less than stated size
	9.8	99.1
	6.0	96.7
	3.5	89.4
	1.55	52.6
	0.93	39.0
	0.52	7.5

MMAD 1.40 µm, σg 2.21

MMAD Mass median aerodynamic diameter
σg Standard geometric deviation

TABLE 2

(Particle size distribution of ACTICIDE 45 - continued)

Group 3

Sample	Time taken	Stage	Cut-off size (µm)	Amount collected (mg)	% of total
PSD 1	0h : 45m	3	9.8	0.04093	1.4
		4	6.0	0.11701	3.9
		5	3.5	0.34171	11.5
		6	1.55	1.04870	35.2
		7	0.93	0.29156	9.8
		8	0.52	0.90778	30.5
		Filter	0.0	0.22849	7.7
			Totals	2.97618	100.0
PSD 2	1h : 45m	3	9.8	0.05162	1.8
		4	6.0	0.12857	4.5
		5	3.5	0.30330	10.7
		6	1.55	1.01728	35.8
		7	0.93	0.33618	11.8
		8	0.52	0.82136	28.9
		Filter	0.0	0.18461	6.5
			Totals	2.84292	100.0

Overall means Cut-off size (µm)	Cumulative % less than stated size
9.8	98.4
6.0	94.2
3.5	83.1
1.55	47.6
0.93	36.8
0.52	7.1

MMAD 1.56 µm, σg 2.34

MMAD Mass median aerodynamic diameter
σg Standard geometric deviation

TABLE 2

(Particle size distribution of ACTICIDE 45 - continued)

Group 4

Sample	Time taken	Stage	Cut-off size (µm)	Amount collected (mg)	% of total
PSD 1	0h : 45m	3	9.8	0.05485	1.3
		4	6.0	0.14830	3.6
		5	3.5	0.47600	11.5
		6	1.55	1.49560	36.1
		7	0.93	0.53090	12.8
		8	0.52	1.22650	29.6
		Filter	0.0	0.21261	5.1
			Totals	4.14476	100.0
PSD 2	1h : 45m	3	9.8	0.05109	1.3
		4	6.0	0.13845	3.4
		5	3.5	0.38806	9.6
		6	1.55	1.59175	39.4
		7	0.93	0.45720	11.3
		8	0.52	1.18075	29.2
		Filter	0.0	0.23716	5.9
			Totals	4.04446	100.1

Overall means	Cut-off size (µm)	Cumulative % less than stated size
	9.8	98.8
	6.0	95.3
	3.5	84.7
	1.55	47.0
	0.93	34.9
	0.52	5.5

MMAD 1.58 µm, σg 2.21

MMAD Mass median aerodynamic diameter
σg Standard geometric deviation

Section A 6.1.4-01 Annex Point IIA VI.6.1.4		Acute dermal irritation Skin irritation in the rabbit	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] Skin Irritation to the Rabbit of Acticide 45, [redacted] unpublished	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	THOR GmbH, Germany	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. EPA FIFRA 81-5 which complies with OECD 404	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Other: ACTICIDE 45	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	[redacted]	
3.1.2.1	Description	Amber liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable during the study	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	13-15 weeks 3.2 to 4.0 kg	
3.2.6	Number of animals per group	six	
3.2.7	Control animals	No. Untreated skin areas served as control.	
3.3	**Administration/ Exposure**		
3.3.1	Application	dermal	

Section A 6.1.4-01 **Annex Point IIA VI.6.1.4**		**Acute dermal irritation** Skin irritation in the rabbit	
3.3.1.1	Preparation of test substance	ACTICIDE 45 as it is.	
3.3.1.2	Test site and Preparation of Test Site	10 cm^2 of clipped skin of dorso-lumbar region	
3.3.2	Occlusion	Semi-occlusive	
3.3.3	Vehicle	Propylene gycol	
3.3.4	Concentration in vehicle	45% a.i.	
3.3.5	Total volume applied	0.5 ml	
3.3.6	Removal of test substance	Washing with water	
3.3.7	Duration of exposure	4 hours	
3.3.8	Postexposure period	14 days	
3.3.9	Controls	Yes	X
3.4	**Examinations**		
3.4.1	Clinical signs	Daily	
3.4.2	Dermal examination		
3.4.2.1	scoring system	Erythema and eschar formation: No erythema . 0 Very sIight erythema (barely perceptible) 1 WeII-defined erylhema 2 Moderate In severe erythemia 3 Severe erythema (beet redness) to slight eschar formation (injuries in deptli) 4 Oedema formation: No oedema 0 Very slight oedema (barely perceptible) 1 SIight oedema (edges of area weIIdefined by definite raising) 2 Moderate oedema (raised approximately 1 milIl imetre) 3 Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure) 4	
3.4.2.2	Examination time points	On Day 1 30 min after 4 hour exposure. On day 2, 3, 4 (equivalent to 24, 48, 72 hours). Additioanl observations on Day 5 through 14.	
3.4.3	Other examinations	Any other lesion not covcred hy his scoring System was describcd.	
3.5	**Further remarks**		
		4 RESULTS AND DISCUSSION	

Section A 6.1.4-01 **Annex Point IIA VI.6.1.4**		**Acute dermal irritation** Skin irritation in the rabbit	
4.1	**Average score**		
4.1.1	Erythema	WeIl-defined erythema with severe oedema was evident at all trealmenl sites following the removal of the dressings (Day 1). By Day 2, necrosis and chemical burns with slight to moderate oedema had developed at all sites. Similar reactions persisted and were still visible at five sites on Day 14. Reactions at one site ameliorated by Day 8 (moderate to severe erythema with slight oedema and regressed futher by Day 10 with weIl-defined erythema and very slight oedema present. Similar reaetions were visible on Day 4. This response was accompanied by desquamation of the stratum corneum (sloughing) on Days 8 and 9 and by hyperkeratinisation on Days 10 to 14.	
4.1.2	Oedema		
4.2	**Reversibility**	Not fully reversible within 14 days post dose observation.	
4.3	**Other examinations**		
4.4	**Overall result**	A single semi-occlusive application of ACTICIDE 45 to intact rabbit skin for four hours elicted persistent severe dermal irritation.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	*Per-guideline study with technical grade product (45 % OIT in propylene glycol).*	
5.2	**Results and discussion**	Product [redacted] is highly irritating.	
5.3	**Conclusion**	The active substance OIT is corrosive (causes burns)	
5.3.1	Reliability	1	
5.3.2	Deficiencies	no	
		Evaluation by Competent Authorities	
		Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date			
Materials and Methods		*Study date: 1991* *3.3.9: Untreated skin areas served as controls.*	
Results and discussion			
Conclusion			
Reliability		*1*	
Acceptability		*Acceptable*	
Remarks		*In agreement with the applicant's assessment.*	
		COMMENTS FROM ...	
Date			
Materials and Methods			

Section A 6.1.4-01 Annex Point IIA VI.6.1.4	Acute dermal irritation Skin irritation in the rabbit	
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A 6.1.4-1: Table for skin irritation study – mean scores for six rabbits

TABLE 1

Dermal reactions elicited by Acticide 45

Rabbit Number and Sex	E = Erythema O = Oedema	Day													
		1*	2	3	4	5	6	7	8	9	10	11	12	13	14
613♀	E O	2 4	4A 3	4A 3	4A 3	4A 3	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2
614♀	E O	2 4	4A 2	4A 3	4A 3	4A 3	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2
615♀	E O	2 4	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 1	4A 1	4A 1	4A 1	4A 1	4A 1
616♀	E O	2 4	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 1	4A 1	4A 1	4A 1	4A 1	4A 1
624♀	E O	2 4	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	4A 1	4A 1	4A 1	4A 1	4A 1	4A 1
626♀	E O	2 4	4A 2	4A 2	4A 2	4A 2	4A 2	4A 2	3B 2	3B 2	2C 1	2C 1	2C 1	2C 1	2C 1

* Approximately 30 minutes after removal of the dressing
A Necrosis
B Sloughing
C Hyperkeratinisation

Section A 6.1.4-02 **Annex Point IIA VI.6.1.4-2**	**Acute eye irritation** **Eye irritation in the rabbit**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [X]**	
Detailed justification:	Scientifically unjustified Risk of serious damage to eyes is predictable without testing. According to OECD 405 no testing is needed because of existing animal data showing corrosive effects on skin (see section A 6.1.4-01). Other justification Ethical reasons: The responsible use of as few as possible animals is expected by customers as well as demanded by legal regulations and political organizations.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*11/03/2009*	
Evaluation of applicant's justification	*Acceptable*	
Conclusion	*Agree with applicant*	
Remarks	*Support applicant's pragmatic approach to minimising animal use and suffering.*	
	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 A 6.1.5-01 Annex Point IIA VI.6.1.5		Skin sensitisation Local lymph node assay (LLNA) in mice	
		6 REFERENCE	**Official use only**
6.1	**Reference**	[redacted] 2003, ACTICIDE OIT: Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens), [redacted] unpublished	
6.2	**Data protection**	Yes	
6.2.1	Data owner	THOR GmbH, Germany	
6.2.2	Companies with letter of access		
6.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.	
		7 GUIDELINES AND QUALITY ASSURANCE	
7.1	**Guideline study**	Yes, OECD 429	
7.2	**GLP**	Yes	
7.3	**Deviations**	No	
		8 MATERIALS AND METHODS	
8.1	**Test material**	ACTICIDE OIT	
8.1.1	Lot/Batch number	[redacted]	
8.1.2	Specification	technical grade	
8.1.2.1	Description	clear yellow-brown liquid	
8.1.2.2	Purity	[redacted]	
8.1.2.3	Stability	unknown and therefore excluded from the Statement of Compliance	
8.1.2.4	Preparation of test substance for application	0.25%, 0.5%, 1%, 2.5% and 5% ACTICIDE OIT in acetone:olive oil, 4:1 (v/v)	
8.1.2.5	Pretest performed on irritant effects	Yes	
8.2	**Test Animals**		
8.2.1	Species	Mouse	
8.2.2	Strain	[redacted]	
8.2.3	Source	[redacted]	
8.2.4	Sex	female	
8.2.5	Age/weight at study initiation	8-12 weeks, 16.4-19.8 g	
8.2.6	Number of animals per group	4 (5 groups)	
8.2.7	Control animals	Yes , 2 groups per 4 animals	

Section A 6.1.5-01 **Annex Point IIA VI.6.1.5**		**Skin sensitisation** Local lymph node assay (LLNA) in mice	
8.3	**Administration/ Exposure**	LLNA: non-Adjuvant	
8.3.1	Induction schedule	day 1, 2 and 3 (topical induction)	
8.3.2	Way of Induction	Topical: Application of 0.25 µL/ear on the dorsal surface of both ears at 0.25%, 0.5%, 1%, 2.5% and 5% (w/v) ACTICIDE OIT in acetone:olive oil, 4:1 (v/v) on three consecutive days	
8.3.3	Concentrations used for induction	See 3.3.2 Selection of concentrations was based on a pre-test with 0% (vehicle), 0.25%, 0.5%, 1%, 2.5% and 5% (w/v) ACTICIDE OIT in acetone:olive oil (25 µL/ear on three consecutive days).	
8.3.4	Concentration Freunds Complete Adjuvant (FCA)	Not applicable	
8.3.5	Challenge schedule	Not applicable On day 6 mice received a 250 µL i.v. injection of 19.9 µCi ^{3}H-thymidine (group 1-4) or 19.1 µCi ^{3}H-thymidine (group 5-7) in phosphate-buffered saline.	
8.3.6	Concentrations used for challenge	Not applicable	
8.3.7	Rechallenge	Not applicable	
8.3.8	Scoring schedule	About 5 hours after application of ^{3}H-thymidine, mice were sacrificed and cell suspensions from the auricular lymph nodes were prepared.	
8.3.9	Removal of the test substance	Not applicable	
8.3.10	Positive control substance	5%, 10%, 25% (w/v) α-hexylcinnamaldehyde (HCA) in acetone:olive oil 4:1	
8.4	**Examinations**		
8.4.1	Pilot study	Yes Test with 0.1%, 0.25%, 0.5%, 1%, 10%, 25%, 50% (w/v) and 100% (undiluted) test item in action:olive oil, 4:1 (v/v) in 2 mice/group	
8.5	**Further remarks**	Radioactivity in the lymph nodes cell suspensions was determined as an indicator of proliferative changes (skin sensitisation). The stimulation index (SI) as the ratio of treated vs. control animal values was calculated. In case of an SI ≥3, the test compound is considered a skin sensitiser and the EC_3 (estimated concentration resulting in a 3-fold SI) was determined for comparison of the relative skin sensitisation potential between chemicals.	
		9 RESULTS AND DISCUSSION	
9.1	**Results of pilot studies**	One day after application in one animal dosed at 10% and 25% test item showed severe swelling and moderate erythema at both dosing sites. Animals dosed with 50% and 100% test substance died. No irritation effects were seen at other dose groups.	
9.2	**Results of test**		
9.2.1	24h after challenge	Not applicable	
9.2.2	48h after challenge	Not applicable	

Section A 6.1.5-01 **Annex Point IIA VI.6.1.5**	**Skin sensitisation** Local lymph node assay (LLNA) in mice	
9.2.3 Other findings	No test item related clinical signs were observed in any animal of the control group and the 1% dose group. In the mice treated with 0.5% test item slight swelling was observed in all animals at both test sites about 1 hour after first application. On the second application day slight erythema (2.5% group) and general erythem and slight swelling (5% group) were seen at both test sites in all animals of the higher dose groups. All signs remained till the end of the study. The body weights were unaffected by treatment. The levels of radioactivity were slightly higher than controls at 0.25% and the stimulation index was well below 3. In all other treated groups was the stimulation index above 3 (see table A6_1_5-2).	
9.3 Overall result	ACTICIDE OIT is considered to be a skin sensitiser in this test with a calculated EC3 = 0.46% (w/v)	
	10 APPLICANT'S SUMMARY AND CONCLUSION	
10.1 Materials and methods	Evaluation of the skin sensitisation potential in a local lymph node assay in mice (topical application; determination of proliferative activity in the draining lymph node); no relevant deviation from guideline (OECD No. 429)	
10.2 Results and discussion	ACTICIDE OIT revealed skin sensitisation potential.	
10.3 Conclusion	Classification for skin sensitisation (R43) is considered required for ACTICIDE OIT according to Directive 2001/59/EC (adaptation of 67/548/EEC).	
10.3.1 Reliability	1	
10.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*31/03/2009*	
Materials and Methods		
Results and discussion		
Conclusion		
Reliability	*1*	
Acceptability	*Acceptable*	
Remarks	*In agreement with the applicant's assessment.*	

Section A 6.1.5-01 **Annex Point IIA VI.6.1.5**	**Skin sensitisation** Local lymph node assay (LLNA) in mice	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A6.1.5-1 Detailed information including induction/challenge/scoring schedule for skin sensitisation test

Inductions	**LLNA**		**Observations/Remarks**
	day of treatment	application	
Induction 1	1	topical	slight swelling at 0.5% ACTICIDE OIT at both application sites
Induction 2	2	topical	slight erythema (2.5% group) and general erythem and slight swelling (5% group) at both application sites
Induction 3	3	topical	
scoring	6	Collection of lymph nodes 5 hours after application with ^{3}H thymidine	All skin reactions remained till end of test

Table A6.1.5-2 Result of skin sensitisation test

	Negative control		**Test groups**					**Positive control**
ACTICIDE OIT	0%	0%	0.25%*****	0.5%*****	1%	2.5%	5%	25% (v/w)HCA
DPM per LN	581	574	1007	1834	2672	6954	6111	3732
SI	-	-	1.8*****	3.2*****	4.6	12.0	10.5	7.1
			EC3 = 0.46% (w/v)					

* The values were used for calcuationg the EC3

Section A6.1.5/02 Skin sensitisation

Annex Point IIA6.1.5 *(Local Lymph Node Assay)*

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] 2006, N-(n-octyl) malonamic acid: local lymph node assay, [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2	Letter of access	/
1.1.1	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD 429 and US EPA OPPTS 870.2600
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	N-(n-octyl) malonamic acid (NNOMA)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	The test substance is a metabolite of OIT.
3.1.2.1	Description	Waxy white solid
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	Stable refrigerated
3.1.2.4	Preparation of test substance for application	Dilutions for induction: 3, 10 and 30% in acetone:olive oil (4:1)
3.1.2.5	Pretest performed on irritant effects	Not applicable
3.2	**Test Animals**	
3.2.1	Species	Mice
3.2.2	Strain	[redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	9 weeks at test initiation Animals weighed 19-24 g at test initiation.
3.2.6	Number of animals per group	5
3.2.7	Control animals	Yes, acetone:olive oil (4:1) as the vehicle control and 35% hexylcinnamaldehyde in acetone:olive oil (4:1) as the positive control

Section A6.1.5/02 Skin sensitisation

Annex Point IIA6.1.5 *(Local Lymph Node Assay)*

3.3	**Administration/ Exposure**	Non-Adjuvant
3.3.1	Induction schedule	3 induction doses, 25 µl each ear (total volume of 50 µl), once per day for 3 days
3.3.2	Way of Induction	25 µl of either the test substance or vehicle was applied to the dorsum of each ear Open application
3.3.3	Concentrations used for induction	3, 10 and 30% in acetone:olive oil (4:1), NNOMA
3.3.4	Challenge schedule	Not applicable
3.3.5	Concentrations used for challenge	Not applicable
3.3.6	Rechallenge	Not applicable
3.3.7	Scoring schedule	Three days after the last induction dose, each animal received an intravenous injection of 20 µCi of ^{3}H-thymidine. Approximately 5 hours later, the draining lymph nodes were collected and incorporation of the ^{3}H-thymidine was assessed by scintillation counting.
3.3.8	Removal of the test substance	Not applicable
3.3.9	Positive control substance	35% hexylcinnamaldehyde in acetone:olive oil (4:1)
3.4	**Examinations**	
3.4.1	Pilot study	No
3.5	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	Not applicable
2.3	**Results of test**	
4.1.1	24h after challenge, 48h after challenge	Not applicable
4.1.2	Incidence	The positive control, hexylcinnamicaldehyde, responded as expected. Exposure to NNOMA with 3, 10 and 30% resulted in stimulation indices of 1.30, 2.20 and 2.10, respectively.
4.2	**Overall result**	Not a sensitizer

Section A6.1.5/02 **Skin sensitisation**

Annex Point IIA6.1.5 *(Local Lymph Node Assay)*

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

OECD Guideline No. 429, skin sensitisation and US EPA OPPTS 870.2600: Local lymph node assay in CBA/J female mice. There were no guideline deviations.

5.2 Results and discussion

There was no mortality and all animals appeared normal throughout the study. At termination, the lymph nodes from the vehicle control and NNOMA treated mice were normal in size and appearance. There were no statistically significant differences of mean body weights on Day 1 and Day 6 between any of the treatment groups. The positive control, 35% hexylcinnamicaldehyde, resulted in a stimulation index (SI) of 7.26 and was statistically significant when compared to the acetone:olive oil vehicle control group. Exposure to NNOMA with 3, 10 and 30% resulted in stimulation indices of 1.30, 2.20 and 2.10, respectively.

Based on the data of this study, treatment with N-(n-octyl) malonamic acid did not result in a stimulation index of 3 or greater and hence N-(n-octyl) malonamic acid is not considered to have skin sensitizing activity.

5.3 Conclusion

N-(n-octyl) malonamic acid was not a sensitizer under the conditions of this assay.

5.3.1 Reliability

(1) valid without restrictions

5.3.2 Deficiencies

No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*18/06/2008*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	*1*
Acceptability	*Acceptable*
Remarks	*In agreement with the applicant's evaluation.*
	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	